

HIGH PERFORMANCE ANTI – GLIADIN ANTIBODY ASSAY: USE OF DEFINED SYNTHETIC PEPTIDES

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Abstract

Objective: The development of an enhanced performance anti – gliadin antibody (AGA) assay by use of deamidated, fully synthetic peptides on the solid phase.

Methods: Five hundred and ten (510) random normal samples as well as 23 samples from biopsy – proven celiacs, 20 samples from celiacs on gluten – free diet, 5 celiacs on diet but still endomysial positive and 10 samples from first degree relatives were tested for IgA and IgG antibodies to deamidated synthetic gliadin – homologous peptides. These same samples were also tested on standard gliadin *ELISA* assays using native, whole gliadin.

The analysis also included 41 endomysial (EMA) and tissue transglutaminase (tTG) double positives, 26 EMA negative IgA deficient sera, 44 samples tTG IgA negative yet gliadin IgG and/or IgA positive by in house *ELISA*'s and 35 additional samples found to be both tTG and gliadin negative.

Results: For the clinically defined samples the IgA peptide - based assay was more sensitive (70% vs. 57%) and more specific (98.2% vs. 96.9%) compared with the standard method. For IgG, sensitivities were comparable (70% vs. 74%) with the peptide – based test, producing significantly improved specificity (99.9% vs. 78.1 %).

Similar side by side testing of samples submitted to a reference lab showed enhanced sensitivity and specificity compared with the standard in house assay.

The test was further evaluated in a large gastroenterology hospital with samples defined serologically and by biopsy. One hundred and three celiacs classified according to a Marsh II or greater biopsy result as well as 114 disease controls were tested. The gliadin peptide assay produced an IgA sensitivity of 95% and 92% for IgG and a 96% result when combined. Specificity was 98% with 1 of the 2 positives having a Marsh I biopsy and tTG positivity.

Conclusion: Use of fully synthetic, deamidated, gliadin homologous peptides increases the clinical utility of AGA detection in patients suspected of celiac disease. The highly refined solid phase addresses the two greatest deficiencies of current methods – lack of sensitivity and non – specificity. The strong reactivity of antibody from celiac patients to only a few short peptides provides further evidence that the epitope repertoire is highly restricted on the gliadin molecule.

Introduction

Celiac Disease or gluten sensitive enteropathy is a chronic condition whose main features include inflammation and characteristic histologic "flattening" of intestinal mucosa resulting in a malabsorption syndrome. The exact etiology of the disease remains unknown but gliadin, or the alcohol soluble fraction of wheat gluten, is clearly the toxic agent.¹

Detection of anti – gliadin antibodies (AGA) is commonly performed to aid in diagnosis of celiac disease (CD)⁽²⁻⁹⁾. Currently available assays for detection of AGA have two main deficiencies: 1.) relative insensitivity and 2.) high false positive rates, especially when using IgG conjugate.

Recent advances have been made in identifying a limited number of small epitopes on wheat proteins that stimulate the immune system of celiac patients^(7,8). It has also become apparent that deamidated gliadin proteins that have been exposed to the action of the enzyme tissue transglutaminase (tTG) have greatly enhanced antigenicity for the immune cells of CD patients^(8,9). Using these recent break through discoveries, synthetic deamidated peptides of less than 30 amino acids have been designed. Using these novel "designer peptides" an AGA test has been developed with enhanced performance as compared with currently available assays.

Relative Sensitivity vs. In House Assay

Forty-one samples submitted to Focus Diagnostics for celiac serology were found to be double positive for IgA endomysial and tTG antibody. Because of the high positive predictive power of either a tTG or endomysial result individually, these double positives are categorized as "probable celiacs" even though ultimate patient diagnosis was unavailable. These 41 samples were tested for IgA and IgG antibodies to both a standard gliadin and a gliadin peptide. Twenty-six IgA deficient patients samples were also tested.

	# (% positive) IgA		# (% positive) IgG	
	Standard gliadin	Gliadin peptide	Standard gliadin	Gliadin peptide
41 "probable" Celiac Samples	34 (83%)	39 (95%)	34 (83%)	39 (95%)
26 IgA deficient Sera	0	0	7 (27%)	1* (4%)

*This one sample was also found to be tTG IgG positive and is a probable IgA deficient celiac. This same sample was found negative for IgG antibody to native gliadin. The 25 other IgA deficient samples were all found negative for IgG tTG.

The gliadin peptide assays were clearly more sensitive compared with the standard assay using whole native gliadin. Only 1 of the 41 "probable celiacs" samples was found to be negative for both IgA and IgG antibodies to gliadin peptide giving a combined sensitivity of 97.6%.

Further Studies Performed at Focus

Another 44 samples tested at Focus, found to be negative for tTG IgA yet positive for gliadin IgG and/or IgA by standard native antigen method were all found to be negative for both IgG and IgA antibodies against the gliadin peptide.

Another 35 samples tTG IgA and gliadin negative by standard method were found to be negative on the peptide *ELISA* except for 1 sample that was positive at 55 units for IgA. This 1 sample was also found to be endomysial IgA negative and may be a false positive result.

IgA Deficient Celiac Patients

Five biopsy proven IgA deficient celiac patient samples were tested for IgA and IgG antibodies to tissue transglutaminase (tTG), native gliadin and gliadin peptide.

