510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K163177

B. Purpose for Submission:

New device

C. Measurand:

IgA autoantibodies specific for gliadin
IgG autoantibodies specific for gliadin

D. Type of Test:

Immunoassay, semi-quantitative or qualitative

E. Applicant:

IMMCO Diagnostics, Inc.

F. Proprietary and Established Names:

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA
ImmuLisa™ Enhanced 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 uses:

   ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA:

   Enzyme linked immunosorbent assays (ELISA) for the qualitative or semi-quantitative detection of IgA anti-gliadin antibodies in human serum to aid in the diagnosis of patients with celiac disease or dermatitis herpetiformis in conjunction with other laboratory and clinical findings.

   ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

   Enzyme linked immunosorbent assays (ELISA) for the qualitative or semi-quantitative detection of IgG anti-gliadin antibodies in human serum to aid in the diagnosis of patients with celiac disease or dermatitis herpetiformis 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:

   An ELISA microplate reader capable of reading absorbance values at 450 nm. If a dual wavelength microplate reader is available, the reference filter should be set at 450/650 nm. An automatic microplate washer capable of accurately dispensing 200 µL of fluid is also required.

I. Device Description:

Each kit consists of 12 x 8-antigen coated microwell strips, negative control (1x 1.75 mL), positive control (1x 1.75 mL), five assay calibrators (5x 1.75 mL), horseradish peroxidase anti-human IgA or IgG conjugate (1x 15 mL), TMB enzyme substrate (1x 15 mL), stop solution (1x 15 mL), wash buffer (2 vials) and diluent (1x 60 mL). The results are read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL) and reported as positive or negative.

J. Substantial Equivalence Information:

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

   Immco Anti-Gliadin Antibody (AGA) IgA ELISA (IgA-AGA), K964341
   Immco Anti-Gliadin Antibody (AGA) IgG ELISA (IgG-AGA), K964344

2. Comparison with predicate:

Similarities		
Item	**New Device** ImmuLisa™ Enhanced Gliadin (IgA or IgG) Antibody ELISA	**Predicate** Immco Anti-Gliadin Antibody (IgA-AGA or IgG-AGA) ELISA
Intended Use	Enzyme linked immunosorbent assays (ELISA) for the detection and semi-quantitation of IgA or IgG anti-gliadin antibodies in human serum to aid in the diagnosis of patients with celiac disease or dermatitis herpetiformis in conjunction with other laboratory and clinical findings.	Enzyme linked immunosorbent assays (ELISA) for the detection and semi-quantitation of IgA or IgG anti-gliadin antibodies in human serum to aid in the diagnosis of patients with celiac disease and dermatitis herpetiformis.
Assay Type	Manual ELISA	Same
Solid Phase	Polystyrene microplate wells	Same
Capture Antigen	Purified native gliadin antigen	Same
Sample Type	Serum	Same
Assay Format	Semi-quantitative and qualitative	Same
Cutoff	20 EU/mL	Same
Component set	Includes positive control, negative control, calibrators, conjugate, substrate, diluent, wash buffer, stop solution, microplate well.	Same
Incubation Times	Positive and negative controls, diluted patient samples: 30 min. Conjugate: 30 min. Enzyme Substrate: 30 min (in the dark).	Same
Reaction Temperature	Room temperature	Same
Traceability	International Reference Preparation is not available. Results are traceable to in-house standards.	Same
Positive Control	Gliadin IgA or Gliadin IgG antibodies	Same
Signal	Optical density	Same
Instrumentation	Microwell plate reader	Same
Storage	2-8°C	Same

Differences		
Item	**New Device** ImmuLisa™ Enhanced Gliadin IgA or IgG Antibody ELISA	**Predicate** Immco Anti-Gliadin Antibody (IgA-AGA or IgG-AGA) ELISA
Calibrators	Set of five: Values in EU/mL: 160, 80 40, 20, 1	Set of four: Varying EU/mL by kit lot
Labeled Detection Antibody (Conjugate)	Horseradish peroxidase conjugated to goat anti-human IgA or IgG	Alkaline phosphatase conjugated to goat anti-human IgA or IgG

Enzyme Substrate	Tetramethylbenzidene	p-Nitrophenyl phosphate
Stop Solution	Sulfuric acid (H2SO4)	Ethylenediaminetetraacetic acid (EDTA)
Wash Buffer	Powdered or optional liquid concentrate	Liquid concentrate
Linear Range	IgA 7.5 EU/mL – 160 EU/mL IgG 4.6 EU/mL – 160 EU/mL	Not specified
Limit of Detection	IgA 2.6 EU/mL IgG 2.8 EU/mL	Not specified
Sample dilution	1:101	1:51
Reading on spectrophotometer	450 nm	405 nm
Results Interpretation	Negative: < 20 EU/mL Indeterminate: 20–25 EU/mL Positive: > 25 UE/mL	<u>Adults:</u> Both IgA and IgG Negative: ≤ 20 EU/mL Positive: > 20 EU/mL <u>Children:</u> IgA Negative: ≤ 23 EU/mL Positive: > 23 EU/mL IgG Negative: ≤ 28 EU/mL Positive: > 28 EU/mL

K. Standard/Guidance Document Referenced:

CLSI guideline EP05-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline–Second Edition"

CLSI guideline EP06-A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline"

CLSI guideline EP07-A2, "Interference Testing in Clinical Chemistry; Approved Guideline–Second Edition"

CLSI guideline EP09-A2, "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline."

