

# Celiac G+ Antibody Assay for the Detection of Autoantibodies in Celiac Disease

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Celiac disease (CD) affects approximately 1% of the population and may present with varied symptomatic as well as asymptomatic clinical manifestations. Simple methods of detecting CD such as serum antibody tests have helped in the early identification of the disease thus preventing serious complications of the disorder. Our objective is to develop specific and sensitive immunoassays that are reliable in the detection of CD. To this end, immunoassays were developed for the detection of IgG and IgA antibodies to gliadin using synthetic peptides. Over 200 serum samples were included in the study from individuals with CD submitted for endomysial (EMA) and tissue transglutaminase (tTG) antibody tests as well as from disease controls and healthy normals. To examine the reliability of the Celiac G+ antibody test in comparison with EMA, a test with higher sensitivity and specificity, samples with low and high EMA titers were included in the study. Comparative evaluations of the Celiac G+ antibody assay were made with EMA and another commercially available gliadin peptide assay along with tTG antibody assays. The data show that as the EMA levels increased the sensitivity of detection of antibodies to synthetic peptides on both systems increased, reaching 100% at EMA titers greater than 160. The diagnostic performance of the newly developed Celiac G+ synthetic gliadin peptide assay is significantly superior in comparison with another available gliadin peptide immunoassay. Overall, the diagnostic performance of the Celiac G+ assay for IgA and IgG reached a sensitivity of 80% and 90% respectively in comparison with EMA. Similar comparison of the EMA positivity to the other available synthetic peptide immunoassay yielded sensitivities of 59% (IgA) and 75% (IgG). The specificity of the Celiac G+ antibody assay for IgA and IgG was 90–95% as compared to the other similar assay with specificity of 88–90%. In conclusion, the performance of the recently developed Celiac G+ ELISA is superior in both its sensitivity and specificity in comparison with other available synthetic gliadin peptide immunoassays. Furthermore, the IgG Celiac G+ antibody test and IgA tTG antibody test used in combination is an excellent screening algorithm for suspected cases of celiac disease.

**Key words:** celiac disease; gliadin; autoantibody

## Introduction

The advent of serological methods for the detection of antibodies to gliadin, endomysial and tissue transglutaminase have enabled large-scale screening for celiac disease (CD) in Europe and the United States. These studies

suggest that CD is far more prevalent than once thought. Recent serological studies demonstrate incidence of CD between one in 130 and one in 500.<sup>1</sup> Prevalence of CD is much higher in first and second-degree relatives of patients with CD. Many other autoimmune disorders, such as type 1 diabetes, thyroid autoimmunity and other autoimmune disorders are associated with CD. Approximately 5% of patients with type 1 diabetes have CD.<sup>2,3</sup> It has been proposed that early detection of CD may be

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beneficial in such cases as it is believed that adherence to a gluten-free diet may delay the onset of diabetes. If true, this further emphasizes the utility of and need for serum antibody tests in screening the population of those genetically susceptible for CD.

The most common serological tests for the screening of CD are the indirect immunofluorescence (IFA) method of detecting endomysial antibodies and ELISA methods of detecting antibodies to tissue transglutaminase (tTG) and gliadin.<sup>4,5</sup> Because of the limited sensitivity and specificity of gliadin antibody assays, use of this method is more limited than EMA and tTG.

Reliance on EMA and tTG immunoassays, however, may allow cases of IgA deficient celiac disease to go undetected.<sup>6</sup> Both immunoassays may be of limited utility detecting IgG antibodies. Studies show that 1–2% of the general population is IgA deficient and the incidence of CD in IgA deficient subjects is significant, hence there is a need for specific IgG isotype tests. IgG gliadin antibody tests are useful in establishing diagnosis of CD in IgA deficient patients. In addition, some studies question the specificity of tTG assays and the sensitivity of EMA. These issues highlight a need for another immunoassay that by itself or in combination with other immunoassays could be used for the detection of CD even in patients with IgA deficiency with high degree of reliability. Because of the limitations of the existing gliadin immunoassays, gliadin peptide based assays have shown promising results.<sup>7</sup> We have developed a next generation assay for the detection of gliadin antibodies (Celiac G+) employing proprietary technology.

