

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

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

k091522

**B. Purpose for Submission:**

New device

**C. Measurand:**

Anti-gliadin IgA antibodies

Anti-gliadin IgG antibodies

**D. Type of Test:**

Qualitative and semi-quantitative

**E. Applicant:**

IMMCO Diagnostics, Inc.

**F. Proprietary and Established Names:**

ImmuLisa Celiac G+ (Gliadin) IgA Antibody ELISA

ImmuLisa Celiac G+ (Gliadin) IgG Antibody ELISA

**G. Regulatory Information:**

1. Regulation section:

21 CFR 866.5750 Radioallergosorbent (RAST) immunological test system

2. Classification:

Class II

3. Product code:

MST, Antibodies, Gliadin

4. Panel:

Immunology, 82

**H. Intended Use:**

1. Intended use(s):

Enzyme linked immunosorbent assay (ELISA) for the qualitative and semi-quantitative detection of IgA or IgG antibodies to gliadin in human serum to aid in the diagnosis of celiac disease 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 kit contains the following: microwell plate with 96 breakaway microwells coated with deamidated peptide (Gliadin) antigen; assay controls (positive and negative); calibrators (A-E); HRP IgA/IgG conjugated goat anti-human IgA/IgG antibodies; serum diluent; TMB enzyme substrate; stop solution; wash buffer.

**J. Substantial Equivalence Information**

1. Predicate device name(s):

- Quanta Lite™ Gliadin IgA II  
 Quanta Lite™ Gliadin IgG II
2. Predicate 510(k) number(s):  
 k052143  
 k052142
  3. Comparison with predicate:

Parameter	IMMCO Celiac G+ ELISAs	Inova QuantaLite Gliadin II IgA and IgG ELISAs
<b>Similarities</b>		
Intended Use	Enzyme linked immunosorbent assay (ELISA) for the qualitative and semi-quantitative detection of IgA or IgG antibodies to gliadin in human serum to aid in the diagnosis of celiac disease in conjunction with other laboratory and clinical findings.	Same
Methodology	ELISA	Same
Analyte	Anti-Gliadin antibody	Same
Capture antigen	Gliadin (deamidated peptide)	Same
Assay format	Qualitative and semi-quantitative	Semi- quantitative
Component set	Includes positive control, negative control, calibrators, conjugate, substrate, diluent, wash buffer, stop solution, microplate	Same
Conjugate	Horse radish peroxidase (HRP) goat anti-human polyclonal IgA or IgG conjugate	Same
Substrate/Chromogen	TMB (3, 3', 5, 5'-tetramethylbenzidin)	Same
Positive control	Human serum positive for gliadin IgA or IgG antibody	Same
Negative control	Human serum	Same
Stop solution	H <sub>2</sub> SO <sub>4</sub>	Same
Screening dilution	1:101	Same
Cutoff	20 EU/mL	20 units/mL
Interpretation of results	Negative <20 EU/mL, Intermediate (borderline) 20-25 EU/mL Positive >25 EU/mL	Negative <20 U/mL, Weak positive 20-30 U/mL, Strong positive >30 U/mL
Reading	450nm on spectrophotometer	same
Storage	2-8°C	same

Parameter	IMMCO Celiac G+ ELISAs	Inova QuantaLite Gliadin II IgA and IgG ELISAs
Differences		
Positive Control	Acceptance range printed on vial IgA 75-150 EU/mL IgG 40-80 EU/mL	No value/range assigned (IFU indicates value $\geq 1.0$ OD for acceptance of assay)
Calibrators	Set of 5 concentrations (IgA) 1, 20, 80, 160, 320 EU/mL (IgG) 1, 20, 40, 80, 160 EU/mL	Single, 25units. Value in arbitrary units (both assays)
Linear Range	(IgA) 8.9-320 EU/mL (IgG) 2.2-160 EU/mL	Not applicable
Limit of Detection	(IgA) 8.9 EU/mL (IgG) 2.2 EU/mL	Not applicable

**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 test is performed as a solid phase immunoassay. Microwells are coated with deamidated gliadin peptides followed by a blocking step to reduce non-specific protein binding during the assay run. Controls and patient sera are incubated in the antigen coated wells to allow specific antibodies present in the serum to bind to the antigen. Unbound antibodies and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled goat anti-human IgA or IgG conjugate to the microwells. Unbound 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, which is proportional to the concentration of the antibody, is read by a spectrophotometer at 450 nm. Results are reported as positive or negative with concentration in arbitrary unit values.

