510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k094060

B. Purpose for Submission:
New device

C. Measurand:
Anti-human Tissue transglutaminase (h-tTG) IgA

D. Type of Test:
Semi-quantitative chemiluminescent immunoassay (CIA)

E. Applicant:
INOVA Diagnostics, Inc.

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

G. Regulatory Information:
1. Regulation section:
   21 §CFR 866.5660, Multiple Autoantibodies Immunlogical 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 IgA: 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 and dermatitis herpetiformis.
   QUANTA Flash™ h-tTG IgA Calibrators: The QUANTA Flash™ h-tTG IgA Calibrators are intended for use with the QUANTA Flash™ h-tTG IgA chemiluminescent immunoassay (CIA) on the BIO-FLASH™ instrument. Each calibrator establishes a point of reference for the working curve that is used to determine Chemiluminescent Units (CU) values in the measurement of IgA anti-h-tTG antibodies in serum.

QUANTA Flash™ h-tTG IgA Controls: The QUANTA Flash™ h-tTG IgA Controls are intended for quality control purposes of the QUANTA Flash™ h-tTG IgA 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 IgA Kit (Catalog Number 701100) 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 IgA: 1 vial of isoluminol conjugated monoclonal anti-human IgA 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 IgA Kit also contains Resuspension Buffer: 1 vial of phosphate buffered saline, with glycerol, protein (bovine) stabilizer, and preservative (sodium azide).

QUANTA Flash™ h-tTG IgA Calibrators (Catalog Number 701101) 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 IgA Controls (Catalog Number 701102) 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):
   QUANTA Lite™ h-hTG IgA ELISA

2. Predicate 510(k) number(s):
   k011566

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	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 and dermatitis herpetiformis.	Same
Assay Type	Semi-quantitative immunoassay	Same
Analyte Detected	Human IgA anti-tissue transglutaminase autoantibodies	Same
Cutoff between positive and negative	20 units	Same
Sample Matrix	Serum	Same

Differences		
Item	Device	Predicate
Antigen	Recombinant human tissue transglutaminase from baculovirus	Native human red blood cell tissue transglutaminase
Assay Technology	Chemiluminescent Immunoassay (CIA) utilizing magnetic particles	Enzyme-linked Immunosorbent Assay (ELISA)
Conjugate	Isoluminol conjugated monoclonal anti-human IgA	Horse radish peroxidase conjugated goat anti-human IgA
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.	Single point determination for unit calculations, 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 IgA 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 IgA 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 IgA (known as Tracer IgA) 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 IgA anti-h-tTG antibodies bound to the h-tTG on the beads.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
      Intra- and inter-assay: Testing was performed in accordance with CLSI EP5-A2. Precision of the QUANTA Flash™ h-tTG IgA assay was evaluated by running 7 patients with values across the entire reportable range of the assay and with several samples close to the cutoff of 20 CU. Patients 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.

Sample	N	Mean (CU)	Within-Run SD	Within-Run % CV	Between-Run SD	Between-Run % CV	Between-Day SD	Between-Day % CV	Total SD	Total % CV
1	100	8.7	0.3	**3.5%**	0.2	**2.5%**	0.0	**0.0%**	0.3	**4.0%**
2	100	22.9	0.6	**2.6%**	0.7	**3.0%**	0.3	**1.3%**	1.0	**4.2%**
3	100	25.1	0.5	**2.2%**	0.8	**3.2%**	0.5	**1.8%**	1.1	**4.2%**
4	100	40.5	1.2	**2.9%**	1.5	**3.6%**	0.0	**0.0%**	1.8	**4.5%**
5	100	69.6	1.5	**2.2%**	2.5	**3.6%**	1.0	**1.4%**	3.1	**4.4%**
6	100	342.1	10.6	**3.1%**	9.9	**2.9%**	4.6	**1.3%**	15.2	**4.4%**
8	84	3476.1	224.3	**6.5%**	277.4	**8.0%**	171.6	**4.9%**	395.8	**11.4%**

Lot-to-lot: Patient samples were tested using three different lots of QUANTA

Flash™ h-tTG IgA reagent cartridges. All patient samples met the acceptance criteria of <15% CV across the reagent cartridge lots.

| Sample | Reagent Cartridge Lot | | | Mean | SD | %CV |
| | 1 | 2 | 3 | | | |
	Avg CU	Avg CU	Avg CU			
2	950.9	998.1	976.0	975.0	24	2%
3	278.7	277.7	264.2	273.5	8	3%
5	90.7	86.4	87.1	88.1	2	3%
6	203.1	218.5	223.2	214.9	10	5%
8	702.0	671.0	650.6	674.6	26	4%
9	667.1	652.3	654.2	657.9	8	1%
10	80.2	74.7	75.9	76.9	3	4%
11	204.9	161.6	185.6	184.0	22	12%
12	2412.3	2553.7	2627.0	2531.0	109	4%

b. *Linearity/assay reportable range:*
Positive serum samples were selected to cover the entire range of the assay, including one with low reactivity (30 CU), one with moderate reactivity (75 CU), two with high reactivity (100 and 500 CU) and two with very high reactivity (2000 CU and 3800 CU). Dilutions were made by combining the positive sample with a 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	1.9-45.4	1.0 (0.95-1.05)	0.93 (-0.26-2.2)	1.000
2	2.8-118.3	1.03 (0.99-1.06)	0.68 (-1.38-2.7)	1.000
3	3.0-141.3	0.99 (0.95-1.02)	1.05 (-1.46-3.56)	1.000
4	17.6-724.7	1.02 (0.98-1.06)	7.81 (-7.25-22.87)	1.000
5	2.2-2699.0	1.05 (1.0-1.09)	31.18 (-18.39-80.76)	1.000
6	10.0-5428.0	0.92 (0.86-0.98)	-100.9 (-267.2-65.5)	0.990

