The QUANTA Flash™ Chemiluminescent Immunoassays for Celiac Disease Show Excellent Analytical and Clinical Performance Characteristics

Peter Martis, Priscilla Carrión, Hoi Yan Li, Rufus W. Burlingame, Gabriella Lakos INOVA Diagnostics, Inc., San Diego CA, USA.

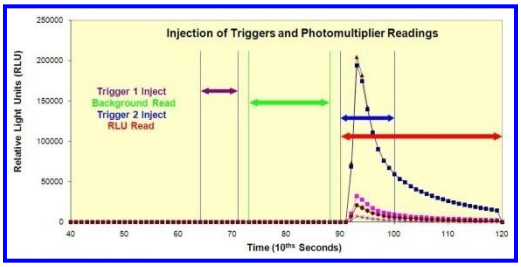
Poster presented at the
24th Annual AMLI Meeting: 13-16th August 2011, San Diego, CA, USA

Objective: To evaluate the analytical and clinical performance of four new chemiluminescent immunoassays (CIAs) for the diagnosis of Celiac Disease (CD) under the QUANTA Flash™ product line: QUANTA Flash h-tTG IgA, h-tTG IgG, DGP IgA, and DGP IgG. This new product line has been developed for the random-access, fully automated BIO-FLASH® instrument.

Introduction: Celiac disease is characterized by chronic inflammation of the intestinal mucosa and villous atrophy on biopsy.[1-2] Currently, serological testing has been suggested for screening patients suspected of having CD, as well as for monitoring dietary compliance.[3-6] Assays using deamidated gliadin peptides (DGPs) have higher diagnostic accuracy for celiac disease than standard anti-gliadin.[5-6] Because a significant proportion of celiac patients have selective IgA deficiency,[7] a sensitive screening strategy for at risk populations includes testing for both IgA and IgG anti-h-tTG and anti-DGP antibodies.

Methods: The precision of the assays was assessed according to CLSI guideline EP5-A2. The lower limit of detection (LoD) and the linearity of the reportable range were determined in accordance with CLSI guidelines EP17-A and EP6-A, respectively. Clinical sensitivity and specificity of the four QUANTA Flash assays were determined using samples from more than 700 clinically characterized subjects. The patient population consisted of CD patients (not on gluten-free diet), healthy subjects, non-CD patients (including infectious disease and inflammatory bowel disease patients), and for h-tTG IgG only, a group of known CD patients with selective IgA deficiency.

Results: The QUANTA Flash h-tTG IgA, h-tTG IgG, DGP IgA and DGP IgG assays have the same cut-off value (20 CU). Their dynamic range is much wider than that of the ELISA methods for the same analytes. The reportable ranges show dilution linearity for all four antibodies throughout the total span of the calibration curve. The within-run, between-run and total %CVs in all four CD assays were less than 12.2%. The clinical sensitivity was found to be 92.8% for h-tTG IgA, 49.7% for h-tTG IgG, 68.1% for DGP IgA, and 81.3% for DGP IgG, respectively. The clinical specificity values were 98.1% for h-tTG IgA, 98.2% for h-tTG IgG, 99.6% for DGP IgA, and 98.2% for DGP IgG, respectively. The h-tTG IgG test was positive in six out of seven IgA-deficient CD patients. All tests have high diagnostic efficiency, characterized by area under the ROC curve values of 0.99 for h-tTG IgA, 0.93 for h-tTG IgG, 0.92 for DGP IgA, and 0.93 for DGP IgG, respectively.

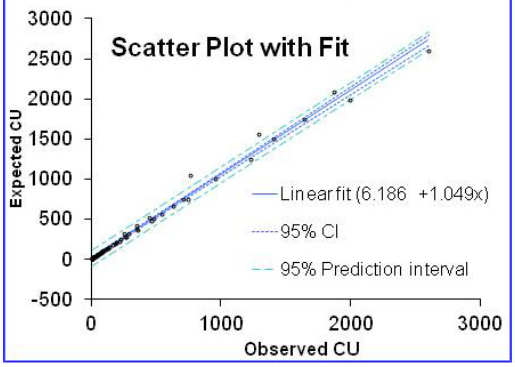


n	64
R^2	0.99
Adjusted R^2	0.99
SE	49.18

Linearity
QUANTA Flash h-tTG IgA

Term	Coefficient	95% CI	SE	t statistic	DF	p
Intercept	6.186	-8.486 to 20.858	7.3397	0.84	62	0.4026
Slope	1.049	1.026 to 1.072	0.0113	92.76	62	<0.0001

