510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K183313

B. Purpose for Submission:

New devices

C. Measurands:

IgA autoantibodies specific for tissue transglutaminase (tTG)
IgG autoantibodies specific for tissue transglutaminase (tTG)

D. Type of Test:

Semi-quantitative and qualitative (anti-tTG IgA) and qualitative (anti-tTG IgG) manual immunoassays

E. Applicant:

EUROIMMUN US INC.

F. Proprietary and Established Names:

Anti-tissue Transglutaminase ELISA (IgA)
Anti-tissue Transglutaminase ELISA (IgG)

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5660, Multiple autoantibodies immunological test system

2. Classification:

   Class II

3. Product code:

   MVM

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended uses:

   The Anti-tissue Transglutaminase ELISA (IgA) test kit is intended for the qualitative or semi-quantitative determination of IgA class antibodies against tissue transglutaminase in human serum and EDTA plasma (K3-EDTA, Li+-heparin, Na+-citrate). It is used as an aid in the diagnosis of gluten-sensitive enteropathy (celiac disease) and dermatitis herpetiformis Duhring, in conjunction with other laboratory and clinical findings.

   The Anti-tissue Transglutaminase ELISA (IgG) test kit is intended for the qualitative determination of IgG class antibodies against tissue transglutaminase in human serum and EDTA plasma (K3-EDTA, Li+-heparin, Na+-citrate). It is used as an aid in the diagnosis of gluten-sensitive enteropathy (celiac disease), in conjunction with other laboratory and clinical findings.

2. Indications for use:

   See above

3. Special conditions for use statement:

   For prescription use

4. Special instrument requirements:

   An ELISA microplate reader capable of reading absorbance values at 450 nm with a reference filter between 620–650 nm. An automatic microplate washer capable of dispensing 200 µL of fluid is recommended.

I. Device Description:

Anti-tissue Transglutaminase ELISA (IgA)
The device is packaged as a kit containing: 12 microplate strips each containing eight antigen-coated wells and frame; three calibrators (2, 20, and 200 RU/mL); human serum-based IgA positive and negative controls; horseradish peroxidase (HRP)-conjugated rabbit anti-human IgA; ready-to-use sample buffer; 10x wash buffer concentrate; 3,3',5,5'-tetramethylbenzidine (TMB) substrate; and sulfuric acid stop solution.

Anti-tissue Transglutaminase ELISA (IgG)
The device is packaged as a kit containing: 12 microplate strips each containing eight antigen-coated wells and frame; one cut-off calibrator (20 RU/mL); human serum-based IgG

positive and negative controls; horseradish peroxidase (HRP)-conjugated rabbit anti-human IgG; ready-to-use sample buffer; 10x wash buffer concentrate; 3,3',5,5'-tetramethylbenzidine (TMB) substrate; and sulfuric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device names and predicate 510(k) numbers:

   INOVA QuantaLite h-tTg (human tissue transglutaminase) IgA, K011566
   INOVA QuantaLite h-tTg (human tissue transglutaminase) IgG, K011570

2. Comparison with predicate:

Anti-tissue Transglutaminase ELISA (IgA)

Similarities		
Item	Device	Predicate
Intended Use	The anti-tissue Transglutaminase ELISA (IgA) test kit is intended for the qualitative or semi-quantitative determination of IgA class antibodies against tissue transglutaminase in human serum and EDTA plasma (K3-EDTA, Li+-heparin, Na+-citrate). It is used as an aid in the diagnosis of gluten-sensitive enteropathy (celiac disease) and dermatitis herpetiformis Duhring, in conjunction with other laboratory and clinical findings.	An Enzyme Linked Immunosorbant Assay (ELISA) for the semi-quantitative detection of IgA antibodies to tissue transglutaminase (endomysium) in human serum. Detection of these antibodies is an aid in diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis.
Assay Type	ELISA	Same
Sample Type	Serum	Same
Labeled Detection Antibody (Conjugate)	Horseradish peroxidase conjugated to goat anti-human IgG	Same
Enzyme Substrate	3,3',5,5'-tetramethylbenzidene (TMB)	Same
Signal/Wavelength	Optical density, 450nm	Same
Instrumentation	Microwell plate reader	Same
Storage	2–8°C	Same

