



QUANTA Plex™ Celiac IgA Profile: Detection of IgA anti-h-tTG, anti-DGP and Selective IgA Deficiency on the Luminex™ Flow Analyzer

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ABSTRACT

Objective: Develop a complete screen for celiac disease using an addressable laser bead immuno-assay (ALBIA) for the simultaneous semi-quantitative detection of IgA anti-human tissue transglutaminase (h-TTG) and anti-deamidated gliadin peptide (DGP) antibodies, and detection of selective IgA deficiency, in human serum.

Methods: Purified recombinant h-TTG and synthetic DGP are each bound to different, fluorescently "colored" beads, as are the control antigens anti-IgA and IgA. The 4 different antigen coated beads are mixed together and put into wells of a microwell plate under conditions that preserve the antigens in their immunologically reactive state. Pre-diluted controls and diluted patient sera are added to separate microwells, allowing any h-TTG and DGP antibodies present to bind to the immobilized antigens. Free IgA will also bind to the anti-IgA bead. Then an anti-human IgA conjugated to a fluorescent probe is added to each microwell. A second incubation allows the anti-human IgA fluorescent conjugate to bind to any patient antibodies that have become attached to the antigens on the beads. The samples are then measured in the Luminex™ flow analyzer. Each antibody can be semi-quantitated by comparing the fluorescent intensity of the patient sample with the fluorescence of the corresponding calibrator. Furthermore, the ratio of the anti-IgA bead to the IgA bead will identify patients with selective IgA deficiency.

Results: The two QUANTA Plex™ tests in the Celiac IgA Profile, anti-h-TTG and anti-DGP, compared very well with the corresponding ELISAs. Only 3.0% of the tests were discrepant (58 of 1,908 tests) between the two types of assays. The anti-IgA/IgA LU ratio is a sensitive indicator of the amount of total serum IgA. Based on serial dilutions of samples with known amounts of total serum IgA, the results of testing 5 known celiac disease patients with selective IgA deficiency, and examining the results of more than 900 other samples, this test is 100% sensitive in detecting samples with selective IgA deficiency.

Conclusion: The ALBIA technique employed by the QUANTA Plex™ Celiac IgA Profile has virtually identical clinical sensitivity and specificity for anti-h-TTG and anti-DGP antibodies as the corresponding ELISAs. The anti-IgA and IgA control beads detected selective IgA deficiency in all 5 known cases. This result indicates the need for follow-up IgG anti-h-TTG and anti-DGP antibody testing. This multiplex technology is useful for screening people for celiac disease because it contains the three most common screening tests in a single well.

INTRODUCTION

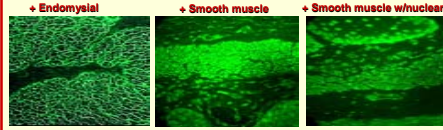
Celiac disease and dermatitis herpetiformis, the two recognized forms of gluten sensitive enteropathies (GSE), are characterized by chronic inflammation of the intestinal mucosa and flattening of the epithelium, or positive "villos atrophy", on biopsy.^{1,3} All GSE patients have an increased risk of lymphoma.⁴ Originally, a series of multiple intestinal biopsies was used to diagnose celiac disease and related disorders.⁴ More recently, serological testing has been suggested for screening patients with suspected GSE as well as for monitoring dietary compliance.^{5,6} Several studies have demonstrated that IgA endomysial antibody (EMA) tests have over 99% specificity for GSE.^{5,6} The endomysial antigen has been identified as the enzyme tissue transglutaminase (tTG).⁷ Recombinant human-tTG (h-TTG) may have certain advantages in solid phase immunoassays compared with the more traditional guinea pig liver antigen. Recent work has revealed that gliadin reactive antibodies from patients with celiac disease bind a very limited number of specific epitopes on the gliadin molecule.^{8,10,11} These studies further reveal that selective deamidation of gliadin by the celiac-associated enzyme tTG results in enhanced binding by anti-gliadin antibodies. Assays using deamidated gliadin peptides (DGPs) have higher diagnostic accuracy for celiac disease than standard anti-gliadin and even anti-tTG assays.^{7,8,12} Additionally, these antibodies are of interest to follow disease activity over time and for monitoring adherence to a gluten-free diet.⁷ Because a significant proportion of celiac patients have selective IgA deficiency,¹³ they do not have IgA antibodies reactive with h-TTG or DGP. Thus, a sensitive screening strategy for at risk populations includes testing for IgA anti-h-TTG and anti-DGP as well as for total serum IgA concentration. We have implemented these three screening tests in one assay on the Luminex™ flow analyzer, as was done for other multiplexed immunoassays.¹⁴

METHODOLOGY

A complete screen for celiac disease in one well requires detection of IgA anti-h-TTG and anti-DGP antibodies, as well as the ability to identify selective IgA deficient patients for further IgG anti-h-TTG and anti-DGP antibody testing. The ability of the QUANTA Plex™ Celiac IgA Profile to detect antibodies against h-TTG and DGP was evaluated by comparison to INOVA QUANTA Lite™ ELISA kits measuring the same antibodies. Samples with sufficient volume that gave discrepant results between comparable tests were then tested for IgA EMA by IFA. The slides were read by someone blinded as to the ELISA and Luminex™ results.

To show substantial equivalence for determining a qualitative level (sufficient or deficient) of total serum IgA, we analyzed the sera from 5 patients who had celiac disease and were IgA deficient. We also obtained 39 sera whose total serum IgA level was determined by nephelometry, and performed serial dilutions of them to determine the absolute amount of IgA that corresponded to our qualitative result.

