510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k111414

B. Purpose for Submission:
New assay

C. Measurand:
IgG and IgA anti-deamidated gliadin peptide (DGP) antibodies

D. Type of Test:
Semi-quantitative chemiluminescent immunoassay

E. Applicant:
INOVA Diagnostics Inc.

F. Proprietary and Established Names:
QUANTA Flash™ DGP Screen
QUANTA Flash™ DGP Screen Calibrators
QUANTA Flash™ DGP Screen Controls

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5750 – Radioallergosorbent (RAST) Immunological Test System
   21 CFR §862.1150 – Calibrator
   21 CFR §862.1660 – Quality Control Material (Assayed and Unassayed)
2. Classification:
   Class II (Assay and calibrator)
   Class I (Control)
3. Product code:
   MST – Antibodies, Gliadin
   JIX – Calibrator, Multi-Analyte Mixture
   JJX – Single (Specified) Analyte Controls (Assayed and Unassayed)
4. Panel:
   Immunology (82)
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   The QUANTA Flash™ DGP Screen is a chemiluminescent immunoassay (CIA) for the semi-quantitative detection of IgG and IgA anti-deamidated gliadin peptide (DGP) antibodies in human serum on the BIO-FLASH™ instrument. It is an aid in the diagnosis of celiac disease as well as dermatitis herpetiformis in conjunction with clinical findings and other laboratory tests.

   The QUANTA Flash™ DGP Screen Calibrators are intended for use with the QUANTA Flash™ DGP Screen 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/IgA anti-DGP antibodies in serum.

   The QUANTA Flash™ DGP Screen Controls are intended for quality control

purposes of the QUANTA Flash™ DGP Screen 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:

1. QUANTA Flash™ DGP Screen 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 synthetic DGP coated magnetic particles preserved in buffer solution.
   - Assay Buffer: 1 vial of Tris-buffered saline with protein stabilizers (sodium azide and chloramphenicol) and surfactant.
   - Tracer IgG/IgA: 1 vial of isoluminol conjugated monoclonal anti-human IgG/IgA antibodies in phosphate buffered saline with protein (bovine) stabilizer and preservative (sodium azide).
2. QUANTA Flash™ DGP Screen Calibrators contain four vials (two each of calibrator 1 and 2) containing human antibodies to DGP in a Tris-buffered saline solution with chloramphenicol and sodium azide. Each vial contains sufficient material for 4 uses. Each calibrator establishes a point of reference on a 6-point lot specific master curve that is used for unit calculations. The master curve is stored on the instrument for the life of the reagent lot.
3. QUANTA Flash™ DGP Screen Controls contain four vials (two each of Negative and Positive Controls) containing human antibodies to DGP in a Tris-buffered saline solution with chloramphenicol and sodium azide. Each vial contains sufficient material for 15 uses.
4. Additional Required Materials
   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 contain one bottle each of Trigger 1 (the catalyst) and 2 (the oxidant).

J. Substantial Equivalence Information:

1. Predicate device name(s) and Predicate k number(s):
   QUANTA Lite™ Celiac DGP Screen, k062708
2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	A chemiluminescent immunoassay (CIA) for the semi-quantitative detection of IgG and IgA anti-deamidated gliadin peptide (DGP) antibodies in human serum on the	Same

Similarities		
Item	Device	Predicate
	BIO-FLASH™ instrument. It is an aid in the diagnosis of celiac disease as well as dermatitis herpetiformis in conjunction with clinical findings and other laboratory tests.	
Assay Type	Semi-quantitative immunoassay	Same
Analyte Detected	Human IgG and IgA anti-DGP antibodies	Same
Cutoff between positive and negative	20 CU: Negative < 20 CU Positive ≥ 20 CU	Same
Sample Matrix	Serum	Same
Antigen	Synthetic DGP	Same

Differences		
Item	Device	Predicate
Assay Technology	Chemiluminescent Immunoassay (CIA) utilizing magnetic particles	Enzyme-linked Immunosorbent Assay (ELISA)
Conjugate	Isoluminol conjugated monoclonal anti-human IgG and IgA	Horse radish peroxidase conjugated goat anti-human IgG and IgA
Signal Detected	Luminescence (visible light)	Absorbance at 450nm
Calibration and unit calculation	Instrument specific working curve based off a 6-point lot specific master curve used for unit calculations; stored on the instrument for the life of the reagent lot.	Single point determination for unit calculations, run each time the assay is run.

