510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k101644

B. Purpose for Submission:
New assay

C. Measurand:
Human Anti-Tissue Transglutaminase (tTG) Immunoglobulin G (IgG) antibodies

D. Type of Test:
Semi-quantitative chemiluminescent immunoassay

E. Applicant:
INOVA Diagnostics, Inc.

F. Proprietary and Established Names:
QUANTA Flash h-tTG IgG
QUANTA Flash™ h-tTG IgG Calibrators
QUANTA Flash™ h-tTG IgG Controls

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5660, Multiple Autoantibodies Immunological Test System
   21 CFR §862.1150, Calibrator
   21 CFR §862.1660, Single (specified) analyte controls (assayed and unassayed)
2. Classification:
   Class II (Assay and calibrator)
   Class I (Controls)
3. Product code:
   MVM, Autoantibodies, endomysial (tissue transglutaminase)
   JIX, Calibrator, Multi-Analyte Mixture
   JJX, Single (specified) analyte controls (assayed and unassayed)
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   QUANTA Flash™ h-tTG IgG:
   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 celiac patients with selective IgA deficiency.
   QUANTA Flash™ h-tTG IgG Calibrators:
   The QUANTA Flash™ h-tTG IgG Calibrators are intended for use with the QUANTA Flash™ h-tTG IgG chemiluminescent immunoassay (CIA) on the BIO-FLASH™ instrument. Each calibrator establishes a point of reference for the working curve that is used to determine values in the measurement of IgG

anti-h-tTG antibodies in serum.
QUANTA Flash™ h-tTG IgG Controls:
The QUANTA Flash™ h-tTG IgG Controls are intended for quality control purposes of the QUANTA Flash™ h-tTG IgG chemiluminescent immunoassay (CIA) kit run on a BIO-FLASH™ instrument.

2. Indication(s) for use:
   Same as Intended Use
3. Special conditions for use statement(s):
   For prescription use only
4. Special instrument requirements:
   BIO-FLASH™ Instrument System (k083518)

I. Device Description:

QUANTA Flash™ h-tTG IgG Kit contains one reagent pack (cartridge) with sufficient material for 100 tests. Each reagent pack contains the following sealed reagent tubes:

- Microparticle Reagent: 1 vial of recombinant human tissue transglutaminase coated magnetic particles preserved in a sugar and protein mixture.
- Assay Buffer: 1 vial of Tris-buffered saline with protein stabilizers and surfactant. Preservatives: sodium azide & chloramphenicol.
- Tracer IgG: 1 vial of isoluminol conjugated monoclonal anti-human IgG antibody in phosphate buffered saline with protein (bovine) stabilizer. Preservative: sodium azide.
- Sample Buffer: 1 vial of phosphate buffered saline with surfactant. Preservative: sodium azide.

The QUANTA Flash™ h-tTG IgG Kit also contains Resuspension Buffer: 1 vial of phosphate buffered saline, with glycerol, protein (bovine) stabilizer, and preservative (sodium azide).

QUANTA Flash™ h-tTG IgG Calibrators contains: four vials (two each of calibrator 1 and 2) containing human antibodies to h-tTG in a Tris-buffered saline solution with EDTA and sodium azide. Each vial contains sufficient material for 4 uses.

QUANTA Flash™ h-tTG IgG Controls contains: four vials (two each of Negative and Positive Control) containing human antibodies to h-tTG in a Tris-buffered saline solution with EDTA and sodium azide. Each vial contains sufficient material for 15 uses.

Additional Required Materials (available from INOVA Diagnostics, Inc.)

a. BIO-FLASH™ Instrument and Software System
b. BIO-FLASH™ System Rinse contains four 5 liter bottles of phosphate buffered saline with Tween-20 and sodium azide.
c. BIO-FLASH Triggers contains one bottle each of Trigger 1 (the catalyst) and 2 (the oxidant).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   BINDAZYME Human Anti-Tissue Transglutaminase IgG ELISA
2. Predicate K number(s):
   k040466
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	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 celiac patients with selective IgA deficiency.	BINDAZYME™ Human IgG Anti-Tissue Transglutaminase EIA Kit is designed for the *in vitro* measurement of specific IgG autoantibodies against tissue transglutaminase (tTG) present in human serum, as an aid in the diagnosis of Celiac Disease.
Assay Type	Semi-quantitative immunoassay	Same
Analyte Detected	Human IgG anti-tissue transglutaminase autoantibodies	Same
Sample Matrix	Serum	Same
Antigen	Recombinant human tissue transglutaminase	Same