Differences		
Item	Device	Predicate
Assay Format	Semi-quantitative and qualitative	Semi-quantitative only
Capture Antigen	Purified recombinant human tissue transglutaminase	Native human red blood cell tissue transglutaminase
Conjugate	Peroxidase-labeled anti-human IgA (rabbit)	Peroxidase-labeled anti-human IgA (goat)
Calibrators	Set of three: Values in RU/mL: 2, 20, 200	One calibrator Value in U/mL
Assay Range	2–200 RU/mL	Not specified
Controls	Positive and Negative Control	Low Positive, High Positive, and Negative Control
Results Interpretation	Semi-Quantitative: Negative: < 20 RU/mL Positive: ≥ 20 RU/mL Qualitative Ratio: Negative: < 1 Positive: ≥ 1	Semi-Quantitative: Negative: ≤ 20 U/mL Weak Positive: 20-30 U/mL Positive: > 30 U/mL

Anti-tissue Transglutaminase ELISA (IgG)

Similarities		
Item	Device	Predicate
Assay Type	ELISA	Same
Labeled Detection Antibody (Conjugate)	Horseradish peroxidase conjugated to goat anti-human IgG	Same
Enzyme Substrate	3,3',5,5'-tetramethylbenzidene (TMB)	Same
Signal/Wavelength	Optical density, 450nm	Same
Instrumentation	Microwell plate reader	Same
Storage	2–8°C	Same

Differences		
Item	Device	Predicate
Intended Use	The Anti-tissue Transglutaminase ELISA (IgG) test kit is intended for the qualitative determination of IgG class	An Enzyme Linked Immunosorbant Assay (ELISA) for the semi-quantitative detection of IgG antibodies to tissue

Differences		
Item	Device	Predicate
	antibodies against tissue transglutaminase in human serum and EDTA plasma (K3-EDTA, Li+-heparin, Na+-citrate). It is used as an aid in the diagnosis of gluten-sensitive enteropathy (celiac disease), in conjunction with other laboratory and clinical findings.	transglutaminase (endomysium) in human serum. Detection of these antibodies, in conjunction with IgA antibodies, is an aid in diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis. This test is intended for providing added sensitivity when testing IgA deficient patients.
Sample Type	Serum, Plasma (K3-EDTA, Li+-heparin, Na+-citrate	Serum
Assay Format	Qualitative	Semi-quantitative only
Capture Antigen	Purified recombinant human tissue transglutaminase	Native human red blood cell tissue transglutaminase
Conjugate	Peroxidase-labeled anti-human IgA (rabbit)	Peroxidase-labeled anti-human IgA (goat)
Calibrators	One cut-off calibrator (20 RU/mL)	One calibrator Value in U/mL
Assay Range	Not applicable	Not specified
Controls	Positive and Negative Control	Low Positive, High Positive, and Negative Control
Results Interpretation	Qualitative (Ratio): Negative: < 1 Positive: ≥ 1	Semi-Quantitative: Negative: ≤ 20 U/mL Weak Positive: 20-30 U/mL Positive: > 30 U/mL

K. Standard/Guidance Document Referenced:

CLSI guideline EP6-A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline"

L. Test Principle:

Controls, calibrators and diluted patient sera are added to separate wells, allowing any anti-tTG antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgA or anti-human IgG conjugate is added to each well.

After washing away any unbound conjugate, specific enzyme substrate (TMB) is then added to the wells. After stopping the enzymatic reaction, the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm.

Semi-quantitative results for the anti-tTG IgA assay are determined from a series of three calibrators (2, 20, and 200 RU/mL). Values less than 20 RU/mL are considered negative results while values ≥ 20 RU/mL are considered positive.

Qualitative results for the anti-tTG IgA and IgG assays are determined using a ratio of the absorbance of the sample to the absorbance of the cut-off calibrator (20 RU/mL). The ratio is determined by dividing the optical density (OD) of the sample or control by the OD of the cut-off calibrator. A ratio < 1.0 is negative while a ratio ≥ 1.0 is positive.