Patient	IgA			IgG		
	tTG	Gliadin	Gliadin Peptide	tTG	Gliadin	Gliadin Peptide
D1	1	2	1	12	121	121
D2	2	2	2	61	107	65
D3	1	2	2	67	121	115
D4	3	3	2	21	27	39
D5	5	3	4	41	24	35

All 5 samples were negative for IgA antibodies by all 3 assays (cut off is 20 units). Four of five were tTG IgG positive and all 5 were found positive for IgG antibodies with both the standard anti-gliadin and anti-gliadin peptide assays.

Clinical Sensitivity & Specificity vs. Standard Gliadin *ELISA*

Samples clinically defined as either celiac positive patients on a gluten-free diet (GFD), celiac positive patients on a gluten-free diet that are still endomysial positive or a first degree relative of a celiac patient were tested on the QUANTA Lite™ Gliadin IgA and IgG II *ELISA* and a standard gliadin *ELISA* test kit. A summary of the clinically defined samples plus the normal range are provided below.

Clinical Sensitivity and Specificity – IgA

Patient Group	number of patients	Number Positive (%)	
		Gliadin IgA (Standard)	Gliadin IgA II Peptide
Celiac Positive	33	16 (48%)	22 (67%)
Celia Positive, GFD	30	4 (13%)	4 (13%)
Celiac Positive, GFD, EMA Positive	5	3 (60%)	4 (80%)
1st relatives	20*	2 (10%)	1 (5%)
Celiac IgA Deficient	5	0	0
Healthy Normals	520	17 (3.3%)	11 (2.1%)

Clinical Sensitivity and Specificity – IgG

Patient Group	number of patients	Number Positive (%)	
		Gliadin IgG (Standard)	Gliadin IgG II Peptide
Celiac Positive	33	25 (76%)	21 (64%)
Celia Positive, GFD	30	12(40%)	5 (17%)
Celiac Positive, GFD, EMA Positive	5	4 (80%)	3 (60%)
1st relatives	20*	11 (55%)	0
Celiac IgA Deficient	5	5 (100%)	5 (100%)
Healthy Normals	520	114 (22%)	3 (0.6%)

*All 20 of these 1st degree relatives were endomysial and tTG negative

Clinical Sensitivity & Specificity

Sensitivity was calculated based on testing 103 Celiacs with a biopsy score of Marsh II or greater. Specificity was determined by testing 114 disease controls.

	Gliadin Peptide IgA	Gliadin Peptide IgG	IgG and/or IgA
	Sensitivity	95%	92%
Specificity	98%	98%	98%*

* One of the 2 "false positives" had a Marsh I biopsy and was also tTG positive.

It was also determined that the IgA Gliadin Peptide assay had a 94% correlation with tTG.

Precision/Reproducibility

Intra-assay performance for QUANTA Lite™ Gliadin IgA and IgG II *ELISA* was evaluated by testing 9 specimens a total of 5 times each. The results are summarized in Tables 1 and 3 below:

Inter-assay variation was assayed by testing, in duplicate, a panel of 5 specimens and the kit high positive control (HPC), twice daily (once in the morning and once in the afternoon) for 3 days. The results are summarized in Tables 2 and 4 below:

Table 1: Intra-assay Performance of QUANTA Lite™ Gliadin IgA II *ELISA*

	1	2	3	4	5	6	7	8	9
Mean units	23.9	39.3	78.2	97.0	142.3	153.2	5.0	4.8	4.9
SD	0.5	0.8	2.5	1.8	1.7	5.0	0.4	0.4	0.3
CV%	1.9	2.1	3.2	2.1	1.2	3.3	7.3	8.9	6.4

Table 2: Inter-assay Performance for QUANTA Lite™ Gliadin IgA II *ELISA*

	HPC	A	B	C	D	E
Mean units	126.0	24.2	34.4	164.1	7.0	7.6
SD	1.5	4.0	0.7	5.1	0.6	1.0
CV%	1.2	16.4	2.0	3.1	8.9	13.8

Table 3: Intra-assay Performance of QUANTA Lite™ Gliadin IgG II *ELISA*

	1	2	3	4	5	6	7	8	9
Mean units	22.4	28.3	62.5	55.5	35.7	59.7	3.7	4.0	3.8
SD	0.9	1.4	2.0	1.7	1.0	2.0	0.3	0.4	0.3
CV%	3.9	5.1	3.9	3.1	2.9	3.4	8.1	10.0	8.4

Table 4: Inter-assay Performance for QUANTA Lite™ Gliadin IgG II *ELISA*

	HPC	A	B	C	D	E
Mean units	110.6	57.0	89.0	113.8	5.2	5.7
SD	4.1	1.9	3.0	3.4	0.3	0.6
CV%	3.7	3.3	3.4	3.0	5.2	9.8

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Conclusions

- The new gliadin peptide *ELISA*'s proved superior in sensitivity and specificity compared with standard gliadin *ELISA* assays.
- Gliadin peptide *ELISA* results agree much better with tissue transglutaminase and endomysial assays.
- The IgG version of the kit has vastly superior specificity yet still detects all IgA deficient celiacs tested thus far.
- The new peptide *ELISA* assays are robust and the highly refined peptide antigens provide for a precise and reproducible result.
- Celiac patients recognize a highly restricted number of epitopes on the gliadin molecule.