

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM**

A. 510(k) Number:

k140691

B. Purpose for Submission:

New assay and instrument

C. Measurand:

Autoantibodies to deamidated gliadin peptide (DGP) IgG and IgA classes
Autoantibodies to tissue transglutaminase (tTG) IgG and IgA classes

D. Type of Test:

Multiplexed Immunoassay

E. Applicant:

SQI Diagnostic Systems, Inc.

F. Proprietary and Established Names:

SQI Ig_plex Celiac DGP Panel

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5750: Radioallergosorbent (RAST) immunological test system

21 CFR §866.5660: Multiple autoantibodies immunological test system

21 CFR §862.2570: Instrumentation for Clinical Multiplex Test Systems

2. Classification:

Class II

3. Product code:

MST: Antibodies, gliadin

MVM: Autoantibodies, Endomysial (Tissue transglutaminase)

NSU: Instrumentation for Clinical Multiplex Test Systems

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

The Ig_plex Celiac DGP Panel is an in vitro diagnostic test for the semi-quantitative detection of the IgA and IgG immunoglobulin classes of antibodies to deamidated gliadin peptide (DGP) and tissue transglutaminase (tTG) in human serum. The test is intended for use in clinical laboratories as an aid in the diagnosis of celiac disease in conjunction with other laboratory and clinical findings, and requires the sqid-X system.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

For use on the sqid-X system

I. Device Description:

1. Microarray plate: 1 package, storage 2-8°C

The Ig_plex Celiac DGP Panel microarray plate consists of an array of capture replicate spots, covalently bound to the surface of coated glass within each well of a 96-well SBS compliant assay plate. All wells have the identical eight by eight grid containing:

- Eighteen (18) capture spots each of DGP and tTG
- Six (6) capture spots each of human IgG control and human IgA/IgG mix control
- Four (4) spots of Dy649-BSA
- Twelve (12) blanks

The control IgG and IgG/IgA captures are used to confirm that the reporter mix is performing within specification and for other internal quality checks. The fluorescently tagged BSA spots are used as “Landing Lights” for the spot finding algorithm’s grid registration. The spots are approximately 200µm in diameter. The outer plate holder is labeled with a barcode indicating the control number which the system reads to identify the assay type and lot number.

2. Reporter mix: mouse or goat anti-human antibodies, fluorescently labeled with Cy5 or Cy3 1 amber bottle, store $\leq -15^{\circ}\text{C}$

3. Standards: Eight (8) 2.0 mL tubes, store $\leq -15^{\circ}\text{C}$
4. Positive Control #1: Human One (1) 2.0 mL tube, store $\leq -15^{\circ}\text{C}$
5. Positive Control #2:- Human One (1) 2.0 mL tube, store $\leq -15^{\circ}\text{C}$
6. Negative Control: One (1) 2.0 mL tube, store $\leq -15^{\circ}\text{C}$
7. Sample Diluent: Three (3) Translucent bottles, store $\leq -15^{\circ}\text{C}$
8. Wash Buffer Concentrates: Four (4) Translucent bottles, store $2-8^{\circ}\text{C}$
9. Ig_plex software: The Ig_plex configuration software contains several modules: washer, scanner, spot finding algorithm, data analysis algorithm, report generation algorithm, and the user interface.
10. The sqid-X system consists of the following components:

Component	Description	Manufacturer
FLAIR™ Scanner	High resolution plate imaging instrument	Sensovation AG
ELx405UCWVST™ Plate Washer	Microarray plate washing instrument	BioTek Instruments Inc.
Drying Station	Microarray plate final drying station	SQI Diagnostics Systems Inc.
ME 4C NT Vario	Vacuum pump for drying microarray	VacuuBrand Inc.
Microplate Shaker	Plate shaker used during incubation steps	VWR
Computer System	Computer running Microsoft Windows™ XP with peripherals	Hewlett Packard, Microsoft etc.
sqid-X software	Ig_plex Celiac DGP panel configuration	SQI Diagnostics Systems Inc.

The system combines manual liquid handling (samples and reagents) including sample pipetting into the plate, with automated steps for washing, scanning, data analyses and reporting. Results for each patient sample are obtained simultaneously and independently for each of the four Ig_plex Celiac DGP Panel markers: DGP IgA, DGP IgG, tTG IgA and tTG IgG using the data from one well containing one sample of the patient's serum. The system uses an integrated hardware and software solution to create a procedure that is semi-automated. The system is configured with assay specific software to perform the automated steps of the assay protocol.

J. Substantial Equivalence Information:

1. Predicate device names and numbers:

Assays:

INOVA Diagnostics Inc. Quanta Lite™ Gliadin IgA II ELISA (k052143); Quanta Lite™ Gliadin IgG II ELISA (k052142); Quanta Lite™ h-tTG IgA ELISA (k011566) and Quanta Lite™ h-tTG IgG ELISA (k011570)

Instrument: SQI Diagnostics SQiDworks Diagnostics Platform (k102490).