M. Performance Characteristics:

1. Analytical performance:

All results presented below met the manufacturer's pre-determined acceptance criteria.

a. Precision:

Anti-tissue Transglutaminase ELISA (IgA)

Imprecision:
Precision performance was evaluated by testing a panel consisting of six patient sera with levels of anti-tTG IgA antibodies that cover the analytical measuring range. The samples were assayed in duplicate, twice a day, for 20 days with one reagent lot for a total of 80 replicates per sample. The semi-quantitative concentration (RU/mL) was determined and the repeatability, between-run, within-day, between-day, and total precision were calculated:

Sample	Mean (RU/ml)	Repeatability		Between-Run		Within-Day		Between-Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	6.21	0.48	7.7%	0.37	6.0%	0.61	9.8%	0.87	14.0%	1.06	17.1%
2	15.11	0.58	3.9%	0.68	4.5%	0.89	5.9%	0.62	4.1%	1.08	7.2%
3	25.95	0.96	3.7%	1.82	7.0%	2.06	8.0%	0.00	0.0%	2.06	8.0%
4	23.92	1.32	5.5%	1.38	5.8%	1.91	8.0%	0.54	2.2%	1.99	8.3%
5	108.72	3.30	3.0%	3.67	3.4%	4.93	4.5%	5.17	4.8%	7.14	6.6%
6	163.88	5.98	3.7%	4.74	2.9%	7.64	4.7%	5.00	3.0%	9.12	5.6%

The qualitative reproducibility of the assay was evaluated using the same data set but by calculating the qualitative ratio of the sample OD to the OD of the cut-off calibrator:

Sample	Mean Ratio	Range of Ratios	Expected Result	Repeatability	
				Positive/ Negative	% Correct
1	0.39	0.31–0.52	Neg	0/80	100
2	0.78	0.62–0.92	Neg	0/80	100
3	1.15	1.00–1.28	Pos	80/0	100
4	1.10	0.90–1.28	Pos	77/3	96
5	3.25	2.60–3.79	Pos	80/0	100
6	4.65	4.20–5.41	Pos	80/0	100

Lot to Lot Imprecision:

To evaluate lot-to-lot imprecision, a panel of six samples with levels of anti- tTG IgA antibodies spanning the AMR was tested with three replicates per run, two runs per day, with three different lots over the course of five days (a total of 90 replicates per sample). The results are summarized in the table below:

Sample	Mean RU/ml	Repeatability		Between-Run		Between-Day		Within-Lot		Between-Lot		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	5.70	0.70	12.3%	0.55	9.6%	0.34	6.0%	0.96	16.8%	0.20	3.6%	0.98	17.2%
2	14.92	0.57	3.8%	0.93	6.2%	0.64	4.3%	1.26	8.5%	0.00	0.0%	1.26	8.5%
3	24.24	1.47	6.1%	1.84	7.6%	0.00	0.0%	2.36	9.7%	0.92	3.8%	2.53	10.5%
4	22.89	1.16	5.1%	1.39	6.1%	1.01	4.4%	2.07	9.1%	0.85	3.7%	2.24	9.8%
5	105.35	3.40	3.2%	4.89	4.6%	3.55	3.4%	6.93	6.6%	1.26	1.2%	7.04	6.7%
6	162.46	5.67	3.5%	10.63	6.5%	3.66	2.3%	12.59	7.8%	0.00	0.0%	12.59	7.8%

Qualitative performance between lots was calculated by the qualitative ratio of the sample OD to the cut-off calibrator:

			Lot 1 Repeatability		**Lot 2 Repeatability**		**Lot 3 Repeatability**		**Total**	
	Mean Ratio	**Expected Result**	**Positive/ Negative**	**% Correct**	**Positive/ Negative**	**% Correct**	**Positive/ Negative**	**% Correct**	**Positive/ Negative**	**% Correct**
1	0.35	Neg	0/30	100	0/30	100	0/30	100	0/90	100
2	0.77	Neg	0/30	100	0/30	100	0/30	100	0/90	100
3	1.11	Pos	30/0	100	30/0	100	29/1	97	89/1	99
4	1.07	Pos	30/0	100	29/1	97	24/6	80	83/7	80
5	3.20	Pos	30/0	100	30/0	100	30/0	100	90/0	100
6	4.68	Pos	30/0	100	30/0	100	30/0	100	90/0	100