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

1. Analytical performance:

a. *Precision/Reproducibility:*

Patient samples were used to perform the precision studies. Studies included 3 between-day runs and a within-run study of six replicates. The results are summarized in the table below:

Kit	S #	Mean (EU/mL)	Total Imprecision		Between days		Within run	
			SD (EU/mL)	CV%	SD (EU/mL)	CV%	SD (EU/mL)	CV%
Celiac G+ IgA Assay	1	15.38	1.713	11.1%	2.474	16.2%	0.572	3.7%
	2	22.09	1.649	7.5%	2.011	9.1%	1.392	6.3%
	3	27.23	1.792	6.6%	1.932	7.1%	1.807	6.7%
	4	37.08	2.565	6.9%	3.043	8.2%	2.277	6.2%
	5	79.94	6.027	7.5%	8.438	10.7%	2.686	3.3%
	6	180.68	5.734	3.2%	7.852	4.3%	3.205	1.8%
	7	186.77	7.926	4.2%	10.837	5.9%	3.872	2.1%
	8	341.97	9.786	2.9%	10.389	3.0%	10.137	3.0%
Celiac G+ IgG Assay	1	13.97	0.827	5.9%	0.884	6.4%	0.757	5.3%
	2	23.10	1.274	5.5%	0.327	1.4%	1.821	7.8%
	3	33.81	2.071	6.1%	2.552	7.6%	1.696	5.0%
	4	91.83	3.472	3.8%	4.504	5.0%	1.723	1.9%
	5	116.49	4.360	3.7%	5.813	5.1%	1.349	1.1%
	6	120.44	3.008	2.5%	2.713	2.3%	2.770	2.3%
	7	179.74	4.661	2.6%	5.461	3.1%	3.998	2.2%

G+ Kit	Sample	Qualitative Calculated Reproducibility (EU/mL)					
		R1	R2	R3	R4	R5	R6
IgA	1	15.33	15.63	16.42	15.53	14.73	15.13
		Negative	Negative	Negative	Negative	Negative	Negative
	2	20.7	21.89	20.60	24.07	22.19	23.28
		Positive	Positive	Positive	Positive	Positive	Positive
	3	28.45	25.56	29.24	26.06	24.77	28.05
		Positive	Positive	Positive	Positive	Positive	Positive
IgG	1	12.11	11.40	12.81	13.23	10.42	11.12
		Negative	Negative	Negative	Negative	Negative	Negative
	2	23.66	26.20	28.60	20.99	20.99	24.93
		Positive	Positive	Positive	Positive	Positive	Positive
	3	41.42	39.87	39.03	41.71	41.71	40.72
		Positive	Positive	Positive	Positive	Positive	Positive

*Linearity/assay reportable range:* Three clinical samples were diluted in 8 equidistant dilutions and run in duplicates. Data is summarized in the table below:

Test Range (EU/ml)	Slope (95% CI)	Y-intercept (95% CI)	R <sup>2</sup>	% recovery (obtained/expected)
<b>IgA</b>				
7.2 to 70.3	0.971 (0.907 to 1.035)	0.468 (-2.415 to 3.349)	0.9958	97.3 to 106.9
4.4 to 136	0.987 (0.942 to 1.033)	2.203 (-1.518 to 5.924)	0.9979	88.9 to 103.3
1.1 to 309.6	0.993 (0.909 to 1.076)	-4.609 (-18.092 to 11.808)	0.9946	94.4 to 114.8
<b>IgG</b>				
5.2 to 75.8	1.011 (0.975 to 1.047)	-0.499 (-2.170 to 1.171)	0.9987	97.3 to 105.7
4.8 to 93.9	1.006 (0.946 to 1.067)	1.161 (-2.217 to 4.538)	0.9963	90.9 to 102
1.2 to 169.8	1.034 (0.992 to 1.076)	0.9027 (-3.196 to 5.002)	0.9971	86.0 to 102.3

The claimed assay range of IgA is 8.9 to 320 EU/mL.

The claimed assay range for the IgG is 2.2 to 160 EU/mL.

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

Calibrators and controls: There is no recognized reference material for anti-gliadin antibody. The results are reported in arbitrary units (EU/mL). New lots are tested and compared with normal sera, clinical samples and internal standards.