2. Comparison with predicate:

Similarities- All devices					
Item	Device	Predicates			
	SQI Ig_plex Celiac DGP Panel	Quanta Lite™ Gliadin IgA II (k052143)	Quanta Lite™ Gliadin IgG II (k052142)	Quanta Lite™ h-tTG IgA (k011566)	Quanta Lite™ h-tTG IgA (k011570)
Intended Use	Detection of autoantibodies to aid in the diagnosis of celiac disease.	Same	Same	Same	Same
Assay format	Semi-quantitative, manual washing and preparation	Same	Same	Same	Same
Sample	Human serum	Same	Same	Same	Same
Assay substrate	96-well plate	Same	Same	Same	Same
Calibration	On each plate	Same	Same	Same	Same

Differences- All devices					
Item	Device	Predicates			
	SQI Ig_plex Celiac DGP Panel	Quanta Lite™ Gliadin IgA II (k052143)	Quanta Lite™ Gliadin IgG II (k052142)	Quanta Lite™ h-tTG IgA (k011566)	Quanta Lite™ h-tTG IgA (k011566)
Sample Dilution	1:151	1:101	1:101	1:101	1:101
Automation	Semi-automated	Manual	Manual	Manual	Manual
Technology	Microarray-based Fluorescence detection	ELISA	ELISA	ELISA	ELISA
Multiplexed assay	Yes	No	No	No	No
Shelf life Stability	6 months	Not stated in months	Not stated in months	One year	One year
Controls/Standards	Eight standards, three controls: Negative, Positive #1 and Positive #2	Three controls: Negative High positive, low positive			

Device-specific similarities and differences		
Item	Ig_plex Celiac Panel DGP IgA	Quanta Lite™ Gliadin IgA II (k052143)
Analyte	Deamidated gliadin peptide (DGP) IgA antibodies	Gliadin IgA antibodies
Capture antigen	Synthetic DGP	Similar
Measuring range	8.0–110.0 U/mL	Not specified
Cut-off	15.0 U/mL	20.0 U/mL

Device-specific similarities and differences		
Item	Ig_plex Celiac DGP Panel DGP IgG	Quanta LITE™ Gliadin IgG II (k052142)
Analyte	Deamidated gliadin peptide (DGP) IgG antibodies	Gliadin IgG antibodies
Capture antigen	Synthetic DGP	Similar
Measuring range	9.0–120.0 U/mL	Not specified
Cut-off	13.0 U/mL	20.0 U/mL

Device-specific similarities and differences		
Item	Ig_plex Celiac DGP Panel tTG IgA	Quanta Lite™ h-tTG IgA (k011566)
Analyte	Tissue transglutaminase (tTG) IgA antibodies	Human tissue Transglutaminase (h-tTG) IgA antibodies
Capture antigen	Recombinant human tTG	Native human red blood cell tTG
Measuring range	16.0–140.0 U/mL	Not specified
Cut-off	20.0 U/mL	20.0 U/mL

Device-specific similarities and differences		
Item	Ig_plex Celiac DGP Panel tTG IgG	Quanta Lite™ h-tTG IgG (k011570)
Analyte	Tissue transglutaminase (tTG) IgG antibody.	Human tissue Transglutaminase (h-tTG) IgG antibodies
Capture antigen	Recombinant human tTG	Native human red blood cell tTG
Measuring range	24.0–100.0 U/mL	Not specified
Cut-off	36.0 U/mL	20.0 U/mL

Item	Sqid-X platform	SQIDworks Diagnostics Platform
Sample, Reagent and Plate Handling	Manual	Automated
Detector	Multichannel fluorescence LED camera scanner	Multichannel fluorescence CCD camera scanner

K. Standard/Guidance Document Referenced (if applicable):

1. CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline
2. CLSI EP06-A: Evaluation of the Linearity of Quantitative Analytical Methods
3. CLSI EP07-A2: Interference Testing in Clinical Chemistry, Approved Guideline
4. CLSI EP09-A2: Method Comparison Bias Estimate using Patient Samples
5. CLSI C28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline- Third Edition
6. CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation, Approved Guideline

L. Test Principle:

The system is a multiplex immunoassay analyzer that semi-automates the protocol of a specific Ig_{plex} assay from plate washing to reporting of all assay markers for each individual patient sample. The system combines manual liquid handling (samples and reagents) with automated steps for washing, scanning, data analyses and reporting.

The system uses graphical user interface software to lead the operator through manual sample, reagent, and plate handling operations. The software controls the instruments, analysis and reporting using the same core software as the predicate instrument. The sqid-X software also provides access to maintenance procedures.

The system controls the washer to ensure that the same wash programs are used every time and in the correct sequence. Once the assay's biochemical reactions are complete and the plate is placed in the scanner, the instrument automatically performs a multi-wavelength fluorescent scan of each well in the microarray plate, analyzes the data, and generates a report containing results for all assay markers. The sqid-X system also includes numerous internal quality checks.

Results for each patient sample are obtained simultaneously for each of the four Ig_{plex} Celiac DGP Panel markers: DGP IgA, DGP IgG, tTG IgA and tTG IgG using the data from one well containing one sample of the patient's serum. The system does not interpret the results with respect to diagnosis; it simply reports each result independently. The biochemical reactions and analysis for the measurements are found in 4.5 Principle of Operation following the description of the device components.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The sample sets used in these studies together covered the measuring range for each assay, although not every sample contained all four analytes. The specific number of samples for each element is described below. The manufacturer’s acceptance criterion is that all reproducibility results should have % CVs of ≤10%.

i. Within-Run (intra-assay) Reproducibility Testing

Negative and positive samples covering the measuring range for each device, including at least 10% of total samples within ±25% of the cut-off for each assay, were run in replicates of twelve (12) on one kit. A total of four (4) kits were run on different days.

