

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

A. 510(k) Number:

k102490

B. Purpose for Submission:

New assay

C. Measurand:

Anti- tissue transglutaminase IgA and IgG autoantibodies

D. Type of Test:

Qualitative, microarray based multiplex assay

E. Applicant:

SQI Diagnostics Systems Inc.

F. Proprietary and Established Names:

IgX PLEX™ Celiac Qualitative Assay

G. Regulatory Information:

1. Regulation section:
21 CFR§ 866.5660 – Multiple autoantibodies immunological test system
2. Classification:
Class II
3. Product code:
MVM - Autoantibodies, Endomysial (Tissue Transglutaminase)
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
The IgX plex™ Celiac Qualitative Assay is an in vitro diagnostic test for the qualitative detection of the IgA and IgG immunoglobulin classes of anti-tissue transglutaminase antibody in 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 SQiDworks™ Diagnostics Platform.
2. Indication(s) for use:
See Intended Use above
3. Special conditions for use statement(s):
For prescription use only.
4. Special instrument requirements:
SQiDworks™ Diagnostics Platform

I. Device Description:

The IgX PLEX Celiac Qualitative Assay is supplied as two boxes requiring separate storage conditions: the Refrigerator Box must be refrigerated at 2-8°C, and the Freezer Box must be stored in a -15°C or lower freezer. A microarray plate and wash buffer concentrate are stored refrigerated while the Reporter mix, Calibrators, Positive Control #1, Positive Control #2, Negative Control, and Sample Diluent are

stored frozen. The microarray plate consists of an array of protein and antibody replicate spots, covalently bound to the surface of coated glass within each well of a standard 96-well assay plate. All plates have the identical configuration of microarray elements, containing:

- 21 capture spots for tTg.
- Internal normalization curve and control spots for internal consistency confirmation.

The outer plate holder is labeled with a barcode indicating the assay type and lot number which is read by the platform. The reporter mix is comprised of diluted fluorescently labeled mouse monoclonal antibodies to human immunoglobulin G and A (marker antibody). Each type of marker antibody is labeled with a dye in a different spectral range allowing for the detection of both sub-classes in one well. Each signal is analyzed to provide the end result for each analyte. The system reports each result independently.

The SQiDworks Diagnostics Platform is a multiplex immunoassay instrument that fully automates the process of a specific IgX PLEX Assay from serum transfer to reporting of all assay markers for each individual patient sample. The instrument automates the entire immunoassay protocol from end-to-end including sample pipetting, serum dilution, incubation, washing, and drying. Once the assay’s biochemical reactions have completed, the instrument automatically performs a multi-color fluorescent scan of each well in the microarray, analyzes the data, and generates a report containing results for all assay markers. The SQiDworks Diagnostics Platform also includes numerous internal quality checks and user safety features with fail-safe and interlock mechanisms.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Aeskulisa™ tTG-A
Aeskulisa™ tTG-G
2. Predicate K number(s):
k042644
3. Comparison with predicate:

Characteristics	IgX PLEX™ Celiac Qualitative Assay anti-tTG-IgA	Aeskulisa™ tTG-A (K042644)
Intended Use	An in vitro diagnostic test for the qualitative detection of the IgA and IgG immunoglobulin classes of anti-tissue transglutaminase antibody in 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.	A solid phase enzyme immunoassay for the semiquantitative and qualitative detection of IgA antibodies against tissue transglutaminase (tTG) in human serum. The assay is an aid in the diagnosis of celiac disease (gluten-sensitive enteropathy) and should be used in conjunction with other serological tests and clinical findings. For <i>in vitro</i> diagnostic use

