CLINICAL DIAGNOSIS OF CELIAC DISEASE BIOMARKERS: SENSITIVITY AND SPECIFICITY IN A NOVEL 4-PLEX PLANAR MICROARRAY ASSAY

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Abstract

Simultaneous multiplex detection of recognized gluten-sensitive enteropathic Celiac disease biomarkers improves diagnosis. A novel multiplex serological assay simultaneously measured four Celiac disease auto-antibody biomarkers, Gliadin IgA, Gliadin IgG together with tTG IgA and tTG IgG, using SQI's IgX PLEX™ assay. Testing 10 microliter clinically diagnosed samples (n=342), the multiplex planar microarray fluorescent immunoassay offers qualitative /semi-quantitative and positive/negative results for four biomarkers in each sample well of a 96-well, Celiac assay microarray plate. Immunoglobulin specific fluorescent tagged markers captured on microarray spots are read in a microarray scanner. Each microarray is processed with reference to quality control and calibration incorporated into every sample [1].Celiac disease human serum samples and normal controls were tested and results compared with predicate and conventional immunoassays. Based on ROC analysis, the study demonstrated that the IgX PLEX test achieved Gliadin IgA 47.6% sensitivity with 98.1% specificity; Gliadin IgG at 61.9% sensitivity with 94.6% specificity; tTG IgA 90.5% sensitivity at 100% specificity; and tTG IgG 52.4% sensitivity at 98.1% specificity. In a method comparison study, clinical truth discrepant result resolution indicated that, when compared to predicate assays, the percentage of accurate IgX PLEX Celiac assay detection resulted in increases of 13.3% for Gliadin IgA, 57.5% for Gliadin IgG, 37.5% for tTG IgA and 8.3% for tTG IgG.

Introduction

Celiac disease is an immune-mediated small bowel disease which develops in genetically predisposed individuals by ingestion of wheat gluten or related proteins from rye and barley. Celiac diagnosis is based on clinical symptoms and serological testing of anti-endomysium antibodies, IgA and IgG antibodies to gliadin and tissue tansglutaminase (tTG) followed by biopsy of small intestine [2,3]. SQI's unique automated multiplex microarray fluorescent immunoassay offers the qualitative/semi quantitative analyses of Gliadin-IgA, IgG tTG-IgA, IgG antibody, in a single test. SQI's multiplex automated assay screens 76 patient samples on a 96-well microtiter-formatted plate, all wells with quality control elements.

Clinical cut-off values: Determined for IgX PLEX Celiac assay- Gliadin IgA:4.03U/mL, Gliadin IgG:4.29U/mL, tTG IgA:1.78U/mL, and tTG IgG:2.92U/mL.

References:

1. Clin Rev Allergy Immunol.2009 Dec 9. [DOI 10.1007/s12016-009-8189-z;] Lea et al.
2. J Pediatr Gastroenterol Nutr 1990: 10:435-442 Calabuig et al.
3. Curr Opin Gastroenterol 2006: 22:674-679, Fasano A.

Methods

Development of the assay

The printed native gliadin and tissue transglutaminase microspots capture autoimmune antibodies present in celiac patients. Fluorescence-labeled anti-human antibodies were used to detect the analytes captured on the microarray plates The control spots are also printed in each well. The in-house developed assay standards were traceable to an FDA-approved predicate assay kit. All tests were performed on an automated SQiDworks™ Diagnostics System. 10 µl of sample was diluted and incubated for 60 minutes at room temperature in wells of the SQI test plates. Wells were washed and reacted with fluorescence-labeled reporter markers. After incubation, wells were washed again, dried and read in a microarray scanner. The responses were further analyzed. The final results (U/mL) were calculated against a standard curve.

Results

Method comparison: 75 clinically diagnosed celiac patient samples and 29 normal samples tested. For gliadin, Quanta Lite™ Gliadin IgA and IgG ELISA test (INOVA Diagnostics, Inc.) were used and for tTG, AESKULISA ® tTG A and tTG G ELISA test (AESKU, Inc.) were used to confirm agreement with predicate methods (Table 1).

Table 1 Relative Sensitivity and Specificity of the IgX PLEX Celiac Assay

n = 102		Relative Sensitivity (%)	Relative Specificity (%)	Total Agreement (%)
Gliadin	IgA	93.4	81.5	87.8
	IgG	72.7	89.7	79.0
tTG	IgA	97.3	100.0	98.3
	IgG	87.0	91.4	88.5

Precision: The coefficients of variance (CVs) for the precision within run were found to be 4.2% to 11.5% (Table 2).
Reproducibility: The reproducibility (CVs) was found to be 5.6% to 12.5%(Table 2).

Table 2 Precision Reproducibility

	U/mL (%CV)			
	Gliadin IgA	Gliadin IgG	tTG IgA	tTG IgG
Precision within run n=4	16.0 (7.7%)	36.9 (4.9%)	2.7 (11.5%)	9.1 (2.2%)
	3.4 (6.6%)	11.1 (4.2%)	1.53 (8.8%)	3.6 (8.7%)
	12.6 (4.9%)	8.6 (6.5%)	7.0 (6.4%)	3.3 (5.4%)
Reproducibility between runs n=12	7.1 (10.4%)	36.8 (5.6%)	6.7 (12.5%)	3.3 (8.5%)
	11.8 (9.3%)	11.8 (9.1%)	2.4 (9.7%)	4.2 (10.8%)

Sensitivity and Specificity: 342 European and North American subjects (111 celiac disease patients, 37 biopsy confirmed and on gluten free diet (GFD), 58 other autoimmune diseases, and 136 normal controls) tested. Assay demonstrated improved sensitivity * (Table 3) and specificity ** (Table 4).

Table 3 Sensitivity of IgA & IgG Gliadin and tTG

Celiac disease group	Number of patients	Number positive (%)			
		Gliadin IgA	Gliadin IgG	tTG IgA	tTG IgG
Clinically positive	83	57 (69%)	42 (51%)	55 (66%)	37 (45%)
EMA positive	28	25 (89%)*	23 (82%) *	28 (100%) *	24 (86%) *
Biopsy confirmed (GFD)	37	10 (27%)	9 (24%)	16 (43%)	5 (14%)

Table 4 Specificity of IgA & IgG Gliadin and tTG

Disease group	Number of patients	Number negative (%)			
		Gliadin IgA	Gliadin IgG	tTG IgA	tTG IgG
Healthy normal	136	127 (93%)	129 (95%)	134 (99%) **	135 (99%) **
Crohn's disease	24	24 (100%)	14 (58%)	23 (96%)	24 (100%)
Lactose intolerance	7	4 (57%)	7 (100%)	7 (100%)	7 (100%)
Rheumatoid arthritis	8	5 (63%)	7 (88%)	8 (100%)	6 (75%)
Ulcerative colitis	10	5 (50%)	7 (70%)	10 (100%)	9 (90%)
Systemic Lupus Erythematosus	9	6 (67%)	6 (67%)	8 (89%)	4 (44%)

Conclusions:

1. Clinical performance of the IgX PLEX Celiac assay was equivalent to FDA cleared predicate assays.
2. Automated IgX PLEX celiac assays provide simultaneous, multiple normalized results for Gliadin IgA, IgG, and tTG IgA, IgG per patient test well.