Anti-tissue Transglutaminase ELISA (IgG)

Qualitative Repeatability:

Repeatability was evaluated by testing a panel consisting of six patient sera with levels of anti-tTG IgG antibodies at different concentrations. The samples were assayed in duplicate, twice a day, for 20 days with one reagent lot, a total of 80 replicates per sample.

Sample	Mean Ratio	Range of Ratios	Expected Result	Repeatability	
				Positive/ Negative	% Correct
1	0.13	0.09–0.16	Neg	0/80	100
2	0.65	0.45–0.78	Neg	0/80	100
3	0.86	0.63–1.09	Neg	5/75	93.8
4	1.13	0.98–1.36	Pos	76/4	95
5	1.93	1.74–2.18	Pos	80/0	100
6	4.25	3.65–5.05	Pos	80/0	100

Lot to Lot Imprecision:
To evaluate lot-to-lot imprecision, a panel of six samples with levels of anti-tTG IgG antibodies at different concentrations was tested with three replicates per run, two runs per day with three different lots over the course of five days (a total of 90 replicates per sample). The results are summarized in the table below:

	Mean Ratio	Expected Result	Lot 1 Repeatability		Lot 2 Repeatability		Lot 3 Repeatability		Total	
			Positive/ Negative	% Correct	Positive/ Negative	% Correct	Positive/ Negative	% Correct	Positive/ Negative	% Correct
1	0.14	Neg	0/30	100	0/30	100	0/30	100	0/90	100
2	0.72	Neg	0/30	100	0/30	100	0/30	100	0/90	100
3	0.93	Neg	0/30	100	18/12	40	5/25	83.3	23/67	74.4%
4	1.32	Pos	30/0	100	30/0	100	30/0	100	90/0	100
5	2.21	Pos	30/0	100	30/0	100	30/0	100	90/0	100
6	4.80	Pos	30/0	100	30/0	100	30/0	100	90/0	100

b. Linearity/assay reportable range:

Anti-tissue Transglutaminase ELISA (IgA)
A set of 11 sample dilutions was made by diluting a high positive sample (> 200 RU/mL) and a moderately high sample (87.8 RU/mL) with different proportions of negative sera. The dilutions were tested in quadruplicate and the results were evaluated by a linear and polynomial regression analysis. The linear regression analysis of the samples showed the samples were linear within the range defined by the calibrators. Although the 2nd order polynomial regression coefficient was significant (p-value <0.05) for both samples, the amount of non-linearity was acceptable. The linear regression analyses yielded $y = 1.00x - 0.03$ for the high sample and $1.08x - -10.4$ for the moderately high sample. The assay range was defined as 2 to 200 RU/mL.

Anti-tissue Transglutaminase ELISA (IgG)
Not applicable

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

Anti-tissue Transglutaminase ELISA (IgA) and Anti-tissue Transglutaminase ELISA (IgG):

Traceability: A recognized standard or reference material for anti-tTG antibodies is not available. The assay is calibrated in relative arbitrary units (RU/ml) in semi-quantitative mode. Alternatively, results may be determined as a ratio of the cut-off calibrator (i.e., 20 RU/mL calibrator) then defined as positive or negative in qualitative mode.

Stability studies:
Real-time stability studies were conducted using three production lots of all kit reagents. The studies support a 12-month unopened stability claim when stored at +2°C to +8°C. Other studies support that the reconstituted wash buffer is stable for up to 28 days. Opened reagents stored at +2°C to +8°C are stable for six months for the anti-tTG IgA assay, and for 12 months for the anti-tTG IgG assay.