		only.
Assay Format	Qualitative	Qualitative and Semi-Quantitative
Capture antigen	Recombinant human tissue-Transglutaminase	Recombinant human tissue-Transglutaminase and Gliadin-specific peptides
Technology	Microarray-based Fluorescent detection- Automated	ELISA-Manual
Assay substrate	96-well microarray plates	96 well microtiter plates
Sample	Serum	Serum
Calibration	On each plate	Same
Cut-off	2.8 U/mL	15.0 U/mL
Multiplexed Assay	Yes	No
Characteristics	IgX PLEX™ Celiac Qualitative Assay anti-tTG-IgG	Aeskulisa™ tTG-G (K042644)
Intended Use	An <i>in vitro</i> diagnostic test for the qualitative detection of the IgA and IgG immunoglobulin classes of anti-tissue transglutaminase antibody in 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.	A solid phase enzyme immunoassay for the semiquantitative and qualitative detection of IgG antibodies against tissue transglutaminase (tTG) in human serum. The assay is an aid in the diagnosis of celiac disease (gluten-sensitive enteropathy) and should be used in conjunction with other serological tests and clinical findings. For <i>in vitro</i> diagnostic use only.
Assay Format	Qualitative	Qualitative and Semi-Quantitative
Capture antigen	Recombinant human tissue-Transglutaminase	Recombinant human tissue-Transglutaminase and Gliadin-specific peptides
Technology	Microarray-based Fluorescent detection-Automated	ELISA-Manual
Assay substrate	96-well microarray plates	96 well microtiter plates
Sample	Serum	Serum
Calibration	On each plate	Same
Cut-off	4.9 U/mL	15.0 U/mL
Multiplexed Assay	Yes	No

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

CLSI EP12-A2: “User Protocol for Evaluation of Qualitative Test Performance”
 GP10-A: Assessment of Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots

L. Test Principle:

Anti-tissue transglutaminase antibodies present in the serum sample or control materials bind to immobilized human tissue-Transglutaminase to form antigen-antibody complexes. Unbound antibody and other substances in the sample are washed from the wells. Next, anti-human IgA and anti-human IgG antibodies labeled

with fluorescent dye are added as a mix. Excess reporter antibodies are washed away, and excess wash buffer is removed from the wells. Each well is scanned for each of two dye wavelengths. The intensity of the generated signal is proportional to the amount of IgG, and IgA antibodies bound to the printed spots in the wells.

The IgX PLEX™ Celiac Qualitative Assay reports qualitative results for the IgG and IgA immunoglobulin classes of anti-gliadin and anti-tissue transglutaminase antibodies separately. Positive and Negative determinations are calculated based on analyte specific cutoff values, but the internal values for each result are not reported. Concentrations at or below the cutoff are determined to be negative.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The day-to-day, lot-to-lot, and site-to-site qualitative reproducibility of the IgX PLEX™ Celiac Qualitative Assay was evaluated. CLSI EP12-A2: “User Protocol for Evaluation of Qualitative Test Performance” was followed as closely as possible. In the tables below, ‘agreement’ refers to the percentage of samples that agreed with the correct (known) result.

A panel of one clear negative, one clear positive, one sample representing borderline positive and one sample representing borderline negative for each analyte were used for the day-to-day precision reproducibility analysis.

Day-to-day Reproducibility for anti-tTG-IgA: Cut-off 2.8 U/mL

	Negative Sample	Borderline Sample below cut-off	Borderline Sample above cut-off	Positive Sample
Mean (U/mL)	<0.90	1.98	4.49	6.10
% above/below cut-off	NA	-29.4	60.4%	NA
Number positive	0	9	109	116
Number Negative	117	110	3	0
Total Samples	117	119	112	116
Agreement	100%	92.4%	97.3%	100%

Day-to-day Reproducibility for anti-tTG-IgG: Cut-off 4.9 U/mL

	Negative Sample	Borderline Sample below cut-off	Borderline Sample above cut-off	Positive Sample
Mean (U/mL)	2.28	3.29	6.96	9.69
% above/below cut-off	NA	-32.8	42.0%	NA

	Negative Sample	Borderline Sample below cut-off	Borderline Sample above cut-off	Positive Sample
Number positive	1	5	118	120
Number Negative	119	115	2	0
Total Samples	120	120	120	120
Agreement	99.2%	95.8%	98.3%	100%

Site-to-site reproducibility was tested at three sites (SQI Diagnostics and two clinical laboratories). For each analyte, two celiac positive (PS1 and PS2) and one negative sample (NS) was tested eight times in a run. Six runs were performed; three different kit lots were each tested twice. There were a total of 48 possible results per sample per analyte; samples with less than 48 possible results were due to ‘no calls’ by the instrument.