Excellent values are slope = 1, intercept = 0, $R^2 > 0.95$

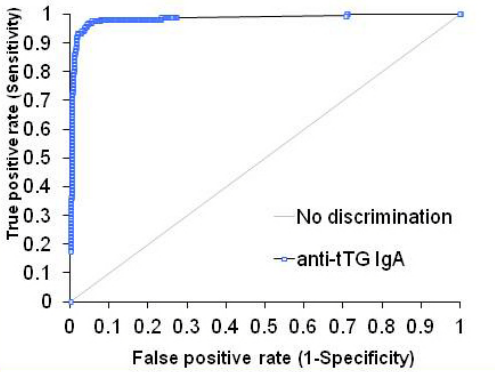


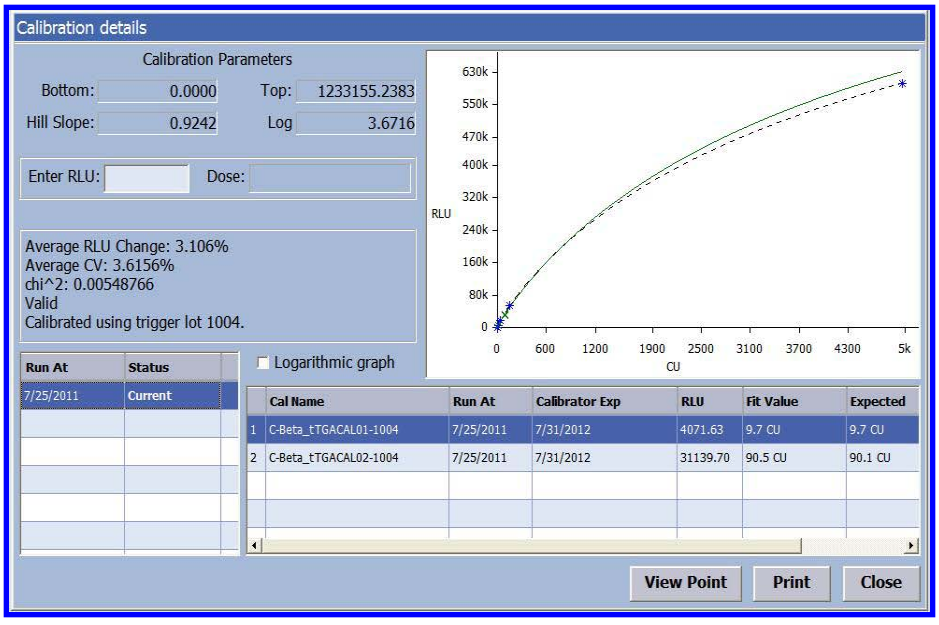
ROC Plot – QUANTA Flash h-tTG IgA

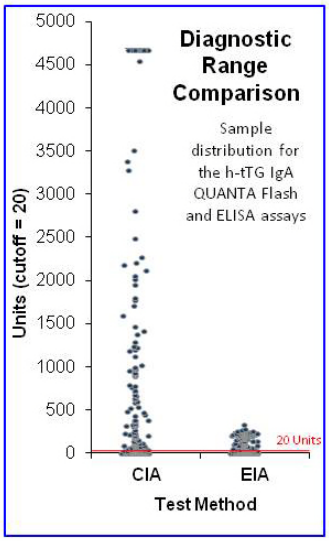
n	817
celiac disease	n
0	650
1	167
Prevalence	0.204

AUC values:
Perfect = 1.0
No discrimination = 0.5

Test	Area	95% CI	SE	Z	p	celiac disease = 1
anti-tTG IgA	0.99	0.97 to 1.00	0.006	85.48	<0.0001	have higher values