DGP IgA		DGP IgG		tTG IgA		tTG IgG	
Mean (U/mL)	%CV						
11.33	3.5%	11.97	3.4%	17.43	2.9%	32.58	4.5%
17.99	2.6%	15.23	3.4%	27.26	3.4%	35.78	3.4%
20.08	4.3%	27.31	2.7%	38.35	4.8%	40.82	4.9%
32.30	3.1%	38.42	2.6%	41.80	2.8%	44.53	5.2%
43.23	2.2%	54.69	3.5%	56.68	3.9%	58.51	3.5%
56.08	4.6%	69.51	2.7%	64.03	3.3%	61.70	5.2%
64.03	6.3%	86.69	3.8%	86.56	1.9%	71.01	2.7%
		117.26	2.3%	125.66	3.1%	78.36	3.2%
						98.78	4.8%

ii. Day-to-Day Reproducibility Testing

Negative and positive samples covering the measuring range for each device, including at least 10% of total samples within ±25% of the cut-off for each assay, were run in replicates of two (2) per run, two (2) runs per day, for 20 non-consecutive days, on one system by two operators (31 runs by operator 1 and 9 runs by operator 2) using one kit lot for a maximum of 80 results per sample per analyte (2x2x20=80).

DGP IgA- Day to day reproducibility								
Mean (U/mL)	Within Run		Between Run		Between Day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV

DGP IgA- Day to day reproducibility								
Mean (U/mL)	Within Run		Between Run		Between Day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
9.13	0.43	4.70%	0.31	3.30%	0.00	0.00%	0.52	5.70%
15.83	0.64	4.00%	0.27	1.70%	0.31	2.00%	0.76	4.80%
20.28	0.70	3.50%	0.98	4.80%	0.00	0.00%	1.21	6.00%
28.47	1.68	5.90%	1.23	4.30%	0.66	2.30%	2.18	7.70%
36.2	2.02	5.60%	1.14	3.20%	0.59	1.60%	2.39	6.60%
47.84	1.83	3.80%	2.59	5.40%	1.16	2.40%	3.38	7.10%
54.63	2.97	5.40%	1.82	3.30%	1.56	2.90%	3.81	7.00%
78.94	4.75	6.00%	3.47	4.40%	1.43	1.80%	6.06	7.70%
104.24	3.80	3.60%	5.39	5.20%	0.00	0.00%	6.60	6.30%

DGP IgG- Day to day reproducibility								
Mean (U/mL)	Within Run		Between Run		Between Day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
15.38	0.71	4.60%	0.47	3.00%	0.70	4.50%	1.10	7.10%
22.09	1.01	4.60%	1.01	4.60%	0.75	3.40%	1.61	7.30%
31.89	1.26	4.00%	0.61	1.90%	0.91	2.90%	1.67	5.20%
41.51	2.02	4.90%	0.61	1.50%	0.69	1.70%	2.22	5.30%
50.08	2.61	5.20%	1.42	2.80%	1.17	2.30%	3.19	6.40%
71.35	2.74	3.80%	2.64	3.70%	0.00	0.00%	3.80	5.30%
85.57	3.22	3.80%	2.34	2.70%	0.00	0.00%	3.98	4.60%
119.12	6.06	5.10%	2.27	1.90%	0.00	0.00%	6.47	5.40%

tTG IgA- Day to day reproducibility								
Mean (U/mL)	Within Run		Between Run		Between Day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
17.29	0.64	3.70%	0.67	3.90%	0	0.00%	0.93	5.40%
24.46	1.00	4.10%	0.62	2.60%	0.53	2.20%	1.29	5.30%
32.35	1.62	5.00%	0.56	1.70%	0	0.00%	1.71	5.30%
35.19	1.38	3.90%	0.74	2.10%	1.71	4.90%	2.32	6.60%
43.29	1.95	4.50%	1.79	4.10%	0	0.00%	2.64	6.10%
60.05	3.26	5.40%	1.42	2.40%	1.21	2.00%	3.75	6.30%
86.24	3.88	4.50%	1.95	2.30%	2.86	3.30%	5.20	6.00%
120.73	7.52	6.20%	1.90	1.60%	2.92	2.40%	8.28	6.90%
136.63	6.42	4.70%	3.29	2.40%	1.84	1.30%	7.44	5.40%

tTG IgG- Day to day reproducibility								
Mean (U/mL)	Within Run		Between Run		Between Day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
27.56	1.04	3.80%	1.19	4.30%	0.69	2.50%	1.72	6.20%
28.22	2.01	7.10%	1.18	4.20%	1.09	3.90%	2.58	9.10%
33.72	2.54	7.50%	0.00	0.00%	1.02	3.00%	2.73	8.10%
40.65	2.30	5.70%	2.19	5.40%	1.39	3.40%	3.47	8.50%
48.39	3.40	7.00%	1.49	3.10%	0.00	0.00%	3.71	7.70%
50.61	3.19	6.30%	0.00	0.00%	1.39	2.70%	3.48	6.90%
78.54	4.84	6.20%	2.00	2.50%	2.99	3.80%	6.03	7.70%
87.78	3.72	4.20%	2.44	2.80%	3.28	3.70%	5.53	6.30%
99.21	6.20	6.20%	3.51	3.50%	3.22	3.20%	7.82	7.90%

iii. Lot-to-Lot Reproducibility Testing

Negative and positive samples covering the measuring range for each device, including at least 10% of total samples within $\pm 25\%$ of the cut-off for each assay, were run in replicates of five (5) per kit, two (2) kits per lot, from three (3) kit lots were tested on one instrument by one operator for a maximum of 30 results (5x2x3) per sample per analyte. Ten (10) results per sample per analyte, per lot were evaluated.