Differences		
Item	Device	Predicate
Cutoff	20 units	6 units
Assay Technology	Chemiluminescent Immunoassay (CIA) utilizing magnetic particles	Enzyme-linked Immunosorbent Assay (ELISA)
Conjugate	Isoluminol conjugated monoclonal anti-human IgG	Horse radish peroxidase conjugated rabbit anti-human IgG
Signal Detected	Luminescence (visible light)	Absorbance at 450nm
Calibration and unit calculation	Instrument specific working curve based off a 5 point lot specific master curve used for unit calculations; stored on the instrument for 30 days.	Five calibrators run each time the assay is run.

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

Interference Testing in Clinical Chemistry; Approved Guideline (CLSI EP 7-A)
Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (CLSI EP09-A2)
Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (CLSI EP5-A)
Evaluation of the Linearity of Quantitative Analytical Methods (CLSI EP6-P2)
Protocols for Determination of Limits of Detection and Limits of Quantitation (CLSI

EP17-A)

L. Test Principle:

The principles of the QUANTA Flash™ h-tTG IgG assay are similar to many other solid phase indirect immunosorbent assays. In this case the solid phase is paramagnetic beads and the detecting reagent is an isoluminol-conjugated anti-human IgG monoclonal antibody. Specifically, a patient's serum is diluted with sample dilution buffer in a disposable cuvette. A small amount of this patient dilution is combined with assay buffer and h-tTG beads in a second cuvette, and mixed. This reaction cuvette is incubated for 9 ½ minutes at 37°C. The cuvette is then exposed to a small magnet that holds the beads in place, the liquid is aspirated, and the beads 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, rather isoluminol conjugated monoclonal anti-human IgG (known as Tracer IgG) is added to the beads in the cuvette, and mixed. Again, the cuvette is incubated for 9 ½ minutes at 37°C. Three wash steps, as described in the first wash step above, are performed on the beads. In the fourth wash step, no liquid is added to the beads after the aspiration.

The cuvette is then placed in a light-tight luminometer and the beads are exposed to a catalyst and an oxidizing agent. These two reagents, or "Triggers", cause the isoluminol to produce a flash of visible light. The light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH™ optical system. The RLU are proportional to the amount of bound isoluminol conjugate, which in turn is proportional to the amount of IgG anti-h-tTG antibodies bound to the h-tTG on the beads.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>
   a. Precision/Reproducibility:
   Imprecision of the assay was evaluated in accordance with CLSI EP5-A2. Precision of the QUANTA Flash™ h-tTG IgG assay was evaluated by running seven patient samples, three were close to the cutoff of 20 CU. Samples were run using duplicate aliquots, twice a day, for 25 days over a 34 day period on one reagent lot, except for patient 8, which was run for 21 days over a 30 day period. The total number of replicates for each sample was 88.

		Within-Run		Between-Run		Between-Day		**Total**	
Sample	**Mean (CU)**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**
1	781.6	26.9	**3.4%**	44.9	**5.7%**	21.6	**2.8%**	56.7	**7.2%**
2	46.7	1.7	**3.5%**	2.7	**5.9%**	1.5	**3.3%**	3.5	**7.6%**
3	21.0	0.7	**3.3%**	1.1	**5.1%**	0.8	**4.0%**	1.5	**7.2%**
4	25.0	0.8	**3.3%**	1.3	**5.3%**	1.0	**4.1%**	1.9	**7.4%**
5	34.0	1.1	**3.1%**	2.0	**5.9%**	1.8	**5.4%**	2.9	**8.6%**
6	20.6	0.8	**3.9%**	1.2	**5.7%**	0.6	**2.8%**	1.5	**7.4%**
7	13.8	0.6	**4.1%**	0.6	**4.2%**	0.4	**2.8%**	0.9	**6.6%**

To investigate the imprecision of the assay at the extreme ends of the assay, calibrator materials were tested as samples in triplicate per run for five days (one run per day). The total number of replicates was 45.

