

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

**A. 510(k) Number:**

k091520

**B. Purpose for Submission:**

New device

**C. Measurand:**

Anti-human Tissue transglutaminase (hu-tTg) IgA antibodies  
Anti-human Tissue transglutaminase (hu-tTG) IgG antibodies

**D. Type of Test:**

Qualitative and semi quantitative ELISA

**E. Applicant:**

IMMCO Diagnostics, Inc.

**F. Proprietary and Established Names:**

ImmuLisa™ Celiac tTG rHuman Tissue Transglutaminase IgA Antibody ELISA  
ImmuLisa™ Celica tTG rHuman Tissue Transglutaminase IgG Antibody ELISA

**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):  
Enzyme linked immunoassays (ELISA) for the qualitative and semi-quantitative detection of anti-human Tissue Transglutaminase IgA or IgG antibodies in human serum to aid in the diagnosis of gluten sensitive enteropathy / celiac disease (CD) in conjunction with other laboratory and clinical findings.
2. Indication(s) for use:  
Same as intended use
3. Special conditions for use statement(s):  
For prescription use only
4. Special instrument requirements:  
ELISA microtiter plate reader capable of measuring OD at 450 nm

**I. Device Description:**

Each device contains: microplate with individual breakaway microwells coated with either recombinant human tTG IgA or IgG antibodies, assay controls (positive and negative), a ready to use set of 5 calibrators, HRP goat anti-human IgA or IgG conjugate, serum diluent, TMB enzyme substrate, stop solution, and powder wash buffer.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
 ImmuLisa hu-tTG IgA Antibody ELISA  
 ImmuLisa hu-tTG IgG Antibody ELISA  
 ImmuGlo Endomysial Antibody IFA
2. Predicate 510(k) number(s):  
 k032571  
 k040095  
 k912551
3. Comparison with predicate:

<b>SIMILARITIES</b>		
	<b>Device</b>	<b>Predicate</b>
Item	IMMCO ImmuLisa Celiac tTG IgA & IgG ELISA	IMMCO ImmuLisa hu-tTG IgA & IgG ELISA
Intended Use	Enzyme linked immunoassays (ELISA) for the qualitative and semi-quantitative detection of anti-human Tissue Transglutaminase IgA/IgG antibodies in human serum to aid in the diagnosis of patients with gluten sensitive enteropathy/celiac disease (CD) in conjunction with other laboratory and clinical findings.	Same
Methodology	ELISA	Same
Analyte	Anti-human tissue transglutaminase(tTG) IgA and IgG antibodies	Same
Capture antigen	Human tTG (rh-tTG)	Same
Detection antibody	Goat anti-human IgA or IgG conjugate	Same
Positive Control	Human serum positive for tTG IgA and IgG Antibodies	Same
Negative control	Human serum	Same
Cutoff	20 EU/mL	Same
Storage	2-8° C	Same

<b>DIFFERENCES</b>		
	<b>Device</b>	<b>Predicate</b>
Item	IMMCO ImmuLisa Celiac tTG IgA and IgG ELISA	IMMCO ImmuLisa hu-tTG IgA and IgG ELISA
Assay format	Semi-quantitative and qualitative	Semi-quantitative
Enzyme Conjugate	Horseradish peroxidase (HRP)	Alkaline phosphate
Substrate/Chromogen	TMB	pNPP

Calibrators	Set of 5. Values in EU/mL: Calibrator E: 1 Calibrator D: 20 Calibrator C: 40 Calibrator B: 80 Calibrator A: 160	Set of 4, Values in EU/mL Calibrator D: ~20 Calibrator C: ~40 Calibrator B: ~80 Calibrator A: ~160
Screening dilution	1:101	1:51
Reading	450 nm on spectrophotometer	405 nm on spectrophotometer
Linear Range	IgA 4-160 EU/mL IgG 2.9-260 EU/mL	20-160 EU/mL 20-80 UU/mL
Limit of Detection	IgA 4 EU/mL IgG 2.9 EU/mL	5.7 EU/mL 4.3 EU/mL

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

CLSI EP-17A “Protocols for Determination of Limits of Detection and Limits of Quantitation;

CLSI EP-6A “Evaluation of the Linearity of Quantitative Analytical Methods”