Lot-to-lot reproducibility							
DGP IgA		DGP IgG		tTG IgA		tTG IgG	
Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.
9.35	9.4%	11.74	5.1%				
11.90	7.2%	15.42	5.8%	19.54	5.8%	29.58	6.6%
15.04	6.0%	17.98	4.7%	32.60	6.7%	34.40	8.1%
16.65	6.9%	33.08	6.3%	38.05	6.1%	43.83	6.8%
18.66	4.7%	46.42	4.6%	44.31	6.8%	50.13	9.5%
20.07	5.0%	74.15	6.7%	60.49	6.1%	62.01	7.6%
59.91	5.7%	85.92	6.0%	95.28	4.5%	65.06	7.5%
65.01	7.1%	116.14	4.5%	135.20	6.8%	103.74	8.4%

iv. Instrument-to-Instrument Reproducibility Testing

Two studies were performed. In the first study, the same samples from the lot-to-lot study were tested in replicates of five (5) per kit, two (2) kits per lot,

from three (3) kit lots on three (3) instruments at three (3) different sites (one internal, one external in Canada and one external in USA) by three (3) operators for a maximum 30 results (5x2x3) per instrument per analyte and 90 results (5x2x3x3) per sample per analyte.

Instrument-to-instrument Study #1							
DGP IgA		DGP IgG		tTG IgA		tTG IgG	
Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.
8.58	9.6%	16.61	9.3%	19.22	5.5%	29.52	9.9%
16.64	7.8%	32.52	8.0%	32.12	6.4%	47.76	9.8%
18.73	7.5%	88.87	6.9%	46.76	8.6%	51.00	10.1%
21.01	7.9%	123.18	6.0%	65.78	6.4%	63.68	9.9%
61.71	9.0%			177.91	5.7%	105.62	8.6%

In study two, a set of fourteen (14) celiac samples with results near the cut-off for one or more analytes were tested at a single site in duplicates on each of three instruments for a maximum of six (6) results per sample. Three kits from the same lot were used. A minimum of four (4) samples were reported per analyte. Two of the three instruments used in the study were used in the original instrument-to-instrument study (SQX1 and SQX 2) and the third instrument is a new one (SQX 3). All three runs were performed by one operator at SQI.

Instrument-to-instrument Study #2							
DGP IgA		DGP IgG		tTG IgA		tTG IgG	
Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.	Mean (U/mL)	%C.V.
15.37	6.27	13.63	7.18	24.64	6.30	35.49	6.46
15.81	6.06	11.41	3.63	23.35	4.18	29.34	8.91
9.77	6.43	15.75	5.73	23.59	4.40	33.11	7.07
18.85	3.93	17.90	5.56	17.69	2.91	37.53	5.61
		16.69	4.57			30.59	6.19
		17.82	2.86			50.12	2.84

b. Linearity/assay reportable range:

i. Linearity

Serial dilutions of high positive clinical samples in normal serum were assayed across each analyte range. A minimum of seven levels with three replicates at each level

were evaluated. Because the instrument reports values below the cut-off as “<” instead of with a number, the concentration of analyte in normal serum was arbitrarily assigned a value below the cut-off so expected concentrations could be calculated. Using the analyte concentration values for different dilutions, a graph of observed (measured) values vs. expected concentrations was plotted. The claimed upper limit is rounded off to the nearest tenth of the result.

Linearity						
Analyte	Sample	Test Range (U/mL)	R ²	Intercept	Slope	Linear Range (U/mL)
DGP IgA	S1	6.80–115.01	0.994	-3.427	1.030	8.0 – 110.0
	S2	9.27–73.86	0.992	-1.846	0.977	
	S3	6.34–44.87	0.992	-0.614	0.995	
DGP IgG	S1	11.18–129.70	0.997	-1.506	1.036	9.0 – 120.0
	S2	5.20–86.34	0.992	-2.302	1.033	
	S3	5.84–60.09	0.996	-0.466	0.991	
tTG IgA	S1	10.42–142.72	0.996	1.879	1.015	16.0-140.0
	S2	11.37–94.50	0.996	0.082	1.013	
	S3	11.89–94.7211	0.991	0.697	1.045	
tTG IgG	S1	13.07–103.60	0.991	2.996	1.017	24.0 – 100.0
	S2	12.19–87.89	0.995	1.331	1.016	
	S3	11.71–82.17	0.984	4.226	1.013	

ii. Analytical Measuring Range:

The measuring range was defined as lower and upper limit of reporting range:

- DGP IgA: 8.0–110.0 U/mL
- DGP IgG: 9.0–120.0 U/mL
- tTG IgA: 16.0–140.0 U/mL
- tTG IgG: 24.0–100.0 U/mL

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

i. Controls and Calibrators

a. Assay Standards (Calibrators)

There are no international standard materials available for celiac anti-DGP IgA and IgG, and anti-tTG IgA and IgG. The calibrators were assigned with arbitrary units. The raw material for the standards is celiac positive patient

serum sample with high titers for anti-DGP IgA and IgG and anti-tTG IgA and IgG.