Lot	Sample	CU	With-in Run Range	Total
1	Standard 1	3.8	1.3% – 6.1%	4.9%
	Standard 5	2560	0.5% - 3.3%	2.7%
2	Standard 1	3.8	1.2% - 10.7%	5.0%
	Standard 5	2560	0.1% - 6.5%	4.1%
3	Standard 1	3.8	0.9% - 10.7%	6.5%
	Standard 5	2560	0 – 12.5%	7.5%

Lot-to- Lot variation was evaluated by testing several clinical samples with two lots of the assay. Lot RP0002 was tested 24 times over 12 days while RP0003 was tested six times on one day.

Lot:	**RP0002**	**RP0003**			
Sample	**Avg CU**	**Avg CU**	**Average**	**St Dev**	**% CV**
G~9	19	13	16	3.8	23%
G~4	20	20	20	0.1	0%
G~6	57	56	56	0.9	2%
G~2	85	82	83	2.1	3%
G~8	95	98	96	1.9	2%
G~1	145	135	140	7.2	5%
G~7	169	191	180	15.4	9%

b. Linearity/assay reportable range:

Seven serum samples were selected to cover the entire range of the assay (see table below for concentrations). Dilutions were made by combining the selected sample with a known negative serum sample, as described in CLSI EP6-A. The observed values were graphed against the calculated values and linear regression was performed.

Sample	**Test Range (CU)**	**Slope (95% CI)**	**Y-intercept (95% CI)**	**R^2**
1	8.5 – 286.6	1.02 (0.97-1.057)	2.20 (-5.2 - 9.6)	0.99
2	10.0 – 191.7	1.03 (0.99-1.06)	-2.63 (-12.0 – 6.8)	0.98
3	54.5 – 1624.8	1.05 (0.99 – 1.10)	-6.40 (-52.2 – 39.4)	0.99
4	6.2 – 40.3	1.09 (0.97-1.22)	-1.25 (-4.14 -1.63)	0.98
5	5.1 – 7.8	1.37 (0.70 - 2.05)	-2.80 (-7.08 - 1.48)	0.93
6	16.0 – 468.0	1.03 (1.00 – 1.07)	-12.7 (-21.0 - -3.50)	1.00
7	857 – 3407.0	1.23 (0.98 – 1.48)	-880.6 (-1481 – -280)	0.96

The reportable range of the assay is defined by the lowest and highest points on the master calibration curve. The lowest point is 3.8 CU while the top point is 2560.0 CU. A separate experiment showed that the assay does not appear to demonstrate a hook effect at very high (above the measuring range) concentrations.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability: There is no reference standard for IgG anti-human tissue transglutaminase antibodies. Calibrators and controls are assigned values based on a 20 unit cutoff between positive and negative during assay development.
Calibrators: The QUANTA Flash™ h-tTG IgG assay utilizes a predefined lot-specific Master Curve that is stored in the reagent pack barcode. The QUANTA Flash™ Calibrators are designed to produce an instrument-specific Working Curve from the parameters of the Master Curve. The two calibrator values are assigned using in-house standards and a four-parameter Master Curve. The assignment values of the two calibrators are used to create a lot-specific four-parameter logistic curve, using two stored parameters from the Master Curve and two lot-specific parameters based on the calibrator values. Calibrators showed acceptable accelerated stability for 2 weeks at 37^{o}C, translating to at least 1 year of storage at 2-8^{o}C. The calibrators may be stored open for a maximum of 8 hours onboard the instrument.
Controls: Controls are manufactured by diluting human serum containing high-titer IgG anti-h-tTG antibodies into buffer. A target CU value is achieved through trial dilutions on a small scale. Once a dilution is selected, the control is bulked, tested, and adjusted. The product undergoes extensive final validation testing to assign a final value.
Controls showed acceptable accelerated stability for 2 weeks at 37^{o}C, translating to at least 1 year of storage at 2-8^{o}C. Controls may be used up to 15 times, 1 hour per use onboard the instrument.
Reagent Pack Stability: The reagent pack can be stored, unopened, at 2-8°C for 1 year based on accelerated stability testing (2 weeks at 37^{o}C). Opened reagent packs must be stored onboard the instrument, and are stable for 60 days. The working curve is valid for 30 days. Recalibration must be done every 30 days.
Sample Stability: Serum sample stability claims and storage recommendations in the package insert are based on CLSI H18-A3 “Procedures for the Handling and Processing of Blood Specimens; Approved Guideline — Third Edition”.

d. *Detection limit:*
Limit of Blank (LoB) was determined according to EP17-A by testing Sample Buffer with no serum 40 times; the LoB was 1.1 CU. The Limit of Detection (LoD) was determined according EP17-A; the LoD was 1.7 CU.