## Materials and Methods

### Serum

Serum samples from normal subjects were obtained from a commercial source. Sera from patients with CD were referred to the laboratory for serum antibody studies by referring clinicians.

## Immunoassays

EMA antibodies were detected by indirect immunofluorescence on primate smooth muscle or on distal esophagus as per kit instructions (IMMCO Diagnostics). The EMA test was performed at two screening dilutions of 1:2.5 and 1:10 – if positive the end point was determined by testing at two fold serial dilutions. Anti-tTG antibodies of IgA and IgG isotypes were detected by ELISA as per kit instructions (IMMCO Diagnostics). Antibodies to gliadin peptides were detected by ELISA (Celiac G+, IMMCO Diagnostics) using plates coated with proprietary gliadin peptides. For comparison, the tests were also performed on another gliadin peptide based kit (INOVA). The serum dilution for ELISA tests was 1:101.

## Results

The Celiac G+ ELISA was evaluated on sera from patients with suspected CD, disease controls, and healthy normals. These specimens were also tested on another commercially available gliadin peptide kit and for EMA and tTG antibodies. Table 1 summarizes the results of the study comparing the performance of the Celiac G+ and INOVA gliadin peptide with the EMA results. Relative sensitivity and specificity of IgA for Celiac G+ was 80 and 95%, respectively, as compared with 59 and 95% on the Inova Gliadin II kit. A number of EMA positive samples negative on the Inova Gliadin II immunoassay were positive on Celiac G+ (Table 2). IgG Celiac G+ has the desired clinical sensitivity and specificity 90% (Table 1). It is interesting that a combination of IgG Celiac G+ antibody test with IgA tTG was able to detect almost all patients with CD with no false positives (Fig. 1).

## Discussion

Celiac disease is an immunologically mediated disorder initiated by gliadin in wheat and some other cereal proteins. This is one

**TABLE 1.** Comparative Sensitivity and Specificity of Celiac G+ and Other Deamidated Gliadin Peptide Assays in Comparison with EMA

Celiac G+ IgA HRP versus Clinical Result (EMA)				Inova Gliadin II IgA versus Clinical Result (EMA)					
		Clinical Diagnosis					Clinical Diagnosis		
		Positive (n=)	Negative (n=)	Total (n=)			Positive (n=)	Negative (n=)	Total (n=)
HRP	Positive	103	6	109	HRP	Positive	27	10	37
	Negative	26	120	146		Negative	19	86	105
	Total	129	126	255		Total	46	96	142
Relative sensitivity		80%			Relative sensitivity		59%		
Relative specificity		95%			Relative specificity		90%		

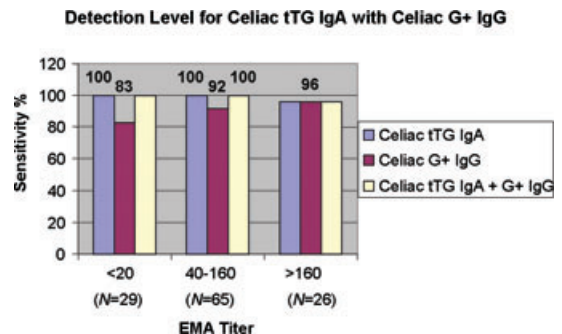
Celiac G+ IgG HRP versus Clinical Result (EMA)				Inova Gliadin II IgG versus Clinical Result (EMA)					
		Clinical Diagnosis					Clinical Diagnosis		
		Positive (n=)	Negative (n=)	Total (n=)			Positive (n=)	Negative (n=)	Total (n=)
HRP	Positive	117	13	130	HRP	Positive	24	6	30
	Negative	13	119	132		Negative	8	44	52
	Total	130	132	262		Total	32	50	82
Relative sensitivity		90%			Relative sensitivity		75%		
Relative specificity		90%			Relative specificity		88%		