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

- 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:

Anti-DGP antibodies present in the serum bind to DGP-coated paramagnetic beads in a disposable cuvette during an incubation step. After washes, isoluminol conjugated monoclonal anti-human IgG and IgA (known as Tracer IgG/IgA) is added to the beads in the cuvette, and incubated. After additional wash steps, the cuvette is placed in a luminometer and the beads are exposed to a catalyst and an oxidizing agent. 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 anti-DGP IgG

and IgA antibodies in serum that was bound to the DGP 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™ DGP Screen assay was evaluated by running samples from 7 patients with values across the entire reportable range of the assay and with several samples close to the cutoff of 20 CU. Samples were run in duplicate, twice a day, for 21 days over a 32 day period on one reagent lot. Results of the study are shown in the table below:

Sample	Mean (CU)	Within Run		Between-Day		Between-Run		Total	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV
1	13.4	0.4	3.0%	0.2	1.9%	0.1	0.9%	0.5	3.6%
2	21.7	0.8	3.8%	0.6	2.7%	0.1	0.5%	1.0	4.7%
3	24.1	1.0	4.3%	0.8	3.3%	0.0	0.0%	1.2	5.0%
4	26.9	1.0	3.9%	0.6	2.1%	0.0	0.0%	1.0	3.9%
5	46.7	1.8	3.9%	1.1	2.4%	0.2	0.4%	2.1	4.6%
6	85.5	3.2	3.7%	2.1	2.5%	0.0	0.0%	3.5	4.1%
7	1354.7	57.7	4.3%	29.0	2.1%	29.4	2.2%	70.9	5.2%

To assess the lot-lot-lot reproducibility of the QUANTA Flash™ DGP Screen assay, 13 patient samples were tested using 3 different lots. Each sample was run once on each lot. The average %CV of the 13 patients ranged between 2% and 14%. The study results are summarized in the table below:

Sample	Lot #1	Lot #2	Lot #3	Average CU	%CV
1	15	16	18	17	9%
2	17	20	17	18	12%
3	29	33	30	31	7%
4	42	40	41	41	2%
5	40	50	44	45	11%
6	43	51	48	47	8%
7	86	114	107	102	14%
8	109	116	101	109	7%
9	116	115	110	113	3%
10	150	159	146	152	4%
11	308	346	319	325	6%
12	343	415	393	384	10%
13	898	731	828	819	10%

b. Linearity/assay reportable range:

Six serum samples were selected to cover the entire range of the assay. Each sample was proportionally diluted with a known negative serum sample and tested. The observed values were graphed against the calculated values and linear regression

was performed. The study results are summarized in the table below:

Sample	Test Range (CU)	Slope (95% CI)	Y-intercept (95% CI)	R^2
1	1.6 to 30.1	0.96 (0.92 to 1.00)	0.08 (-0.65 to 0.81)	1.00
2	1.4 to 67.7	0.86 (0.84 to 0.89)	1.26 (0.40 to 2.12)	1.00
3	4.9 to 292.8	0.96 (0.92 to 1.00)	1.68 (-4.49 to 7.86)	1.00
4	16.4 to 561.0	0.86 (0.83 to 0.89)	-0.37 (-9.71 to 8.97)	1.00
5	30.4 to 1271 .2	0.92 (0.90 to 0.94)	-3.11 (-16.59 to 10.37)	1.00
6	183.8 to 1470.7	1.04 (0.97 to 1.10)	-6.39 (-67.03 to 54.25)	1.00

The claimed reportable range is 0.5 CU to 1461.8 CU.

Non-linearity of very high concentration samples: Two very high concentration samples were tested to assess if there was a hook effect. Each sample was pre-diluted (1:16, 1:8, 1:4, 1:2, and neat) before the standard onboard assay dilution, and then tested according to the standard assay protocol. One sample showed a hook effect in that the RLU did not decrease as expected with dilution. This sample tested above the measuring range for all but the 1:16 pre-dilution. The second sample also did not dilute in a linear fashion and stayed above the measuring range for all but the 1:16 pre-dilution sample.

Dilution Recovery: Four over-range samples were automatically diluted at 1:10 and re-run by the instrument. These samples were also diluted manually, and then tested by the instrument. The differences in recovery between instrument and manual dilutions of each sample ranged from -3 to -9%.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Calibrator and Control traceability: There is no reference standard for IgG/IgA anti-DGP antibodies. Calibrators and controls are assigned values based on a 20 unit cutoff between positive and negative during assay development.

Calibrators: The QUANTA Flash™ DGP Screen 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 up to 8 hours onboard the instrument.

Controls: Controls are manufactured by diluting human serum containing high-titer IgG/IgA anti-DGP 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. Onboard stability testing demonstrated that the controls may be used up to 15 times, 10 minutes 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 63 days. Real time stability studies are ongoing.

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 running immunoglobulin stripped serum diluted at the working dilution of 1:17, 60 times. The LoB is <0.04 CU. The Limit of Detection (LoD) was determined and calculated according EP17-A ; the LoD is 0.04 CU.

e. Analytical specificity:
The effect of endogenous interferents on the performance of the QUANTA Flash™ DGP Screen assay was tested using three samples: a negative (12.1 CU), a weak positive (30.7 CU) and a moderately high positive (183.3 CU). Three different concentrations of interferents were spiked into aliquots of the samples and then tested using the standard assay protocol. The acceptance criteria were 85-115% recovery or ±4 CU (whichever is greater).

