

THE COMBINATION OF A SYNTHETIC DEAMIDATED GLIADIN PEPTIDE (DGP) AND DUAL ISOTYPE CONJUGATE PROVIDES ENHANCED PERFORMANCE FOR DETECTION OF CELIAC DISEASE

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Abstract

Objective: Assess performance of a new serological assay for aiding in the diagnosis of celiac disease and other forms of gluten – sensitive enteropathy such as dermatitis herpetiformis. This new test is in an automatable ELISA format, uses a novel synthetic deamidated gliadin peptide (DGP) as antigen as well as a conjugate that detects both IgA and IgG antibodies.

Methods: Test specificity was evaluated using 81 non-celiac disease controls and 513 normal healthy blood donors. Clinically defined samples included 85 non-diet treated celiacs, 33 celiacs on gluten free diet, 18 first degree relatives of a celiac, 5 IgA deficient celiacs and 18 patients with dermatitis herpetiformis.

Results: Specificity of this new assay with respect to 513 healthy blood donors was 99.2%. Only 4 samples were positive and 2 of these were just over the 20 unit cutoff at 20.1 and 20.2. One of the false positives had high levels of IgA tTG and may actually have been a celiac. Excellent specificity was also observed for 81 non-celiac disease controls. Only 1 out of the 81 was found positive for a specificity of 98.8%. Overall, sensitivity for non-diet treated celiac disease was 95.3% (81 of 85 patients). The assay was found to be more sensitive than both the tTG and EMA assays.

All 5 IgA deficient celiacs tested were positive on the DGP based assay due to the IgG component of the dual specificity conjugate. The new kit also detected 15 of 18 (83%) dermatitis herpetiformis patients. In this same patient group a tTG assay detected 13 out of 18 or 72% and the EMA assay picked up only 11 patients (61%).

Conclusion: The new celiac disease test (QUANTA Lite™ Celiac DGP Screen) using a fully synthetic construct of deamidated gliadin derived B cell epitopes and a dual reactive conjugate that detects both IgA and IgG antibodies seems to out perform the well-established tTG and endomysial assays currently in use. The new test is both more sensitive and specific for detection of celiac disease, dermatitis herpetiformis and IgA deficient celiac disease and may be a cost effective solution for screening at risk populations. The test may be used as a high performance stand alone assay to detect both IgA deficient as well as IgA sufficient celiacs or the test may be used in conjunction with the well established tTG or EMA assays.

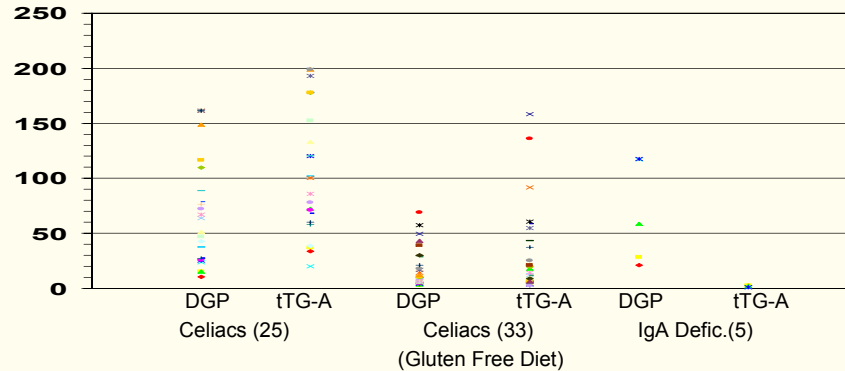
Introduction

It has been shown that synthetic deamidated peptides incorporating major B-cell epitopes of the wheat protein gliadin can form the basis of high performance assays for aiding in diagnosis of celiac disease (1-5). Standardized test kits incorporating synthetic gliadin peptides using IgA or IgG conjugate have proven to be more reliable for aiding in diagnosis of celiac disease compared to kits using whole, native gliadin protein (1,2,4,5) and these newer assays are rapidly replacing gliadin based tests in clinical labs.

These earlier studies have also shown that IgG antibodies directed to the synthetic deamidated peptide are extremely specific for celiac disease in stark contrast to the non-specificity seen with IgG kits using the whole native gliadin protein (1-3). Taking advantage of the high specificity of the IgG antibody assay to deamidated gliadin peptide (DGP) an Elisa assay was developed using DGP as antigen along with a conjugate that uses a blend of both anti-human IgA and IgG antibodies. The goal is to have a single, cost effective, automatable assay that can detect both IgA sufficient and IgA deficient celiac patients with high sensitivity and specificity.

Dermatitis herpetiformis (DH) is a skin disease where two thirds of patients have an enteropathy indistinguishable from celiac disease with the remaining third having features associated with gluten sensitivity. Current serological methods such as EMA and tTG assays exhibit disappointing performance when testing DH patients with sensitivities ranging from only 60-75% (6,7).