Sample stability:
The sponsor recommends following the guidelines in CLSI GP44-A4 for sample storage. Samples should be stored at room temperature (+18°C to +25°C) no longer than 8 hours. If the assay will not be completed within 8 hours, the samples should be refrigerated at +2°C to +8°C. If the assay will not be completed within 48 hours, or will be stored beyond 48 hours, samples should be frozen at −20°C or lower. Samples should not be repeatedly frozen and thawed.

d. *Detection limit:*

Anti-tissue Transglutaminase ELISA (IgA):
The Limit of Blank (LoB) was determined by assaying four negative samples, each tested 15 times for a total of 60 replicates. The limit of detection (LoD) was determined by assaying four low negative sera in three runs with five replicates each, each run performed on a different day. The LoB and LoD for the assay was 0.8 and 1.1 RU/mL, respectively. The Limit of Quantitation (LoQ) was the same as the LoD, 1.1 RU/mL.

Anti-tissue Transglutaminase ELISA (IgG):
Not applicable

e. *Analytical specificity:*

Anti-tissue Transglutaminase ELISA (IgA) and Anti-tissue Transglutaminase ELISA (IgG):
Endogenous interferences were evaluated by testing hemolytic, lipemic, and icteric samples across the assay range. These endogenous interferences showed no influence on the result up to a concentration of 1000 mg/dL for hemoglobin, 2000 mg/dL for triglycerides, and 40 mg/dL for bilirubin in testing with Anti-tissue Transglutaminase

ELISA IgA and IgG. Interferences due to high protein (albumin), cholesterol, and intralipids were not investigated; the package directions instruct the user to interpret the results with caution when testing samples with high levels of these substances.

f. Assay cut-off:

The cut-offs for both assays were established then validated by ROC analyses using sample sets that were independent of the method comparison and clinical studies and included 183 biopsy-confirmed celiac disease patients and 298 control samples with negative biopsies. The ROC analyses demonstrated optimal sensitivity and specificity at the specified cut-offs, described below:

Anti-tissue Transglutaminase ELISA (IgA)

Semi-Quantitative:	Qualitative (Ratio):
Negative: < 20 RU/mL	Negative: < 1
Positive: ≥ 20 RU/mL	Positive: ≥ 1

Anti-tissue Transglutaminase ELISA (IgG)

Qualitative (Ratio):
Negative: < 1
Positive: ≥ 1

2. Comparison studies:

a. Method comparison with predicate device:

Anti-tissue Transglutaminase ELISA (IgA)

A panel of 174 samples was assayed with the EUROIMMUN Anti-tissue Transglutaminase ELISA (IgA) and with the predicate ELISA according to their respective package inserts. The sample cohort contained 30 samples from biopsy confirmed celiac patients, 64 Endomysial Antibody (EMA) / Gliadin Antibody positive patient samples, 10 inflammatory bowel disease (IBD), 10 diabetes mellitus type 1 (T1D), and 60 rheumatic diseases samples.

For semi-quantitative evaluation, 121 samples within the measuring range of the EUROIMMUN Anti-tissue Transglutaminase ELISA (IgA) were included in the method comparison.

		Predicate ELISA		
		Pos	Neg	Total
EUROIMMUN anti-tTG IgA	Pos	67	1	68
	Neg	9	44	53
	Total	76	45	121

Positive Percent Agreement: 88.2% (95% CI 78.7% – 94.4%)
Negative Percent Agreement: 97.8% (95% CI 88.2% – 99.9%)

All samples, including those above or below the AMR, were included in the qualitative method comparison:

		Predicate ELISA		
		Pos	Neg	Total
EUROIMMUN anti-tTG IgA	Pos	90	1	91
	Neg	9	74	83
	Total	99	75	174

Positive Percent Agreement: 90.9% (95% CI 83.4% – 95.8%)
Negative Percent Agreement: 96.0% (95% CI 92.8% –100.0%)

Anti-tissue Transglutaminase ELISA (IgG)
A panel of 174 samples was assayed with the EUROIMMUN Anti-tissue Transglutaminase ELISA (IgG) and with the predicate ELISA according to their respective package inserts. The sample cohort contained 59 samples from biopsy-confirmed celiac patients, 35 Endomysial Antibody (EMA)/Gliadin Antibody positive patient samples, 10 inflammatory bowel disease (IBD), 10 diabetes mellitus type 1 (T1D), and 60 rheumatic disease samples. The correlation between the new device and the predicate for the entire panel is shown in the tables below.