**TABLE 2.** Comparative Evaluation of Celiac G+ with Other Deamidated Gliadin Immunoassays

Celiac G+ IgA HRP versus Inova				
		Inova		
		Positive (n=)	Negative (n=)	Total (n=)
HRP	Positive	24	10	34
	Negative	11	95	106
	Total	35	105	140
Relative sensitivity		69%		Relative agreement
Relative specificity		90%		

Celiac G+ IgG HRP versus Inova				
		Inova		
		Positive (n=)	Negative (n=)	Total (n=)
HRP	Positive	21	11	32
	Negative	4	42	46
	Total	25	53	78
Relative sensitivity		84%		Relative agreement
Relative specificity		79%		



**Figure 1.** Diagnostic sensitivity of IgG-Celiac G+ in combination with IgA-tTG for the detection of Celiac disease.

of the few autoimmune disorders where the etiologic agent initiating the autoimmune disorder is known and elimination of the etiologic agent (wheat and other gliadin containing cereals) from the diet ameliorates the disorder. Because gliadin is shown to be the offending agent, it was given that investigators examine the immune mechanisms against the gliadin. One of the outcomes of these studies was the development of immunoassays for the

detection of antibodies to gliadin and these assays became popular for the serological diagnosis of CD.<sup>8</sup> The limitations were that these assays were not specific and sensitive enough to be utilized in the screening of patients suspected for CD. Other assays such as the immunofluorescence test for endomysial antibodies and tTG, an autoantigen of EMA, were developed and were much more specific and sensitive for CD. However, these methods also have some limitations. While the EMA test has been recognized to be specific and sensitive, its utilization has been limited by its nature as a manual immunohistochemical method requiring skilled laboratory personnel for reading and interpretation. The specificity and sensitivity of tTG assays have been questioned and several publications describe positive tTG antibody results in autoimmune diseases other than CD while the samples are negative for EMA.<sup>9-13</sup> Although intestinal biopsies may not have been performed on these subjects to confirm CD disease status, it is highly likely that the results on the tTG are false positive as EMA results are negative and the high specificity of this test is well established.

Because of increased reliance on serology for the diagnosis of CD and the limitations of existing ELISA methods it is desirable to have other immunoassays that can reliably supplement the existing methods of CD diagnosis. It was recently shown that the gliadin antibodies in the sera of patients with CD recognize deamidated gliadin peptides and the use of deamidated gliadin peptides significantly improves the utility of gliadin immunoassays in CD diagnosis.<sup>14-16</sup> Studies have shown the variability of tTG and deamidated gliadin peptide assay performance.<sup>17,18</sup> We have developed deamidated gliadin immunoassay utilizing a special chemistry to present the proper conformation for antibodies to bind. This assay, referred to as Celiac G+, detects IgG and IgA antibodies. With the sample set chosen for these studies, including challenging samples with low EMA titer, Celiac G+ achieved sensitivity and specificity of 80 and 95% for IgA, respectively, and

90% sensitivity/specificity for IgG. Sensitivity increases as EMA titers increase. Of the two isotypes, IgG provides a higher degree of sensitivity, approaching that observed for IgA-tTG. A combination of IgG-Celiac G+ with IgA-tTG provides an excellent method for screening CD.

In conclusion, Celiac G+ is a very specific and sensitive method of detecting CD. Celiac G+ correlates well with EMA positivity and titer. Celiac G+ provides a strong complement to tTG and EMA antibody tests and will detect IgA deficient CD with reliability.

### Conflicts of Interest

M.J.L., V.R., and V.K. are employees of IMMCODiagnostics. Reagents used herein were produced by IMMCODiagnostics.

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