DGP and tTG IgA Results on Celiac Patients



Twenty five celiacs, 33 celiacs on gluten free diet (GFD) and 5 biopsy proven, IgA deficient celiacs were tested by both tTG and DGP Screen. All 5 IgA deficient were positive on DGP Screen yet negative by tTG. Twenty four of twenty five celiacs were tTG positive. The one negative sample was just under the 20u cutoff at 19.9u. This one negative sample was DGP Screen positive at 23.5 u. Three celiacs were negative by DGP at 10.8, 15.1 and 15.5 units. All three celiacs were tTG positive. Diet treated celiacs tended to have lower antibody levels by DGP with 9 of 33 (27%) being positive while 10 of 33 (30%) were positive by tTG

Precision/Reproducibility

Inter-assay performance for QUANTA Lite™ Celiac DGP Screen was evaluated by testing 9 specimens a total of 5 times each. The results are summarized in the Table below.

Sample #	1	2	3	4	5	6	7	8	9
Mean unit	57.5	51.8	102.0	27.5	121.5	162.0	7.9	4.6	5.3
SD	0.31	1.39	0.92	0.45	1.33	2.77	0.37	0.19	0.14
CV%	0.5	2.70	0.9	1.7	1.1	1.7	4.7	4.1	2.6

Intra-assay performance was assessed by testing in duplicate, a panel of 5 specimens and the kit high positive control and the negative control twice daily for 3 consecutive days. The results are summarized in the Table below.

Sample #	HPC	NC	1	2	3	4	5
Mean unit	110.4	0.13	54.6	48.5	27.5	157.9	9.8
SD	3.36	0.13	1.36	1.86	0.99	3.85	0.57
CV%	3.0	7.90	2.5	3.8	3.6	2.4	5.8

IgA Deficient Celiac Patients

Five biopsy proven IgA deficient Celiac patient samples were tested for IgA and IgG antibodies to tissue transglutaminase (tTG) and by the Celiac DGP Screen assay.

IgA Deficient Sample #	tTG IgA	tTG IgG	Celiac DGP Screen
1	1.1	11.9	117.7
2	1.9	61.4	58.4
3	1.3	67.3	117.2
4	2.7	20.3	20.9
5	2.5	40.8	28.4

All 5 IgA deficient celiacs were positive on the Celiac DGP Screen kit including 1 individual that was negative by IgG tTG.

Dermatitis Herpetiformis: Sensitivity of Established Serologies vs. DGP

DH is a well established gluten sensitive skin disorder presenting with variable degrees of enteropathy. Currently used serologies have unsatisfactory sensitivity for detection of DH, especially for patients with mild enteropathy.

The table below shows the ability of serological methods to detect DH patients with grades of enteropathy ranging from severe (Marsh III c biopsy) to mild (Marsh II biopsy) to no intestinal pathology (Marsh 0). Six patients in each group were tested with the well regarded tTG and EMA tests as well as individual IgG and IgA kits using DGP (Gliadin II) and the QUANTA Lite™ Celiac DGP Screen.

Intestinal Biopsy	# Patients	# POS (%)				
		tTG	EMA	Gliadin II IgA	Gliadin II IgG	DGP Screen
Type 0	6	3	3	3	3	3
Type II	6	4	2	5	5	6
Type IIIc	6	6	6	6	6	6
Total	18	13(72)	11(61)	14(78)	14(78)	15(83)

The most sensitive assays for detection of DH were the 3 DGP based tests with the DGP Screen detecting 83% of the patients including all 12 with mild to severe enteropathy and 3 of the 6 with no evidence of enteropathy. The EMA test only detected 11 or 61% of this cohort.

References

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Summary of Clinical Performance

Patient Group	#	#Pos	(%)
Celiacs	85	81	95.3%
Celiacs on GFD	33	9	27%
1st degree relatives	18	2*	11%
Celiac IgA Deficient	5	5	100%
Dermatitis Herpetiformis	18	15**	83%
Non Celiac Disease Patients	81	1	1.2%
Normals	513	4***	0.8%

Sensitivity – Based on 81 of 85 Celiacs being positive=**95.3%**
Specificity – Based on only 5 of 81 non-celiac disease controls and 513 normals positive = **99%**

- * One of the relatives was 41.3u and was both tTG and EMA +. The second was 20.2u and was both tTG and EMA negative.
- ** Only 13/18 were tTG positive and only 11/18 were EMA positive. Six of the 18 patients had no intestinal involvement (Marsh 0 biopsy).
- *** Of the 4 samples, 1 was 20.1 and another 20.2u. The cutoff is 20u. Another of the 4 was 25.4u and was also strongly positive for tTG and may actually have been from a person with celiac disease

Conclusions

- A single ELISA assay using a synthetic, gliadin derived, deamidated peptide and dual specificity conjugate demonstrates high sensitivity and specificity for celiac disease.
- The new test may outperform the more commonly used endomysial and tTG assays for detection of IgA deficient celiac disease and dermatitis herpetiformis.
- The new test may be used as a cost-effective stand alone front line screening assay or in conjunction with tTG or EMA assays.
- Further studies are in progress to assess test performance in pediatric patients and for disease monitoring.