Site-to-site reproducibility

Analyte	Sample	Site 1 – SQI			Site – 2 CLL			Site 3 – MSS		
		Mean (U/mL)	n	% A	Mean (U/mL)	n	% A	Mean (U/mL)/	n	% A
Anti-tTG-IgA c/o = 2.8	PS1	6.89	48	100	6.54	48	100	6.38	48	100
	PS2	8.95	48	100	8.93	48	100	8.70	46	100
	NS	<0.90	48	100	<0.90	48	100	<0.90	48	100
Anti-tTG-IgG c/o = 4.9	PS1	10.51	48	100	10.61	48	100	10.10	48	100
	PS2	17.70	48	100	17.97	47	100	17.30	48	100
	NS	<1.80	47	100	<1.80	48	100	<1.80	47	100

% A = Percent Agreement

The data from the site-to-site reproducibility study was used to report lot-to-lot reproducibility for 48 results per analyte (8 replicates per run, 2 runs per site, at 3 sites).

Lot-to-lot reproducibility:

Analyte		Positive Sample 1			Positive Sample 2			Negative Sample		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
Anti-tTG-IgA	Mean (U/mL)	6.00	6.47	7.34	8.11	8.76	9.72	<0.90	<0.90	<0.90
	Agreement	100%	100%	100%	100%	100%	100%	100%	100%	100%
Anti-	Mean	10.35	10.05	10.83	17.16	17.5	18.32	<1.80	<1.80	<1.80

Analyte		Positive Sample 1			Positive Sample 2			Negative Sample		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
tTG-IgG	(U/mL)									
	Agreement	100%	100%	100%	100%	100%	100%	100%	100%	100%

b. *Linearity/assay reportable range:*
Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Calibrators: The calibrators are not traceable to any recognized standards. Master (in-house) calibrators were prepared from celiac positive patient samples with high titers for anti-tissue transglutaminase IgA and IgG mixed in appropriately predetermined proportions to yield the desired concentration level for both analytes. Relative values were assigned based on testing with predicate systems. The eight standards provided with the kit are traceable to these master calibrators.

Controls: The positive and negative control samples are derived from human sera clinically diagnosed as positive and normal respectively. The platform treats the controls as samples and does not perform any quality assessment based on the results. Although expected results for each applicable analyte of each control are provided, each lab is expected to follow their own quality procedures for assessing controls.

Kit Stability: A real-time stability study of unopened kits is on-going; current data supports a nine-month shelf life at recommended storage conditions. Real-time stability studies are continuing. An accelerated stability study supported an 11 month shelf life. After opening, the kit is stable for the duration of the run; the directions for use states that a plate with unused wells from a previous run must never be reused.

Serum sample stability: Serum samples were found stable at room temperature for 6 hours and for 1 week at 4°C.

d. *Detection limit:*
Not applicable.

e. *Analytical specificity:*
Interference by normally occurring endogenous substances was assessed. A normal sample and several celiac disease samples with low or intermediate levels were evaluated with different levels of endogenous interferents above physiological ranges. The table below lists the levels (mg/mL) of interferents where the difference between non-spiked results and spiked results relative is $< \pm 15\%$:

	Bilirubin	Hemoglobin	Triglycerides	Human IgG
	(mg/mL)			
tTg- IgA	3.0	50.0	100.0	10.0
tTg- IgG	3.0	100.0	100.0	10.0

The specificity of the antibodies was studied by testing samples from 229 patients with other autoimmune diseases. In data not shown here, the sponsor demonstrated that the unusual specificity of the SLE and Sjorgen's Syndrome (SS) samples might be due to unusual sample handling practices by the sample vendor; a second study with differently sourced samples demonstrated no cross reactivity with either assay. The results below combine the results of both studies. The sponsor included a limitation about potential cross-reactivity by SLE and SS samples in the directions for use.