**L. Test Principle:**

The Celiac tTG IgA and IgG ELISA tests are performed as solid phase immunoassays (ELISAs). Microwells are coated with rh-tTG antigen followed by a blocking step to reduce non-specific binding during the assay run. Controls, calibrators and patient sera are incubated in the antigen coated wells to allow specific antibodies present in the serum to bind to the rh-tTG antigen. Unbound antibodies and other serum proteins are removed by washing the microwells. Unbound HRP goat anti-human conjugate is removed by washing. Specific enzyme substrate (TMB) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of TMB substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL) or reported as positive or negative.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

A precision study was performed using 7 samples with concentrations across the assay measuring range, The assays were run over successive days using a single operator. Results from 5 runs in duplicate were used to calculate imprecision between days. Results from a run of 10 replicates were used to calculate repeatability. Results are summarized below:

Kit	S #	Mean (EU/mL)	Total Imprecision		Between days		Within run (Repeatability)	
			SD (EU/mL)	CV%	SD (EU/mL)	CV%	SD (EU/mL)	CV%
Celiac tTG IgA Assay	1	10.6	1.086	10.2%	1.135	10.7%	1.095	10.2%
	2	20.4	1.224	6.0%	1.476	7.2%	0.956	4.7%
	3	25.0	1.496	6.0%	1.740	6.9%	1.271	5.1%
	4	34.9	2.075	5.9%	2.099	6.1%	2.110	6.0%
	5	48.7	2.634	5.4%	3.661	7.5%	1.090	2.2%
	6	110.6	4.008	3.6%	4.221	3.8%	3.960	3.6%
	7	134.7	7.253	5.4%	8.728	6.5%	5.904	4.4%
	8	183.7	6.161	3.4%	6.606	3.6%	5.615	3.0%
Celiac tTG IgG Assay	1	15.3	1.100	7.2%	1.283	8.3%	0.929	6.1%
	2	24.3	1.727	7.1%	2.101	8.6%	1.355	5.6%
	3	29.8	1.931	6.5%	2.492	8.4%	1.289	4.3%
	4	60.5	3.046	5.0%	3.647	6.0%	2.507	4.1%
	5	99.1	4.720	4.8%	5.687	5.7%	3.756	3.8%
	6	233.7	10.022	4.3%	11.918	5.0%	7.108	3.1%
	7	263.5	13.061	5.0%	14.124	5.4%	11.794	4.4%

**Qualitative precision:**

The results for the precision studies were recalculated using the qualitative protocol and the results were reported as positive or negative. Ten runs of 4 samples, one in the low negative range, one in the moderate positive range and 2 samples at approximately  $\pm 20\%$  of the assay cutoffs, produced 100% qualitative agreement on both the tTG IgA and IgG assays.

Isotype	Range	1	2	3	4	5	6	7	8	9	10	Mean	SD	%CV
tTG IgA	S1 EU/ml Low Negative	9.6	10.8	11.6	12.8	10.2	9.9	10.1	11.9	10.5	9.4	10.7	1.1	10.2
	S1 Qualitative	Neg	Qualitative Agreement: 100%											
	S2 EU/ml ~Cutoff - 20%	14.0	17.3	17.2	16.7	15.0	15.1	17.2	10.8	14.7	14.2	15.2	2.0	13.3
	S2 Qualitative	Neg	Qualitative Agreement: 100%											
	S3 EU/ml ~Cutoff +20%	26.6	25.6	25	24.3	24.9	22.4	25.3	26.1	23.2	24.5	24.8	1.3	5.1
	S3 Qualitative	Pos	Qualitative Agreement: 100%											
	S4 EU/ml Moderate Positive	48.6	50.2	49.7	47.1	47.3	50.4	48.7	49.1	48.5	48.8	48.8	1.1	2.2
	S4 Qualitative	Pos	Qualitative Agreement: 100%											
tTG IgG	S1 EU/ml Low Negative	12.7	13.0	13.0	12.2	13.0	12.8	13.9	14.6	11.8	12.6	13.0	0.8	6.2
	S1 Qualitative	Neg	Qualitative Agreement: 100%											
	S2 EU/ml ~Cutoff - 20%	18.5	16.2	19.3	14.2	17.0	15.7	16.2	17.2	15.4	16.1	16.6	1.5	9.0
	S2 Qualitative	Neg	Qualitative Agreement: 100%											
	S3 EU/ml ~Cutoff +20%	24.4	22	25.8	24.1	23.4	25.7	26	23.2	22.8	24.2	24.2	1.4	5.6
	S3 Qualitative	Pos	Qualitative Agreement: 100%											
	S4 EU/ml Moderate Positive	59.4	59	63.7	60.5	57.2	63.9	58.1	64.1	59.2	60.4	60.6	2.5	4.1
	S4 Qualitative	Pos	Qualitative Agreement: 100%											