		Predicate ELISA		
		Pos	Neg	Total
EUROIMMUN anti-tTG IgG	Pos	66	4	70
	Neg	8	96	104
	Total	74	100	174

Positive Percent Agreement: 89.2% (95% CI 79.8% – 95.2%)
Negative Percent Agreement: 96.0% (95% CI 90.1% – 98.9%)

b. Matrix comparison:

Anti-tissue Transglutaminase ELISA (IgA)
Twenty-five (25) sample sets consisting of matched serum and potassium EDTA plasma, lithium heparin plasma, and sodium citrate plasma (K3 EDTA, Li+ heparin, Na+ citrate) were evaluated semi-quantitatively and qualitatively.
For the semi-quantitative assessment, 19 sample sets were within the assay range; the range of the serum sample concentrations was 2.61 – 168.46 RU/mL. Passing-Bablok regression was calculated for the comparison of plasma to serum:

RU/mL	**K3EDTA plasma**	**Li-heparin plasma**	**Citrate plasma**
Regression equation	y = 0.93x − 0.26	y = 1.03x + 0.96	y = 1.02x − 1.22
95% CI of intercept	−2.14 – 1.64	0.34 –2.57	−3.57 – 0.46
95% CI of slope	0.88 – 0.99	0.99 –1.05	0.93 – 1.06
Correlation coefficient	0.993	0.995	0.993

All samples were qualitatively assessed. The samples covered the range of the assay; the ratios of the serum values ranged from 0.04 – 4.96 and % agreement was >95% for all three matrices tested. Two samples were discordant: one K3 EDTA plasma sample was negative while the serum sample was positive, and one Li+ heparin sample was positive while the serum sample was negative. Both of these samples were < ±10% of the cut-off ratio. None of the results of citrate plasma samples were discordant with matched serum samples.

Anti-tissue Transglutaminase ELISA (IgG)
A series of 20 sample sets of serum and potassium EDTA plasma, lithium heparin plasma, and sodium citrate plasma (K3 EDTA, Li+ heparin, Na+ citrate) samples were evaluated. The samples covered a range of ratios of the serum values (0.04 – 4.10). None of the results of plasma samples were discordant with matched serum samples.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Clinically characterized serum samples were included in the clinical validation for the EUROIMMUN anti-tTG IgA and IgG ELISAs. Both assays' studies included 102 biopsy-confirmed celiac disease samples (Marsh scores 1 to 3c) and 166 samples from autoimmune diseases that could be expected to be found in the differential diagnosis of celiac disease.

Anti-tissue Transglutaminase ELISA (IgA)
The evaluation of the anti-tTG IgA assay included an additional 45 dermatitis herpetiformis Duhring (DH). The sensitivity was 99.0% (95% CI: 94.7 – 100.0%) for celiac disease and 77.8% (95% CI: 62.9 – 88.8%) for DH. The specificity of the device for disease controls was 97.6% (95% CI: 93.9 – 99.3%). A qualitative assessment of the same sample yielded the same results:

		Celiac Disease[1]				Duhring's[2]		
		Pos	Neg	Total		Pos	Neg	Total
EUROIMMUN anti-tTG IgA	Pos	101	4	105		35	4	36
	Neg	1	162	163		10	162	172
	Total	102	166	268		45	166	209
Sensitivity		99.0% (95% CI: 94.7 – 100.0%)				77.8% (95% CI: 62.9 – 88.8%)		
Specificity		97.6% (95% CI: 93.9 – 99.3%)				97.6% (95% CI: 93.9 – 99.3%)		

[1] DH samples not included in this analysis

[2] Celiac Disease samples not included in this analysis

	Anti-tTG IgA results	
Target Disease Group:	*n*	Positive (%)
Celiac Disease	102	101 (99.0%)
Duhring's dermatitis herpetiformis	45	35 (77.8%)
Non-Target Disease Group:	*n*	Negative (%)
Ulcerative colitis	10	10 (100.0%)
Crohn's Disease	10	10 (100.0%)
Diabetes Mellitus Type 1	30	29 (96.7%)
Autoimmune Thyroid Disease	20	20 (100.0%)
Rheumatoid Arthritis	30	28 (93.3%)
Sjögren's syndrome	21	21 (100.0%)
Systemic lupus erythematosus	30	30 (100.0%)
Systemic sclerosis	15	14 (93.3%)
Specificity	166	162 (97.6%)