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RESULTS – h-TTG

Blood Donors N=278	QUANTA Plex Celiac IgA Profile (h-TTG)		
h-TTG IgA ELISA	Negative	Positive	
99.6% agreement	277	1	
	0	0	

*This sample is also positive on IgA DGP Luminex

Celiac disease N=29	QUANTA Plex Celiac IgA Profile (h-TTG)		
h-TTG IgA ELISA	Negative	Positive	
100% agreement	2	0	
	0	27	

Other Controls N=647	QUANTA Plex Celiac IgA Profile (h-TTG)		
h-TTG IgA ELISA	Negative	Positive	
96.6% agreement	478	1**	
	21*	147	

*Sixteen of these were negative on both IgA DGP ELISA and Luminex. Two others were both EMA negative. One was diagnosed autoimmune hepatitis. The remaining two had no diagnosis.
** This sample is diagnosed celiac disease on a gluten-free diet, and is also IgA DGP Luminex positive.

RESULTS – DGP

Blood Donors N=278	QUANTA Plex Celiac IgA Profile (DGP)		
DGP IgA ELISA	Negative	Positive	
99.3% agreement	275	2*	
	0	1	

*These samples were 88 LU and 4 EU, and 27 LU and 7 EU.

Celiac disease N=29	QUANTA Plex Celiac IgA Profile (DGP)		
DGP IgA ELISA	Negative	Positive	
96.0% agreement	10	0	
	1*	18	

*This sample was moderate positive by ELISA, 40 EU, and high negative by Luminex™, 15 LU.

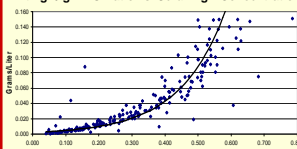
Other Controls N=647	QUANTA Plex Celiac IgA Profile (DGP)		
DGP IgA ELISA	Negative	Positive	
94.0% agreement	498	2**	
	30*	117	

*Eleven were h-TTG IgA negative by both Luminex and ELISA. Sixteen were h-TTG positive by both Luminex and ELISA. However, 9 of these sixteen were already diluted samples used for ELISA screening, and therefore at a greater dilution than that used for the Luminex assay. Three remaining samples were h-TTG Luminex negative but ELISA positive.

**These two samples were also both h-TTG positive by Luminex but negative by ELISA. One is a celiac disease patient on a gluten-free diet. The other has no diagnosis.

RESULTS – IgA DEFICIENCY

Anti-IgA/IgA LU Ratio vs. Serum IgA Concentration



To relate total serum IgA to the anti-IgA/IgA ratio, 39 samples with quantitated serum IgA were serially diluted and plotted. An anti-IgA/IgA LU ratio of 0.5 is equivalent to 0.08 g/L there. A of 954 samples has there are a total of 26 samples with an anti-IgA/IgA LU ratio <0.5.

All 5 celiac patients with IgA deficiency as well as 7 in-house IgG h-TTG positive suspected celiac patients were identified with ratios less than 0.5. Seven more samples were highly diluted for ELISA screening. This leaves 7 of 954 samples also being detected, or less than 1%. Using a ratio of 0.6, only 8 additional samples became positive. Similarly, when the ratio was decreased to 0.4, only 3 of the 26 samples became negative, and none were from the celiac or suspected celiac group. Thus, this robust test is 100% sensitive for detecting patients with selective IgA deficiency.

SENSITIVITY & SPECIFICITY

Clinical Sensitivity for the h-TTG and DGP ELISAs and the Celiac IgA Profile.

All Fbts Controls N=29	ELISA Neg.	ELISA Pos.	Clinical Sensitivity	QUANTA Flex Neg.	QUANTA Flex Pos.	Clinical Sensitivity
h-TTG	2	27	93.1%	2	27	93.1%
DGP	10	19	65.5%	11	18	62.1%

Clinical Specificity for the h-TTG and DGP ELISAs and the Celiac IgA Profile.

All Neg. Controls N=494	ELISA Neg.	ELISA Pos.	Clinical Specificity	QUANTA Flex Negative	QUANTA Flex Positive	Clinical Specificity
h-TTG	484	10	98.0%	483	11	97.8%
DGP	484	10	98.0%	482	12	97.6%

Relative Sensitivity and Specificity for the QUANTA Plex™ Celiac IgA Profile.

All Samples N=954	Both Neg.	Both Pos.	EA Neg. OP Pos.	EA Pos. OP Neg.	Relative Sensitivity	Relative Specificity	Percent Agreement
h-TTG	757	174	2	21	89.2%	99.7%	97.6%
DGP	783	136	4	31	81.4%	99.5%	96.3%

CONCLUSIONS

- The QUANTA Plex™ Celiac IgA Profile is a complete celiac screen in one well. It is an objective, homogeneous, and convenient multiplex based ALBIA for the semi-quantitative detection of IgA anti-h-TTG and anti-DGP antibodies, as well as detection of selective IgA deficiency.
- The QUANTA Plex™ anti-h-TTG and anti-DGP have sensitivities and specificities equivalent to that of ELISA, in the convenience of a multi-plex, no wash assay with only two ½ hour incubations.
- Qualitative detection of selective IgA deficient samples is 100% sensitive.
- The QUANTA Plex™ Celiac IgA Profile is three tests in one, saving labor and space, and reducing chances for technician errors.

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