During development, the raw material samples were diluted to a desired anchorage point, e.g., 2000x, 4000x and 16000x for a U/mL value assignment. These anchorage levels were selected to ensure coverage of the hypothetical cut-off points. A standard curve was constructed using the chosen dilution levels for each. The external standards are eight serial dilutions of a sample derived from human sera containing an appropriate representation of each of the analytes to be reported. Each assay has a specific standard range that is present in the standards; these are encoded in the bar code for each standard. In-house standards are prepared, and are assigned with arbitrary units. For new standards, the repetitiveness of the standard curves and bias of fit versus measured values were considered. Eight standard levels were then selected. Curve fitting algorithms were configured for each analyte. Finally, the standards were validated using celiac positive and normal samples. In this study, cut-off values and sensitivity/specificity of the assay were evaluated and to a large extent were found within industry range.

The positive and negative control samples are derived from human samples. The positive controls were prepared by diluting celiac positive patient serum sample to give results above cut-off for applicable analytes and negative control was prepared by diluting normal human serum sample to give results below cut-off for all four analytes. Expected results for each applicable analyte of each control are provided in the Certificate of Analysis.

b. Antigen

The DGP peptides are a mix of custom sequences, which are conjugated and then lyophilized. Upon receipt, the Certificate of Analysis is confirmed and an incoming acceptance test is performed using an HPLC method to verify the purity and consistency of the peptides.

The tTG is recombinant tissue transglutaminase in a buffer solution. The Certificate of Analysis is verified upon receipt of the material and incoming acceptance testing confirms protein concentration, content, and characteristics using Bradford Protein Assay, Western Blot and SDS-Page methods, respectively.

c. Reporter_mix

The reporter mix is comprised of fluorescently labeled secondary antibodies, Mouse anti-human IgG- Cy5; goat anti-human IgA: Cy3. The same dyes are used for anti-DGP and anti-tTG, but different capture spots in the microarray are analyzed to provide the results for each of the four analytes.

ii. Hook Effect:

High positive samples were selected and tested at a starting dilution of 1:51. Therefore, since the assay's dilution as per package insert is 1:151, at least three fold higher values can be achieved. All of the samples were serially diluted to evaluate any hook effect in the assays.

It was observed that the results were above the assay's reportable range, therefore, during this development study, a manual analysis was performed to see how high above the reportable range the actual values were. The instrument saturated at >200 U/mL for DGP IgG and IgA, so no hook effect was detected. No hook effect was detected at 1354 U/mL for tTG IgA and 835 U/mL for tTG IgG.

iii. Stability:

- a. Sample stability - The sponsor provided data supporting a claim of 1 week stability at 2-8°C.
- b. Shelf life stability - the sponsor provided data supporting a claim of 6 months stability when stored according to the package insert.
- c. Open-vial stability - The directions for use state that the kit is single-use only; therefore, open vial stability is not applicable.

d. *Detection limit:*

i. Limit of Blank (LoB):

Sample diluent from the kit was tested in replicates of seventy three (73) on one kit to determine the software-derived values for each analyte. Values are reported in fluorescence intensity units. All measurements are below the bottom of the AMR.

LoB	DPG IgA	DPG IgG	tTG IgA	tTG IgG
Mean	159.6	192.0	125.2	77.8
STDEV	64.5	37.0	108.4	33.0
LoB	265.6	252.7	303.4	132.1

ii. Limit of Detection (LoD):

Thirteen (13) normal human serum samples were tested in replicates of five (5), on two runs (total n=130) using two different kit lots and two different instruments to determine the software-derived LoD values for each analyte. For analytes, where some replicates reported numerical values, the probability

of values being the same as the intensity values was calculated and reported as a percentage.

	DPG IgA	DPG IgG	tTG IgA	tTG IgG
LoB	265.6	252.7	303.4	132.1
STDEV	116.7	241.6	125.3	196.1
LoD	457.5	650.1	509.4	454.7

iii. Limit of Quantitation (LoQ):

Eleven (11) diluted celiac positive samples (to report three samples per analyte) were tested over five (5) runs, nine (9) replicates per run. Two kit lots were used for a total of ten (10) runs.

Bias was calculated based on the expected value and mean, SD and %CV were calculated for each sample. The claimed LoQs meet the manufacturer's required acceptance criteria.

Analyte	Result for LoQ (U/mL)	LoQ Claim (U/mL)	Bias (%)	CV (%)
DGP IgA	8.25	8.0	0.4	8.9
DGP IgG	9.02	9.0	2.2	6.2
tTG IgA	16.23	16.0	4.5	7.9
tTG IgG	24.30	24.0	1.8	6.0

e. Analytical specificity:

i. Cross-reactivity

This information is contained in the Clinical Sensitivity and Specificity section.

ii. Interference

This study was performed according to the recommendations in CLSI EP7-A2, Interference Testing in Clinical Chemistry, Approved Guideline. Eight celiac positive samples (from the SQI Diagnostics' Sample Bank) and one normal (negative sample) were included in the study in order to report two levels per analyte.

The individual specimens were spiked with three concentrations of the interferents, starting at the maximum concentration and titering down to low levels. The samples were assayed in replicates of n=5 for control and for unspiked, and the mean result reported. Interference was calculated as the analyte recovery in the presence of interferent, relative to the measurement of the analyte

in a control, unspiked sample. The level of non-interference claimed was that level where there was $\leq 15\%$ difference between “control results” and “spiked results.”