Shelf life stability studies were performed on three lots of components/reagents. Accelerated studies were conducted with materials incubated at 37°C. In these conditions, one day is considered equivalent to one month. Based on these studies, IMMCO has established an 18 month shelf life for the product.

Open vial stability was run on 3 lots of Celiac G+IgA and Celiac G+IgG kit reagents. Open kit components are stable up to 90 days, when stored at 2-8°C

Sample stability claim

Specimens should be stored at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen for up to one year. 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:*

Analytical sensitivity was performed according to CLSI EP17-A.

Limit of blank (LoB):

LOB was determined by 60 blank measurements of the kit diluent and averaging the 3<sup>rd</sup> and 4<sup>th</sup> highest value. LoB was calculated as 0.049xOD (7.9 EU/mL) for IgA and 0.051xOD (2.1 EU/mL) for IgG.

Limit of detection (LoD):

For LoD, 6 normal human samples were run in 10 replicates each. The average of the 3<sup>rd</sup> and 4<sup>th</sup> highest value was multiplied by the standard deviation of the 60 run and adding this result to the LoB. LoD was calculated as 0.55xOD (8.9 EU/mL) for IgA and 0.55xOD (2.2 EU/mL) for IgG.

*e. Analytical specificity:*

An interference study by endogenous substances was performed including grossly hemolyzed and lipemic sera. Samples were spiked with hemoglobin

at 2 g/L; bilirubin at 342 µmol/L; RF at 100 EU/mL. The interferent concentrations tested do not demonstrate a significant elevated rate of interference. Results are summarized below:

Celiac G+ IgA						
Sample	Hemoglobin		Bilirubin		RF	
	EU/mL	% Int	EU/mL	% Int	EU/mL	% Int
8.5	9.4	-9.9	11.6	-26.5	11.3	-26.5
25.2	23.7	6.3	23.5	7.5	26.2	7.5
21.7	21.5	0.9	21.4	1.4	23.2	1.4
107.5	103.9	3.5	112.2	-4.2	118.0	-4.2
126.5	122.2	3.5	138.4	-8.5	137.6	-8.5

Celiac G + IgG						
Sample	Hemoglobin		Bilirubin		RF	
	EU/mL	% Int	EU/mL	% Int	EU/mL	% Int
5.0	4.7	5.5	4.6	8.4	5.9	-15.5
22.6	20.4	10.8	20.2	12.1	24.4	-7.5
18.0	19.5	-7.3	18.8	-4.0	22.4	-19.6
74.6	72.0	3.6	78.1	-4.4	82.2	-9.3
105.0	101.4	3.6	115.0	-8.7	114.4	-8.2

Cross reactivity with other autoantibody: 50 potentially cross-reactive specimens from individuals with other autoimmune disorders or positive for other autoantibodies were tested for gliadin antibodies using the Immulisa™ Celiac G+ system.

Condition	n	IgA Positive n (%)	IgG Positive n (%)
Graves Disease	11	0 (0%)	0 (0%)
Hashimoto's Thyroiditis	11	1 (9%)	0 (0%)
ANA positive	9	0 (0%)	0 (0%)
CCP positive	10	0 (0%)	2 (20%)
RF positive	9	1 (11%)	0 (0%)
Total	50	2 (4%)	2 (4%)

*f. Assay cut-off:*

The assay cut-off was established by testing 34 pediatric (age range 1-18y) and 90 adult (age range 20-61y) serum samples from apparently healthy donors obtained from blood bank and other commercial sources. The mean value was < 11 EU/mL for each assay. The mean plus 2 standard deviations of the values of these normal samples was established as the cut off between normal and abnormal results. This value was assigned an arbitrary unit value of 20 ELISA units per milliliter (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:*