	Specificity (%)		
	#	anti-tTG-IgA	anti-tTG-IgG
SLE	40	67.5	47.5
Sjorgen's Syndrome	24	83.3	62.5
Wheat allergy	5	80.0	40.0
IBD-Crohn's Disease	77	98.6	98.7
Rheumatoid Arthritis	64	98.4	98.4
Vasculitis	5	100.0	80.0
Infectious-EBV	3	100.0	66.7
Infectious-syphilis	2	100.0	100.0
Other autoimmune	10	80.0	70.0
Ulcerative Colitis	10	100	90

f. Assay cut-off:

Samples from 136 Celiac diagnosed patients, 121 normal donors and 40 other autoimmune diseases (n=297) were collected from commercial sources and collaborative laboratories and were representative of North American and European populations. They were tested according to standard assay procedure in duplicate on separate plates using two lots of assay kits on two different platforms for the determination of mean values. The CLSI guideline *GP10-A: Assessment of Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots* and *CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance* were followed to calculate the cut-off (decision limit). Cut-offs were chosen based on the optimum combination of sensitivity and specificity. The assay cut-offs chosen are: tTg-IgA 2.8 U/mL and tTg-IgG 4.9 U/mL.

2. Comparison studies:

a. *Method comparison with predicate device:*

Method comparisons consisted of testing 189 positive celiac patient samples and 176 presumptively normal samples with the IgX PLEX Celiac Qualitative Assay and with a commercially available predicate method for each of the analytes. The celiac patient samples were classified using the following characteristics: 91 samples from biopsy-confirmed celiac diagnosed patients (Marsh III criteria; 30 of these 91 were on a gluten free diet), 59 EMA positive pediatric samples, 27 EMA positive adult samples, and 12 celiac diagnosed with no information on biopsy or gluten free diet status. There were three “no-calls” by the instrument for the tTG IgG assay (total sample n = 362):

Method Agreement: IgX PLEX tTG IgA Results

		Predicate IgA			%Agreement and (CI)	
		POS	NEG			
IgX PLEX a-tTG IgA	POS	155	17		Positive	87.6% (81.9-91.6%)
	NEG	22	171		Negative	91.0% (86.0-94.3%)
	Totals	177	188	365	Overall	89.4% (85.8-92.1%)

Method Agreement: IgX PLEX tTG IgG Results

		Predicate IgG			%Agreement and (CI)	
		POS	NEG			
IgX PLEX a-tTG IgG	POS	117	32		Positive	84.2% (77.2-89.3%)
	NEG	22	191		Negative	85.7% (80.4-89.7%)
	Totals	139	223	362	Overall	85.1% (81.1-88.4%)

b. *Matrix comparison:*

Not applicable; this assay is for serum only.

3. Clinical studies:

a. *Clinical Sensitivity and specificity:*

The sensitivity and specificity of the IgX PLEX Celiac Qualitative Assay for both analytes were determined from 628 samples: 70 celiac diagnosed by Marsh III criteria (biopsy confirmed not on gluten free diet), 102 celiac diagnosed Endomysial Antibody (EMA) positive (adult and pediatric), 192 presumptively normal samples, 229 samples from other autoimmune diseases, and 35 other non-celiac samples.

In addition, seven celiac IgA deficient samples were included in the sensitivity analysis for Anti-tTG-IgG. The resulting clinical sensitivities and specificities are shown below:

Sensitivity and Specificity of IgX PLEX a-tTG IgA Results

		Celiac Disease				
		POS	NEG			
IgX PLEX a-tTG IgA	POS	169	25		Sensitivity	98.3% (95.0-95.4%)
	NEG	3	429		Specificity	94.5% (92.0-96.2%)
	Totals	172	454	626	Overall	95.5% (93.6-96.9%)

Sensitivity and Specificity of IgX PLEX a-tTG IgG Results

		Celiac Disease				
		POS	NEG			
IgX PLEX a-tTG IgG	POS	140	50		Sensitivity	80.9% (74.4-86.1%)
	NEG	33	406		Specificity	89.0% (85.8-91.6%)
	Totals	173	456	629	Overall	86.8% (83.9-89.2%)

4. Clinical cut-off:
Not applicable. See assay cut-off discussion above.

5. Expected values/Reference range:
The expected value in the normal population is negative, although a small portion of the population may have anti-tTg autoantibodies without clinical disease.

N. Proposed Labeling:

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

O. Conclusion:

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