Clinical Sensitivity and Specificity					
QUANTA Flash Assay		Diagnosis CD	Not CD	Total	Analysis (95% confidence)
h-tTG IgA (N = 817)	Positive	155	12	167	Sensitivity 93.4% (88.5-96.7%)
	Negative	12	638	650	Specificity 98.2% (96.8-99.0%)
h-tTG IgG (N = 814)	Positive	82	12	94	Sensitivity 49.7% (41.8-57.6%)
	Negative	83	637	720	Specificity 98.0% (96.6-98.9%)
DGP IgA (N = 784)	Positive	62	3	65	Sensitivity 68.1% (57.5-77.5%)
	Negative	29	690	719	Specificity 99.6% (98.7-99.9%)
DGP IgG (N = 705)	Positive	74	11	85	Sensitivity 81.3% (71.8-88.7%)
	Negative	17	603	623	Specificity 98.2% (96.8-99.1%)

Precision				
QUANTA Flash Assay:	h-tTG IgA	h-tTG IgG	DGP IgA	DGP IgG
No. Samples:	7	7	7	8
Range	8.7-3476.1	13.8-781.6	10.5-1927.5	5.8-1781.4
Within-Run %CV	2.2-6.5	3.1-4.1	3.1-5.9	1.9-3.1
Between-Run %CV	2.5-8.0	4.2-5.9	0.0-3.2	0.5-2.5
Total %CV	4.0-11.4	6.6-8.6	7.3-12.2	3.0-4.5

Limit of Detection and Reportable Range				
QUANTA Flash Assay:	h-tTG IgA	h-tTG IgG	DGP IgA	DGP IgG
Lower Limit of Detection	0.3 CU	1.7 CU	1.2 CU	<0.1 CU
Reportable Range	1.9-4965.5 CU	3.8-2560.0 CU	5.2-2367.3 CU	2.8-1936.7 CU
Cutoff	20 CU	20 CU	20 CU	20 CU

Linearity				
QUANTA Flash Assay:	h-tTG IgA	h-tTG IgG	DGP IgA	DGP IgG
No. Samples	6	7	5	6
Range	1.9-5428.0	5.1-3407.0	5.2-2596.8	3.6-2565.4
Slopes	0.92-1.05	1.02-1.37	0.96-1.02	0.94-1.06
r^2	0.99-1.00	0.93-1.00	0.99-1.00	0.99-1.00

Conclusion: The new, fully automated QUANTA Flash h-tTG IgA, h-tTG IgG, DGP IgA, and DGP IgG assays show excellent analytical performance characteristics. Their high clinical sensitivity and specificity make these tests invaluable for the diagnosis and management of Celiac Disease patients.

Reference:

1. Chorzelski TP, et al. IgA class endomysium antibodies in dermatitis herpetiformis and celiac disease. Ann NY Acad Sci 420: 325-334, 1983. **2**. Volta U, et al. Correlation between IgA antiendomysial antibodies and subtotal villous atrophy in dermatitis herpetiformis. J Clin Gastroenterol 14: 298-301, 1992. **3**. Valdimarsson T, et al. Is small bowel biopsy necessary in adults with suspected celiac disease and IgA anti-endomysial antibodies? 100% positive predictive value for celiac disease in adults. Digestive Diseases and Science 41: 83-87, 1996. **4**. Li M, et al. A report on the international transglutaminase autoantibody workshop for celiac disease. Am J Gastro 104: 154-163, 2009. **5**. Sugai E, Vazquez H, Nachman F, et al. Accuracy of testing for antibodies to synthetic gliadin-related peptides in celiac disease. Clin Gastroenterol Hepatol 4: 1112-1117, 2006. **6**. Kaukinen K, et al. Resurrection of gliadin antibodies in coeliac disease. Deamidated gliadin peptide antibody test provides additional diagnostic benfits. Scan J Gastro 42(12): 1428-33, 2007. **8**. Collin P, et al.: Selective IgA deficiency and coeliac disease. Scand J Gastroenterol 27: 367-371, 1992.

Werfen Group | INOVA Diagnostics, Inc.