Anti-tissue Transglutaminase ELISA (IgG)

The evaluation of the anti-tTG IgG assay did not include the DH samples but included an additional 17 IgA-deficient celiac disease samples. The sensitivity was 37.3% (95% CI: 42.4 – 59.7%) for celiac disease and 82.4% (95% CI: 62.9 – 88.8%) for IgA-deficient celiac patients. The specificity of the device for disease controls was 99.4% (95% CI: 96.7 – 100.0%).

		Celiac Disease[1]				IgA-def Celiac[2]		
		Pos	Neg	Total		Pos	Neg	Total
EUROIMMUN a-tTG IgG	Pos	38	165	203		14	165	179
	Neg	64	1	65		3	1	4
	Total	102	166	268		17	166	183
Sensitivity		37.3% (95% CI: 42.4 – 59.7%)				82.4% (95% CI: 62.9 – 88.8%)		
Specificity		99.4% (95% CI: 96.7–100.0%)				99.4% (95% CI: 96.7–100.0%)		

[1] IgA deficient samples not included in this analysis
[2] IgA-sufficient Celiac Disease samples not included in this analysis

	Anti-tTG IgG results	
Target Disease Group:	*n*	Positive (%)
Celiac Disease	102	38 (37.3%)
IgA-deficient Celiac Disease	17	14 (82.4%)
Non-Target Disease Group:	*n*	Negative (%)
Ulcerative colitis	10	10 (100.0%)
Crohn's Disease	10	10 (100.0%)
Diabetes Mellitus Type 1	30	29 (96.7%)
Autoimmune Thyroid Disease	20	20 (100.0%)
Rheumatoid Arthritis	30	30 (100.0%)
Sjögren's syndrome	21	21 (100.0%)
Systemic lupus erythematosus	30	30 (100.0%)
Systemic sclerosis	15	15 (100.0%)
Specificity	**166**	**165 (99.4%)**

4. Clinical cut-off**:**

   See assay cutoff

5. Expected values/Reference range:
   The seroprevalence (as defined as anti-tTG IgA) of celiac disease in the U.S. from National Health and Nutrition Examination Surveys (NHANES) was 0.7% in 2012[1]. To evaluate the incidence of anti-tTG antibodies in normal population, sera from 250 U.S. healthy adult blood donors of a range of ages (17 – 81 years, mean 38 years) and roughly evenly divided by sex (136 male, 114 female) were tested with both assays. A separate panel of 400 normal European healthy blood donors (range 18 – 67 years, mean age 39 years; 251 men, 149 women) was also assessed by both assays:

[1] Rubio-Tapia A et al. (2012). The prevalence of celiac disease in the United States. *American Journal of Gastroenterology*, 107, 1538. doi: 10.1038/ajg.2012.219

Anti-tissue Transglutaminase ELISA (IgA)
There were four positive samples in the U.S. cohort in both the semi-quantitative and qualitative determinations. Thus, the prevalence of the U.S. cohort was 1.6% (95% CI 0.4 – 4.0%). In the semi-quantitative evaluation, the mean value was 6.7 RU/mL and the median value was 3.1 RU/mL. There were three positive samples in the European cohort in both the semi-quantitative and qualitative determinations. Thus, the prevalence of the European cohort was 0.8% (95% CI 0.2 – 2.2%). In the semi-quantitative evaluation, the mean value was 5.4 RU/mL and the median value was 4.2 RU/mL.

Anti-tissue Transglutaminase ELISA (IgG)
None of the samples in either cohort were positive. Thus, the prevalence of the U.S. cohort is 0% (95% CI 0.0 – 1.5%) and the prevalence of the European cohort is 0% (95% CI 0.0 – 0.9%).

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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