Originally 227 serum samples were obtained for the clinical and method comparison studies (117 positive samples from symptomatic and biopsy positive CD patients with positive EMA laboratory results, 90 samples from self-declared “healthy normal subjects” from blood bank and other commercial sources and 20 samples from disease control population of autoimmune disease/autoantibody positive sera (include Graves’ disease, Hashimoto’s Disease, Rheumatoid Arthritis (Rheumatoid Factor/Cyclic Citrullinated Peptides antibody) and antinuclear antibody) from commercial and reference laboratories. Of the CD samples, 65 were from pediatric patients and 5 were IgA deficient patients (2 pediatric and 3 adult samples). Samples below the limit of detection and above the upper limit of claimed linearity were excluded from the analyses which resulted in 156 samples for IgA and 213 samples for IgG comparisons. In addition, 23% of the samples in the IgA and 16% in the IgG method comparison studies were around the cut-off (borderline range  $\pm 5$ EU/mL). Results were analyzed with borderline samples included as positive or negative and are summarized below:

**IgA Method comparison:**

IgA Borderline samples considered positive:				
		INOVA		
		Pos	Neg	Total
IMMCO	Pos	54	27*	81
	Neg	7**	68	75
	Total	61	95	156
Pos agreement		88.5% (95% CI 77.2% - 94.9%)		
Neg agreement		71.6% (95% CI 61.3% - 80.1%)		
Overall agreement		78.2% (95% CI 70.8% - 84.3%)		

\*25 samples were from Celiac patients with positive EMA

\*\*4 samples were from negative disease controls and normals

IgA Borderline samples considered negative:				
		INOVA		
		Pos	Neg	Total
IMMCO	Pos	51	24	75
	Neg	10	71	81
	Total	61	95	156
Pos agreement		83.6% (95% CI 71.5% - 91.4%)		
Neg agreement		74.7% (95% CI 64.6% - 82.8%)		
Overall agreement		78.2% (95% CI 70.8% - 84.3%)		

### IgG Method comparison

IgG Borderline samples considered positive				
		INOVA		
		Pos	Neg	Total
IMMCO	Pos	88	9*	97
	Neg	7**	109	116
	Total	95	118	213
Pos agreement		92.6% (95% CI 84.9% - 96.7%)		
Neg agreement		92.4% (95% CI 85.6% - 96.2%)		
Overall agreement		92.5% (95% CI (87.9% - 95.5%))		

\*6 samples from celiac patients with positive EMA

\*\* 2 from disease control and normal

IgG Borderline samples considered negative				
		INOVA		
		Pos	Neg	Total
IMMCO	Pos	84	5	89
	Neg	11	113	124
	Total	95	118	213
Pos agreement		88.4% (95% CI 83.5% - 90.4%)		
Neg agreement		95.8% (95% CI 92.3% - 96.4%)		
Overall agreement		92.5% (95% CI 89.8% - 93.6%)		

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

The same serum samples described above were also used to determine clinical sensitivity and specificity.

### IgA

IgA Borderline considered positive				
		Diagnosed Celiac Disease		
		Pos	Neg	Total
IMMCO	Pos	78	5	83
	Neg	18	55	73
	Total	96	60	156
Sensitivity		81.3% (95% CI 71.7% - 88.2%)		
Specificity		91.7% (95% CI 80.9% - 96.9%)		
Overall % agreement		85.3% (95% CI 78.5% - 90.2%)		

IgA Borderline considered negative				
		Diagnosed Celiac Disease		
		Pos	Neg	Total
IMMCO	Pos	73	3	76
	Neg	23	57	80
	Total	96	60	156
Sensitivity		76.0% (95% CI 66.0% - 83.9%)		
Specificity		95.0% (95% CI 85.2% - 98.7%)		
Overall % agreement		83.3% (95% CI 76.3% - 88.6%)		

### IgG

IgG Borderline considered positive				
		Diagnosed Celiac Disease		
		Pos	Neg	Total
IMMCO	Pos	94	3	97
	Neg	11	105	116
	Total	105	108	213
Sensitivity		89.5% (95%CI 81.6% - 94.4%)		
Specificity		97.2% (95%CI 91.5% - 99.3%)		
Overall % agreement		93.4% (95% CI 89.3% – 96.2%)		

IgG Borderline considered negative				
		Diagnosed Celiac Disease		
		Pos	Neg	Total
IMMCO	Pos	86	3	89
	Neg	19	105	124
	Total	105	108	213
Sensitivity		81.9% (95% CI 72.9% – 88.5%)		
Specificity		97.2% (95% CI 91.5% – 99.3%)		
Overall % agreement		89.7% (95% CI 84.6% – 93.3%)		

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 that other gastrointestinal disorders are known to induce circulating gliadin antibodies. The assays provide similar negative values for both pediatric and adult populations.

**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.