e. *Analytical specificity:*
Interfering substances: Aliquots of a negative and two positive samples were

mixed with normal sera containing a known concentration of an interferent: bilirubin (9.5 mg/dL), cholesterol (342 mg/dL), triglycerides (643 mg/dL), or a serum hemolyzed by freezing so as to contain a high amount of hemoglobin (estimated as 20 g/dL). Control samples were mixed with a known negative serum. The results of the 1 part sample: 2 part interferent in normal seum dilutions are summarized below:

	Normal		Positive 1*		Positive 2	
	CU	% Control	CU	% Control	CU	% Control
Control	3.8	---	13.4	---	114	---
Bilirubin	3.8	100 %	16.2	123 %	110	96 %
Cholesterol	3.8	100 %	15.0	114 %	106	93 %
Triglycerides	8.5	243 %	19.5	148 %	103	90 %
Hemolyzed	3.8	100 %	12.0	91 %	66	58 %

* Low positive sample (33.1 CU) became negative when diluted with normal (i.e. negative) serum + interferent

A statement indicating that lipemic, icteric, or grossly hemolyzed sera should not be used is included in the package insert.

Analytical Cross-reactivity: Samples from patients with various autoimmune diseases or infectious disease markers were tested in the QUANTA Flash™ h-tTG IgG assay. One patient with CMV, 2 patients with HSV, 1 patient with ANA, and 1 patient with H. pylori IgA were positive for IgG anti-tTG. All other serum samples were negative.

Patient Group	N	anti-tTG IgG positive
CMV	12	1
HSV	17	2
HCV	15	0
RA (CCP/RF)	10	0
ANA	20	1
Crohn's disease	17	0
Ulcerative colitis	9	0
H. pylori IgA	15	1
Hashimoto's Thyroiditis	15	0
Grave's disease	15	0

f. Assay cut-off:

The assay cutoff was determined by testing 446 clinically characterized samples—single bleeds of patients who were clearly positive or negative for celiac disease (and were not on a gluten-free diet). These samples - 117 clinically positive and 329 clinically negative - were used to adjust the cutoff to 20 CU to optimize sensitivity and specificity at 49.6% and 97.6%, respectively, in this training set.

2. Comparison studies:

a. Method comparison with predicate device:
Samples for method comparison analysis included those samples from the clinical validation studies (CD, non-CD, and dermatitis herpetiformis patients) that were within the reportable range of the assay. These samples were tested on both the QUANTA Flash™ h-tTG IgG and on the predicate ELISA.

		Predicate tTG IgG ELISA		
		Positive	Negative	Total
QUANTA Flash™ h-tTG IgG	Positive	37	0	37
	Negative	4	70	74
	Total	41	70	111

Positive agreement (37/41) = 90.2 % (95% C.I. = 76.9 – 97.3%)
Negative agreement (70/70) = 100 % (95% C.I. = 94.9 - 100%)
Overall agreement (107/111) = 96.4 % (95% C.I. = 91.1 – 98.6%)

b. Matrix comparison:
Not applicable; serum is the only indicated matrix for this assay.

3. Clinical studies:
a. Clinical Sensitivity and Specificity:
The clinical validation study included 199 normal blood donors, 71 non-celiac disease controls, 68 samples from an academic study (18 CD and 50 non-CD controls), 23 additional CD samples from the INOVA serum library, and 7 known CD with selective IgA deficiency. These samples were tested with the QUANTA Flash™ h-tTG IgG CIA. The results of this testing are shown below:

		Diagnosis		
		CD	Not CD	Total
QUANTA Flash™ anti-tTG IgG	Positive	24	4	28
	Negative	24	316	340
	Total	48	320	368

Sensitivity (24/48) = 50.0% (95% C.I. = 35.2 – 64.8%)
Specificity (316/320) = 98.4% (95% C.I. = 96.4 - 99.5%)

b. Other clinical supportive data:
The study above contained seven samples from Celiac Disease patients with selective IgA deficiency; six samples tested positive for the QUANTA Flash™ h-tTG IgG.

4. Clinical cut-off**:**
See Assay Cutoff.
5. Expected values/Reference range:
The expected value in the general population is negative. However, as the incidence of CD in the normal population is about 1%, some apparently healthy,

asymptomatic individuals may test positive for tTG antibodies.

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.