Interferent testing concentrations:

Interferent	Interferent Test Level (20x stock)		
	Test Level 1	Test Level 2	Test Level 3
Bilirubin (conjugated)	3.0 mg/mL	1.5 mg/mL	0.8 mg/mL
Hemoglobin	100.0 mg/mL	50.0 mg/mL	25.0 mg/mL
Triglycerides	100.0 mg/mL	50.0 mg/mL	25.0 mg/mL
IgG	10.0 mg/mL	5.0 mg/mL	2.5 mg/mL

There was no interference detected by any of the interfering substances at the concentrations tested.

f. Assay cut-off:

Specimens from 110 celiac diagnosed patients, 126 normal donors and 56 other autoimmune diseases (total =292) were collected from commercial sources and collaborative laboratories representative of North American and European populations. Specimens were tested according to standard procedure, and all specimens were assayed in duplicate on separate kits using two lots of assay kits on two different sqid-X instruments for the determination of mean values. These samples were not used in any other validation assays. The samples and demographic information are presented below:

Condition	N	Gender			Age (years)			Demographic	
		M	F	Unk	Min	Max	Ave.	North America	Europe
Celiac, Biopsy Confirmed	10	4	6	0	21	46	29	10	0
Celiac, EMA Positive (adult)	50	12	38	0	22	90	45	12	38
Celiac, EMA positive (pediatric)	50	16	30	4	0	21	12	10	40
Normal Female Serum	54	0	54	0	20	63	40	54	0
Normal Male Serum	72	72	0	0	18	64	41	72	0
Crohn's Disease	5	2	3	0	26	66	46	5	0

Condition	N	Gender			Age (years)			Demographic	
		M	F	Unk	Min	Max	Ave.	North America	Europe
Ulcerative Colitis	5	3	2	0	17	68	38	5	0
Systemic Lupus Erythematosus	14	2	12	0	24	63	38	10	4
Rheumatoid Arthritis (RA)	22	15	7	0	45	72	58	22	0
Vasculitis	10	0	10	0	26	63	44	0	10

The cut-offs were selected by balancing sensitivity and specificity using ROC curves using Analyze-it v.2.30. The chosen cut-offs for each assay are:

Analyte	Cut-off (U/mL)	Sensitivity (%)	95% CI	Specificity (%)	95% CI
DGP IgA	15.0	85.5	0.775 – 0.915	99.5	0.970 to 1.00
DGP IgG	13.0	94.5	0.885 – 0.980	97.8	0.945 to .994
tTG IgA	20.0	97.3	0.922 – 0.994	97.8	0.945 to .994
tTG IgG	36.0	58.2	0.484 – 0.675	97.8	0.944 to .994

2. Comparison studies:

a. *Method comparison with predicate device:*

229 positive celiac patient samples and 150 non-celiac disease samples including presumptively normal samples were assayed with the Ig_plex Celiac DGP Panel and with the available predicates' ELISA method for each of the analytes. The celiac patient samples were classified using the following characteristics: 147 samples from celiac diagnosed, biopsy confirmed patients, 27 Endomysial Antibody (EMA) positive pediatric patient samples, 45 EMA positive adult patient samples, nine celiac diagnosed without additional information, and one IgA deficient celiac sample. The non-celiac samples included 132 presumptively normal samples and 18 other autoimmune disease samples. The sponsor analyzed the discrepant samples around the cut-off; the majority that were positive in the Ig_plex assays were Celiac-biopsy positive.

i. DGP IgA

		Quanta Lite DGP IgA		Total	PPA and NPA		
		Positive	Negative			% Agreement	95% CI
Ig_plex DGP IgA	Positive	126	9	135	Positive	93.3	87.8–96.5
	Negative	9	173	182	Negative	95.1	90.9– 97.4
	Total	135	182	317	Overall	94.3	91.2–96.4

ii. DGP IgG

		Quanta Lite DGP IgG		Total	PPA and NPA		
		Positive	Negative			% Agreement	95% CI
Ig_plex DGP IgG	Positive	129	19	148	Positive	98.5	94.6–99.6
	Negative	2	176	178	Negative	90.3	85.3–93.7
	Total	131	195	326	Overall	93.6	90.4–95.7

iii. tTG IgA

		Quanta Lite tTG IgA		Total	PPA and NPA		
		Positive	Negative			% Agreement	95% CI
Ig_plex tTG IgA	Positive	133	23	156	Positive	100.0	97.2–100.0
	Negative	0	167	167	Negative	87.9	82.5–91.8
	Total	133	190	323	Overall	92.9	89.5–95.2

iv. tTG IgG

		Quanta Lite tTG IgG		Total	PPA and NPA		
		Positive	Negative			% Agreement	95% CI
Ig_plex tTG IgG	Positive	48	35	83	Positive	94.1	84.1–98.0
	Negative	3	192	195	Negative	84.6	79.3–88.7
	Total	51	227	277	Overall	86.3	81.8–89.9

b. Matrix comparison

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and specificity:*

The study included 378 samples, collected from the following groupings: 128 celiac diagnosed by Marsh III criteria, biopsy confirmed, and 250 samples from other non-celiac autoimmune diseases. Samples were assayed according to the Ig_plex Celiac DGP Panel Instructions for Use. Clinical sensitivity was calculated from celiac disease patients. Clinical specificity was calculated for each disease subgroup and the overall specificity for the total of non-celiac disease patients and normal subject samples were also calculated.