The claimed reportable range is 1.9 CU to 4965.5 CU.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability: There is no reference standard for IgA anti-human tissue transglutaminase antibodies. Calibrators and controls are assigned values based on a 20 units cutoff between positive and negative during assay development.
Calibrators: The QUANTA Flash™ h-tTG IgA 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°C, translating to at least 1 year of storage at 2-8°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 IgA 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 final value is obtained through extensive testing.

Controls showed acceptable accelerated stability for 2 weeks at 37°C, translating to at least 1 year of storage at 2-8°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°C). Opened reagent packs must be stored onboard the instrument, and are stable for 60 days. The working curve is valid for 20 days. Recalibration must be done every 20 days.

d. *Detection limit:*

Limit of Blank (LoB) was determined according to EP17-A by running Sample Buffer with no serum, 40 times, with an average of 0.1 CU. Limit of Detection (LoD) was determined according EP17-A and was found to be 0.3 CU.

e. *Analytical specificity:*

Interfering Substances

Following EP7-A2, sera with specific quantities of hemoglobin (frozen lysed red blood cells to give maximum concentration), bilirubin (29.7 mg/dL), cholesterol (354 mg/dL) or triglycerides (1016 mg/dL) were combined with either negative serum (to evaluate any false positive reactivity) or positive patients (to evaluate any decrease in reactivity). The single negative patient, along with a low and a moderate positive patient were each diluted 1:1 and 1:2 of patient sample to interfering substance. Each serum was also diluted with a known negative sample as a control. The results of the 1:2 dilutions are summarized below:

	Normal		Positive 1		Positive 2	
	CU	% of Control	CU	% of Control	CU	% of Control
Control	5.1	--	27.1	--	71.6	--
Bilirubin	7.2	142%	33.4	123%	78.1	109%
Cholesterol	7.1	138%	30.3	112%	78.9	110%
Triglycerides	5.5	107%	26.8	99%	73.3	102%
Hemolyzed	6.4	125%	26.0	110%	48.0	67%

None of the endogenous compounds tested caused an abnormal increase in signal with the normal patient (small changes in very small numbers give high percentages). With the two positive patients, bilirubin caused a 23% increase in

signal for one patient, while hemolyzed serum caused a 33% decrease in signal for the other patient. A statement indicating that hemolyzed and icteric sera should not be used is included in the direction insert.

Cross-Reactivity:

100+ patient samples with various antibodies to autoimmune or infectious disease markers were tested in the QUANTA Flash™ h-tTG IgA assay. One patient with Grave's disease and one patient with *H. pylori* antibodies were positive for IgA anti-tTG. All other serum samples were negative.

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

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 U/mL to optimize sensitivity and specificity at 92.3% and 98.2%, 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 herpetiformus samples) that were within the reportable range of the assay. These samples were tested on both the QUANTA Flash™ IgA h-tTG and on the predicate ELISA.

		tTG IgA ELISA		Total
		Positive	Negative	
QUANTA Flash™ IgA h-tTG CIA	Positive	69	2	71
	Negative	0	69	69
	Total	69	71	140

Positive agreement (69/69) = 100% (95% C.I. = 94.7 – 100%)

Negative agreement (69/71) = 97.2% (95% C.I. = 90.3 – 99.2%)

Overall agreement (138/140) = 99.1% (95% C.I. = 95.0 – 99.6%)

b. *Matrix comparison:*

Not applicable

3. Clinical studies:
   a. *Clinical Sensitivity and specificity:*
   The clinical validation study included 200 normal blood donors, 71 non-celiac disease controls, 77 samples from a tTG workshop (27 CD and 50 non-CD controls), and 29 additional CD samples form the Inova serum library. These samples were tested with the QUANTA Flash™ h-tTG IgA kit. The results of this testing are shown below:

		Diagnosis		
		CD	Not CD	Total
QUANTA Flash™ h-tTG IgA CIA	Positive	47	6	53
	Negative	3	315	318
	Total	50	321	371

   Sensitivity (47/50) = 94.0% (95% C.I. = 83.5 – 98.7%)
   Specificity (315/321) = 98.1% (95% C.I. = 96.0 – 99.3%)

   b. Other clinical supportive data (when a. is not applicable):
   25 patients with dermatitis herpetiformus were tested with the QUANTA Flash™ h-tTG IgA kit. 20 of these patients were positive for anti-h-tTG IgA antibodies. All five negative patient samples also tested negative with the predicate kit. 19 of the 20 samples testing positive with the QUANTA Flash™ kit also tested positive with the predicate kit.

4. Clinical cut-off:
   See Assay Cutoff.

5. Expected values/Reference range:
   The expected value in the general population is 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.