No interference was detected with bilirubin up to 10 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 1000 mg/dL, cholesterol up to 224.3 mg/dL and with rheumatoid factor IgM up to 500 IU/mL.

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

f. Assay cut-off:
The assay cutoff of 20 CU was determined by testing 446 clinically characterized samples – single bleeds of patients who were clearly positive (n = 117) or negative (n = 329) for celiac disease (and were not on a gluten-free diet).

2. Comparison studies:
   a. Method comparison with predicate device:
   Two hundred and twenty-eight samples within the reportable range of the assay were tested with the QUANTA Flash™ DGP Screen and the predicate method. The samples were collected from normal subjects, diagnosed celiac patients, and patients with other defined diseases. The study results are summarized in the table below:

		DGP Screen ELISA		
		Positive	Negative	Total
QUANTA Flash™ DGP Screen	Positive	30	6	36
	Negative	5	187	192
	Total	35	193	228

Positive agreement (30/35) = 85.7% (95% C.I. = 69.7 – 95.2%)
Negative agreement (187/193) = 96.9% (95% C.I. = 93.4 – 98.9%)
Overall agreement (217/228) = 95.3% (95% C.I. = 91.5 – 97.6%)

In separate analyses, the results of seven IgA deficient samples from patients with diagnosed celiac disease were compared to the predicate and 25 samples from patients with dermatitis herpetiformis were compared to the predicate. The study results are summarized in the tables below

IgA Deficient Celiac Disease (n = 7)		DGP Screen ELISA			Percent Agreement (95% C.I.)
		Positive	Negative	Total	
QUANTA Flash™ DGP Screen	Positive	4	1	5	Positive Agreement 100.0% (39.8 – 100%)
	Negative	0	2	2	Negative Agreement 66.7% (9.4 – 99.2%)
	Total	4	3	7	Total Agreement 85.7% (42.1 – 99.6%)

Dermatitis Herpetiformis (n = 25)		DGP Screen ELISA			Percent Agreement (95% C.I.)
		Positive	Negative	Total	
QUANTA Flash™ DGP Screen	Positive	17	0	17	Positive Agreement 81.0% (58.1 – 94.6%)
	Negative	4	4	8	Negative Agreement 100% (39.8 – 100%)
	Total	21	4	25	Total Agreement 84.0% (63.9 – 95.5%)

b. Matrix comparison:
Not applicable.

3. Clinical studies:
   a. Clinical Sensitivity and specificity:
   The clinical validation study included 23 celiac disease samples from INOVA's serum library, 71 non-celiac disease controls, and 68 samples from an academic study (18 celiac disease and 50 non-celiac disease controls). An additional, separate external study included 93 celiac disease samples and 98 disease controls. These samples were tested with the QUANTA Flash™ DGP Screen. The combined results of this testing are shown in the table below:

		Diagnosis		
		Celiac disease	Non-celiac disease	Total
QUANTA Flash™ DGP Screen	Positive	121	4	125
	Negative	20	215	235
	Total	141	219	360

Sensitivity (121/141) = 85.8% (95% C.I. = 78.9 – 91.1%)
Specificity (215/219) = 98.2% (95% C.I. = 95.4 – 99.5%)

The study above includes seven samples from celiac disease patients known to be selectively IgA-deficient. They were tested with the QUANTA Flash™ DGP Screen and five were positive.

The results of 25 samples from patients diagnosed with dermatitis herpetiformis tested with the QUANTA Flash™ DGP Screen CIA were compared to their clinical diagnosis. Seventeen of the samples were tested positive by the QUANTA Flash™ DGP Screen.

The distribution of the disease control populations used in the clinical validation study is shown below:

Patient Group	n =	DGP Screen Positive
Ulcerative colitis	9	0
Crohn's disease	17	0
Inflammatory bowel disease (unspecified)	5	0
H. pylori infection	27	2
Other gastrointestinal diseases	11	0
Food allergy	9	0
Type I diabetes	14	0
Systemic rheumatic diseases (various)	12	0
Autoimmune thyroid disease	40	0
Viral Hepatitis	13	0
Autoimmune Liver Disease	5	0
Non-CD controls (unspecified, diagnosis not known)	50	2
Total	360	4

4. Clinical cut-off**:**
   See Assay Cutoff above.
5. Expected values/Reference range:
   The expected value in the general population is negative. However, as the incidence of celiac disease in the normal population is about 1%, some apparently healthy, asymptomatic individuals may test positive for DGP antibodies. A study of 351 apparently healthy individuals tested with the QUANTA Flash™ DGP

Screen showed that 2% were positive (7 of 351). The mean concentration was 3.8 CU and the values ranged from <0.5 to 358.8 CU.

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.