The sample distribution included both pediatric and adult subjects:

Disease Code	Disease Category	Age (years)
Celiac Biopsy	Celiac Disease Biopsy Confirmed	4-82
SLE	SLE	21-85
SS	Sjögren's Syndrome	30-76
Wheat Allergy	Wheat Allergy	2 (3 unknown)
IBD (Crohn's Disease)	IBD (Crohn's Disease)	23-92
IBD (Ulcerative Colitis)	IBD (Ulcerative Colitis)	19-68
Lactose Intolerant	Lactose Intolerant	32-60
Osteoarthritis	Osteoarthritis	46-83
Celiac 1 ^o Relative	Celiac 1 ^o Relative	20-59
IgA Def, non-Celiac	IgA Deficient, non-Celiac	60-101
RA	Rheumatoid Arthritis (RA)	45-72
HT	Hashimoto's Thyroiditis	30-57
GT	Grave's Thyroiditis	27-67
Vasculitis	Vasculitis	26-62
EBV Positive	EBV Positive	34-54
Syphilis Positive	Syphilis Positive	18-60
Diabetes Mellitus (Types 1 and 2)	Diabetes Mellitus	39-70
Suspected Celiac, biopsy negative	Suspected Celiac, biopsy negative	20-73
OAD	Other Autoimmune Diseases	20-83
Normal Female	Normal Female	18-66
Normal Male	Normal Male	19-90

The manufacturer's pre-specified acceptance criteria are: Specificity for each analyte should be >95%; Sensitivity should be >72% for DGP IgA, >85% for DGP IgG, >90% for tTG IgA and >45% for tTG IgG.

i. DGP IgA

DGP IgA		Celiac Disease		Total
		Positive	Negative	
Ig_plex	Positive	102	2	104
	Negative	26	248	274
	Total	128	250	378

Sensitivity: 79.7% (95% CI 76.1% - 83.2%)
 Specificity: 99.2% (95% CI 98.6% - 99.8%)

DGP IgA Sample Group	Sample Disease Classification	Number of Samples	Ig_plex		Sens
			TP	FN	
Celiac Disease (Sensitivity)	Celiac Disease Biopsy confirmed	128	102	26	79.7%
			TN	FP	Spec
Non-Celiac Diseases (Specificity)	SLE	30	0	30	100%
	Sjögren's syndrome	14	0	14	100%
	Wheat allergy	4	0	4	100%
	IBD (Crohn's Disease)	24	0	24	100%
	IBD (Ulcerative Colitis)	25	0	25	100%
	Lactose Intolerant	8	1	7	87.5
	Osteoarthritis	10	0	10	100.0%
	Celiac 1 ^o relative	5	0	5	100%
	IgA Deficient, non-Celiac	3	1	2	66.7%
	Rheumatoid Arthritis	26	0	26	100%
	Hashimoto's thyroiditis	20	0	20	100%
	Vasculitis	16	0	16	100%
	EBV positive	6	0	6	100%
	Syphilis positive	7	0	7	100%
	Type 2 Diabetes	2	0	2	100%
	Type I Diabetes	10	0	10	100%
	Grave's Thyroiditis	12	0	12	100%
	Suspected celiac, negative biopsy	10	0	10	100%
Other autoimmune disease	16	0	16	100%	
Total non-celiac diseases		250	2	248	99.2%

ii. DGP IgG

DGP IgG		Celiac Disease		Total
		Positive	Negative	
Ig_plex	Positive	114	1	115
	Negative	14	249	263
	Total	128	250	378

Sensitivity: 89.1% (95% CI 86.3% - 91.8%)

Specificity: 99.6% (95% CI 99.2% - 100.0%)

DGP IgG Sample Group	Sample Disease Classification	Number of Samples	Ig_plex		Sens
			TP	FN	
Celiac Disease (Sensitivity)	Celiac Disease Biopsy confirmed	128	114	14	89.1%
			TN	FP	Spec
Non-Celiac Diseases (Specificity)	SLE	30	0	30	100%
	Sjögren's syndrome	14	0	14	100%
	Wheat allergy	4	0	4	100%
	IBD (Crohn's Disease)	24	0	24	100%
	IBD (Ulcerative Colitis)	25	0	25	100%
	Lactose Intolerant	8	0	8	100%
	Osteoarthritis	10	0	10	100%
	Celiac 1 ⁰ relative	5	0	5	100%
	IgA Deficient, non-Celiac	3	0	3	100%
	Rheumatoid Arthritis	26	1	25	96.2%
	Hashimoto's thyroiditis	20	0	20	100%
	Vasculitis	16	0	16	100%
	EBV positive	6	0	6	100%
	Syphilis positive	7	0	7	100%
	Type 2 Diabetes	2	0	2	100%
	Type 1 Diabetes	10	0	10	100%
	Other autoimmune disease	16	0	16	100%
	Grave's Thyroiditis	12	0	12	100%
Suspected celiac, negative biopsy	10	0	10	100%	
	Total non-celiac diseases	250	1	249	99.6%

iii. tTG IgA

tTG IgA		Celiac Disease		Total
		Positive	Negative	
Ig_plex	Positive	126	0	126
	Negative	2	250	252
	Total	128	250	378