**b. Linearity/assay reportable range:**

Linearity studies were assessed by evaluating three clinical samples of different antibody levels. Each sample was diluted using eight equidistant dilutions, and the actual value was compared to the expected value. The results of the study support a claim that the tTG IgG linear range is 2.9-260 EU/mL, and the tTG IgA linear range is 4-160 EU/mL.

Test Range (EU/mL)	Slope (95% CI)	Y-intercept (95% CI)	R <sup>2</sup>	% recovery (obtained/expected)
IgA				
6.0 to 62.7	0.932 (0.879 to 0.985)	0.926 (-1.240 to 3.091)	0.9943	97.9 to 108.3
3.0 to 157.0	0.947 (0.892 to 1.002)	0.999 (-4.347 to 6.345)	0.9979	94.5 to 107.7
3.3 to 163.6	0.910 (0.824 to 0.957)	3.239 (-1.628 to 8.106)	0.9946	85.0 to 109.5
IgG				
3.0 to 41.9	0.987 (0.970 to 1.004)	0.405 (-0.037 to 0.847)	0.9997	93.5 to 101.2
2.7 to 67.6	0.976 (0.839 to 1.112)	4.303 (-0.854 to 9.459)	0.9801	77.6 to 100.1
2.9 to 260.5	0.800 (0.648 to 0.951)	11.6 (-9.4 to 32.6)	0.9693	85.6 to 122.0

The claimed assay range of IgA is 4.0 (LoD) to 160 EU/mL.

The claimed assay range for the IgG is 2.9 (LoD) to 260 EU/mL.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):* Calibrators are not traceable to any recognized standards. Calibrators are dilutions of pooled serum of tTG antibody from patients with Celiac disease. IMMCO formulates new calibrator and control lots from an array of tTG antibody positive sera obtained from various commercial plasma centers stored at -70°C. The calibrators and controls are taken from different pooled sera. As new lots of calibrators are developed, studies are performed to calibrate values against original calibrators. Each lot of calibrator is also tested in comparison with normal human sera, clinical samples and internal standards. The concentration values of the calibrators are as follows:

Calibrator	IgA Assay Value	IgG Assay Value
Cal A	160 EU/mL	320 EU/mL
Cal B	80 EU/mL	160 EU/mL
Cal C	40 EU/mL	80 EU/mL
Cal D	20 EU/mL	20 EU/mL
Cal E	0 – 1 EU/mL	0 – 1 EU/mL

#### *Stability*

Shelf-life: Stability studies are performed comparing materials stored at 2-8°C with materials stored at 37°C and removed in 3 day intervals. One day stored at 37°C is considered equivalent to one month of real time at 2-8°C. Three lots of components/reagents were tested. Based on these studies, IMMCO established a shelf-life of 18 months.

Open-kit: Opened materials, stored at 2-8°C, were tested in the above fashion, and were found to be stable up to 90 days.

#### Sample stability

Specimens should be stored at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Repeated freezing and thawing of samples should be avoided.

Coated microwell strips are for one time use only. Resealed microwell strips should be stored at 2-8°C until expiration date indicated on the label.

d. *Detection limit:*

The limit of blank (LoB) was calculated using sixty replicates of a serum diluent sample, sorting the results by OD and averaging the 3<sup>rd</sup> and 4<sup>th</sup> highest values.

The limit of detection (LoD) was calculated by taking the SD of 10 replicates of each of 6 low-level (normal) clinical samples (for a total of 60 replicates) and using procedures described in CLSI EP-17A:

	LoB (EU/mL)	LoD (EU/mL)
Celiac tTG IgA	3.7	4.0
Celiac tTG IgG	2.6	2.9

e. *Analytical specificity:*

Cross reactivity: Cross reactivity was tested using 49 clinical samples from individuals with other autoimmune disorders. In addition, data was collected from 14 patients with gastrointestinal disorders. Results demonstrated no significant cross-reactivity.

<b>Condition</b>	<b>IgA Positive</b>		<b>IgG Positive</b>	
	<b>N</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>
Graves Disease	11	0 (0%)	0 (0%)	0 (0%)
Hashimoto's Thyroiditis	10	0 (0%)	0 (0%)	0 (0%)
Ulcerative Colitis	5	0 (0%)	0 (0%)	0 (0%)
Crohns Disease	9	0 (0%)	2 (22%)	2 (22%)
ANA positive*	9	0 (0%)	1 (11%)	1 (11%)
CCP positive**	10	0 (0%)	0 (0%)	0 (0%)
RF positive***	9	0 (0%)	0 (0%)	0 (0%)
<b>Total</b>	<b>49</b>	<b>0 (0%)</b>	<b>1 (2%)</b>	<b>1 (2%)</b>

\*Antinuclear antibodies

\*\*Cyclic Citrullinated Peptides Antibodies

\*\*\*Rheumatoid Factor Antibodies

Interference was studied by mixing sera with known tTG antibody levels with potentially interfering serum samples and studying deviation from expected results. No significant interference was demonstrated for the following substances at the levels indicated: Hemoglobin (2 g/L), Bilirubin (342 µmol/L), and Rheumatoid Factor (100 EU/mL).

<b>Celiac tTG IgA</b>	<b>Hemoglobin</b>		<b>Bilirubin</b>		<b>RF</b>		
	<b>EU/mL</b>	<b>EU/mL</b>	<b>% Int</b>	<b>EU/mL</b>	<b>% Int</b>	<b>EU/mL</b>	<b>% Int</b>
Negative	5.5	5.4	1.9	5.5	0.0	5.2	5.8
Cutoff 1	18.7	20.5	-8.8	20.4	-8.3	21.2	-11.8
Cutoff 2	22.2	23.7	-6.3	23.8	-6.7	21.1	5.2
Positive 1	77.4	73.8	4.9	72.1	7.4	77.9	-0.6
Positive 2	105.5	102.2	3.2	103.3	2.1	101.5	3.9

Celiac tTG IgG Sample	Hemoglobin		Bilirubin		RF	
	EU/mL	EU/mL % Int				
Negative	9.7	8.7 11.5	9.1 6.6	9.2 5.4	9.2 5.4	9.2 5.4
Cutoff 1	21.2	22.6 -6.2	23.5 -9.8	23.3 -9.0	23.3 -9.0	23.3 -9.0
Cutoff 2	23.0	21.8 5.7	22.9 0.6	25.1 -8.2	25.1 -8.2	25.1 -8.2
Positive 1	64.4	62.7 2.7	66.2 -2.7	72.1 -10.7	72.1 -10.7	72.1 -10.7
Positive 2	135.8	139.1 -2.4	125.0 8.6	129.4 4.9	129.4 4.9	129.4 4.9

The package insert states that “Grossly hemolyzed or lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used”..

*f. Assay cut-off:*

The normal range was established by testing 114 samples from apparently healthy donors and non-celiac controls on each assay. The mean value was <10 EU/mL for both the IgA and IgG assay. The mean value (<10 EU/mL) plus 2 standard deviations was established as the cut-off for each assay, and assigned an arbitrary unit value of 20 EU/mL.

<20 EU/mL Negative

20-25 EU/mL Indeterminate (Borderline)

>25 EU/mL Positive

2. Comparison studies:

*a. Method comparison with predicate device:*

The IMMCO Celiac tTG IgA ELISA was tested in comparison to the predicate, using 185 clinical samples within the linear range of the assay, from 93 EMA positive Celiac subjects, 31 disease controls, and 61 healthy normal subjects.