Sensitivity: 98.4% (95% CI 97.3% - 99.5%)
 Specificity: 100.0% (95% CI 100.0% - 100.0%)

tTG IgA Sample Group	Sample Disease Classification	Number of Samples	Ig_plex		Sens
			TP	FN	
Celiac Disease (Sensitivity)	Celiac Disease Biopsy confirmed	128	126	2	98.4%
			TN	FP	Spec
Non-Celiac Diseases (Specificity)	SLE	30	0	30	100%
	Sjögren's syndrome	14	0	14	100%
	Wheat allergy	4	0	4	100%
	IBD (Crohn's Disease)	24	0	24	100%
	IBD (Ulcerative Colitis)	25	0	25	100%
	Lactose Intolerant	8	0	8	100%
	Celiac 1 ^o relative	5	0	5	100%
	IgA Deficient, non-Celiac	3	0	3	100%
	Rheumatoid Arthritis	26	0	26	100%
	Hashimoto's thyroiditis	20	0	20	100%
	Vasculitis	16	0	16	100%
	EBV positive	6	0	6	100%
	Syphilis positive	7	0	7	100%
	Type 2 Diabetes	2	0	2	100%
	Type I Diabetes	10	0	10	100%
	Other autoimmune disease	16	0	16	100%
	Grave's Thyroiditis	12	0	12	100%
	Suspected celiac, negative biopsy	10	0	10	100%
Total non-celiac diseases		250	0	250	100%

iv. tTG IgG

tTG IgG		Celiac Disease		Total
		Positive	Negative	
Ig_plex	Positive	60	3	63
	Negative	68	247	315
	Total	128	250	378

Sensitivity: 46.9% (95% CI 42.5% - 51.3%)

Specificity: 98.8% (95% CI 98.1% - 99.5%)

tTG IgG Sample Group	Sample Disease Classification	Number of Samples	Ig_plex		Sens
			TP	FN	
Celiac Disease (Sensitivity)	Celiac Disease Biopsy confirmed	128	60	68	46.9%
			TN	FP	Spec
Non-Celiac Diseases (Specificity)	SLE	30	1	29	96.7%
	Sjögren's syndrome	14	0	14	100%
	Wheat allergy	4	0	4	100%
	IBD (Crohn's Disease)	24	0	24	100%
	IBD (Ulcerative Colitis)	25	0	25	100%
	Lactose Intolerant	8	1	7	87.5%
	Osteoarthritis	10	0	10	100%
	Celiac 1 ^o relative	5	0	5	100%
	IgA Deficient, non-Celiac	3	0	3	100%
	Rheumatoid Arthritis	26	1	25	96.2%
	Hashimoto's thyroiditis	20	1	19	95.0%
	Vasculitis	16	0	16	100%
	EBV positive	6	0	6	100%
	Syphilis positive	7	0	7	100%
	Type 2 diabetes	2	0	2	100%
	Type 1 diabetes	10	0	10	100%
	Grave's Thyroiditis	12	0	12	100%
	Suspected celiac, negative biopsy	10	0	10	100%
Other autoimmune disease	16	0	16	100%	
	Total non-celiac diseases	250	3	247	98.8%

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range:

Specimens from 328 presumptively normal donors (including 167 males ranging in age from 19 to 90 years, and 161 females ranging in age from 18 to 66 years) were collected from commercial sources. Specimens were tested according to standard procedure, and all specimens were assayed in single replicates. The normal response is negative.

Analyte	DGP IgA	DGP IgG	tTG IgA	tTG IgG
N (% Positive)	4 (1.22%)	10 (3.05%)	2 (0.61%)	2 (0.61%)
Range (U/mL)	<8.00 – 46.30	<9.00 – 55.04	<16.00 – 100.96	<24.00 – 54.33
Negative Result (U/mL)	<15.0	<13.0	<20.0	<36.0
Positive Result (U/mL)	≥15.0	≥13.0	≥20.0	≥36.0

N. Instrument Name:

sqid-X system

O. System Descriptions:

1. Modes of Operation:

Semi-automated: All sample handling and preparation is performed by the user, and adds reagents to each well. The instrument incubates, washes, scans and analyzes the results. The sqid-X software provides processing instructions and analysis which are configured for the Ig_plex Celiac DGP Panel.

2. Software:

FDA has reviewed applicant’s Hazard Analysis and software development processes for this line of product types:

Yes or No

3. Specimen Identification:

Each 96-well plate has a barcode. The user types the sample information into the computer file.

4. Specimen Sampling and Handling:

The system uses graphical user interface software to lead the operator through manual sample, reagent, and plate handling operations. The system controls the washer to ensure that the same wash programs are used every time and in the correct sequence. Once the assay’s biochemical reactions are complete and the plate is placed in the scanner, the instrument automatically performs a multi-wavelength fluorescent scan of each well in the microarray, analyzes the data, and generates a report containing results for all assay markers. Results for each patient sample are obtained simultaneously for each of the four

Ig_plex Celiac DGP Panel markers: DGP IgA, DGP IgG, tTG IgA and tTG IgG using the data from one well containing one sample of the patient's serum. The system does not interpret the results with respect to diagnosis; it reports each result independently. The sqid-X system also includes numerous internal quality checks.

5. Calibration:

The assay has eight calibrators/standards that are aliquoted into specific wells of the 96-well plate. These wells do not have the microarray of antigens/controls that are in the sample wells.

6. Quality Control:

In addition to the results calculation, a set of internal quality control rules are invoked to evaluate the data that is produced. These rules were identified by safety and hazard analysis to mitigate risks such as general failures in the reaction. They include quality control rules for checking the fitness of the standardization curves, as well as thresholds of controls measuring reporter activity. When data is found that does not fit the required control levels or rules, further processing of the data is halted and the value "No Result" is reported. The internal quality control rules, or invalidation rules, are at each level of data processing. Single analyte results in a well may be invalidated due to high CV of the replicate capture spots; results for an entire well may be invalidated due to failing to meet a control threshold, and all results for an analyte on a plate may be invalidated due to an improper standard curve.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.