ImmuLisa™ hu-tTG IgA ELISA with borderline samples considered positive

		ImmuLisa™ hu-tTG IgA ELISA (cut off =20)		
		Positive	Negative	Total
IMMCO CELIAC tTG IgA ELISA	Positive	74	19	93
	Negative	2	90	92
	Total	76	109	185

Positive % Agreement 97.4% (95% CI 90- 99.5%)

Negative % Agreement 82.6% (95% CI 73.9-88.9%)

Overall % Agreement 88.6% (95% CI 83.0-92.7%)

ImmuLisa™ hu-tTG IgA ELISA with borderline samples considered negative

		ImmuLisa™ hu-tTG IgA ELISA (cut off =25)		
		Positive	Negative	Total
IMMCO CELIAC tTG IgA ELISA	Positive	63	23	86
	Negative	0	99	99
	Total	63	122	185

Positive % Agreement	100.0% (95% CI 92.8- 100.0%)
Negative % Agreement	81.1% (95% CI 72.8-87.4%)
Overall % Agreement	87.6% (95% CI 82.0-91.6%)

The Immco Celiac *tTG IgG* ELISA was tested in comparison to the predicate, using 310 clinical samples within the linear range of the assay, from 166 EMA positive Celiac subjects, 53 disease controls, and 91 healthy normal subjects.

**Immulin<sup>TM</sup> hu-tTG IgG ELISA with borderline samples considered positive**

		Immulin <sup>TM</sup> hu-tTG IgG ELISA (Cut off =20)		
		Positive	Negative	Total
IMMCO CELIAC tTG IgG ELISA	Positive	74	21	95
	Negative	31	184	215
	Total	105	205	310

Positive % Agreement	70.5% (95%CI 60.7-78.8%)
Negative % Agreement	89.8% (95% CI 84.6-93.4%)
Overall % Agreement	83.2% (95%CI 78.5-87.1%)

**Immulin<sup>TM</sup> hu-tTG IgG ELISA with borderline samples considered negative**

		Immulin <sup>TM</sup> hu-tTG IgG ELISA (Cut off =25)		
		Positive	Negative	Total
IMMCO CELIAC tTG IgG ELISA	Positive	53	27	80
	Negative	20	210	230
	Total	73	237	310

Positive % Agreement	72.6% (95%CI 60.7-82.1%)
Negative % Agreement	88.6% (95% CI 83.7-92.2%)
Overall % Agreement	84.8% (95%CI 80.3-88.6%)

b. *Matrix comparison:*  
Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and Clinical Specificity:*

For the IgA assay, clinical study included 185 Clinical samples, (including 88 also positive by EMA IgA assay), 5 IgA deficient CD cases, 31 disease controls, and 61 normals. Results are summarized below:

**IgA borderline considered positive (cut-off = 20)**

		Celiac disease		
		Positive	Negative	Total
<b>IMMCO CELIAC tTG IgA ELISA</b>	Positive	88	5	93
	Negative	5	87	92
	Total	93	92	185
Sensitivity		94.6% (95% CI 87.3-98.0%)		
Specificity		94.6% (95% CI 87.2-98.0%)		

IgA borderline considered negative (cut-off = 25)

		Celiac disease		
		Positive	Negative	Total
<b>IMMCO CELIAC tTG IgA ELISA</b>	Positive	85	1	86
	Negative	8	91	99
	Total	93	92	185
Sensitivity		91.4% (95% CI 83.3 - 95.9%)		
Specificity		98.9% (95% CI 93.2 - 99.9%)		

IgG Clinical Study – includes all CD patients (132 CD patients, including samples also positive by EMA - 74 EMA IgG positive) 53 disease controls, 91 normals.

IgG borderline considered positive (cut-off = 20)

		Celiac disease		
		Positive	Negative	Total
<b>IMMCO CELIAC tTG IgA ELISA</b>	Positive	72	7	79
	Negative	60	137	197
	Total	132	144	276
Sensitivity		54.5% (95% CI 45.7-63.2%)		
Specificity		95.1% (95% CI 89.9-97.9%)		

IgG borderline considered negative (cut-off = 25)

		Celiac disease		
		Positive	Negative	Total
<b>IMMCO CELIAC tTG IgA ELISA</b>	Positive	65	4	69
	Negative	67	140	207
	Total	132	144	276
Sensitivity		49.2% (95% CI 40.5 - 58.0%)		
Specificity		97.2% (95% CI 92.6 - 99.1%)		

*b, Other clinical supportive data (when a. is not applicable):*

Not applicable

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range:

The expected value in the normal population is negative. However, the sponsor states, as the incidence of CD in the normal population is about 1%, some apparently healthy, asymptomatic individuals may test positive for tTG antibodies.

**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.