CLSI guideline EP12-A2, "User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline–Second Edition"

CLSI guideline EP17-A2, "Evaluation of Detection Capability for Clinical. Laboratory Measurement Procedures; Approved Guideline–Second Edition"

L. Test Principle:

The test is performed as a solid phase immunoassay. Gliadin antigen is bound to the wells of a polystyrene microwell plate followed by blocking the unreacted sites to reduce non-specific binding. Controls, calibrators and patient sera are added to separate wells, allowing any gliadin antibodies present to bind to the immobilized antigen. Unbound antibodies and other serum proteins are removed by washing the microplate wells. Bound antibodies are detected by adding an enzyme labeled anti-human IgA or IgG conjugate to the microplate wells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. After washing away any unbound conjugate, 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. 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. Results are expressed in ELISA units per milliliter (EU/ml) and reported as positive or negative.

Semi-quantitative results are determined from a series of five calibrators (160 EU/mL, 80 EU/mL, 40 EU/mL, 20 EU/mL, and 1 EU/mL). Values less than 20 EU/mL are considered negative results while values greater than 25 EU/mL are considered positive; results between 20 EU/mL and 25 EU/mL are considered 'indeterminate/borderline'. The sponsor states the following recommendations in the Package Insert: "*Indeterminate/borderline results should be retested and evaluated along with other laboratory methods, such as assays for detection of EMA and/or tTG antibodies*".

Qualitative results are determined using a ratio of the absorbance of the sample to the absorbance of the cut-off calibrator (20 EU/mL). The ratio is multiplied by the concentration of the cut-off calibrator to give a numerical value. Values greater than or equal to 20 EU/mL are considered positive and <20 EU/mL values are considered negative.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   Semi-Quantitative Precision:

   Precision performance was evaluated in accordance with CLSI guideline EP05-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline". A panel consisting of seven patient sera with levels of gliadin antibodies that cover the analytical measuring range was assayed in duplicate, twice a day, for 20 days with one reagent lot (a total of 80 replicates per sample). Testing was performed by two operators using different equipment sets. The first operator performed the assay with a multi-channel pipettor (BioHit), microplate washer (ELx405) and microplate reader (ELx808). The second operator used different instruments from the same type of equipment. All %CV values were within the manufacturer's pre-determined acceptance limit. The results are summarized in the table below for each assay:

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA:

Specimen	Mean (EU/mL)	Within-Run (Repeatability)		Between-Runs		Between-Days		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	3.6	0.4	9.9	0.1	3.1	0.2	6.6	0.4	10.3
2	15.3	0.8	5.3	0.3	7.1	0.6	3.8	0.9	5.6
3	20.2	0.8	4.0	0.4	2.1	0.8	3.9	0.9	4.5
4	23.7	1.0	4.1	0.9	3.9	0.8	3.3	1.4	5.7
5	67.9	1.8	2.6	1.3	1.9	1.3	1.9	2.2	3.2
6	93.2	4.9	5.2	2.1	2.2	4.8	5.1	5.6	5.7
7	143.5	9.3	6.4	4.4	3.1	10.3	7.2	11.0	7.1

ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

Specimen	Mean (EU/mL)	Within-Run (Repeatability)		Between-Runs		Between-Days		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	13.7	0.6	4.1	1.1	8.0	0.6	4.2	1.4	9.9
2	17.4	0.6	3.3	0.9	5.4	0.3	1.8	1.1	6.5
3	19.9	0.7	3.3	0.7	3.6	0.7	3.2	1.2	5.9
4	23.4	0.7	2.8	1.0	4.4	0.5	2.2	1.2	5.3
5	44.8	1.8	4.0	1.6	3.5	0.7	1.6	2.5	5.5
6	96.8	3.5	3.6	7.6	7.8	1.7	1.7	8.5	8.8
7	153.4	6.6	4.3	11.6	7.6	5.1	3.3	13.4	8.7

Qualitative Reproducibility:

Studies were performed under the guidance of CLSI EP12-A2 'User Protocol for Evaluation of Qualitative Test Performance.' Eighty replicates of each patient serum in the negative range, ~20% below cut-off, at ~cut-off, ~20% above cut-off and in the moderate positive range of the assays were performed to determine intra-assay qualitative reproducibility. Results were calculated using single-point (qualitative) analysis as indicated in the product insert. All values for qualitative agreement were within the manufacturer's pre-determined acceptance limit. Results are summarized below.

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA:

Specimen	Mean (EU/mL)	Total replicates	Qualitative Agreement	
			Positive/negative	%
Negative	2.6	80	0/80	100
Cut-off −20%	15	80	0/80	100
Cut-off	20.2	80	41/39	51.3
Cut-off +20%	24.3	80	80/0	100
Moderate Positive	67.5	80	80/0	100
Moderate to High Positive	83.1	80	80/0	100
High Positive	102.8	80	80/0	100

ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

Specimen	Mean (EU/mL)	Total replicates	Qualitative Agreement	
			Positive/negative	%
Negative	13.3	80	0/80	100
Cut-off −20%	17.1	80	0/80	100
Cut-off	19.7	80	46/34	57.5
Cut-off +20%	23.4	80	80/0	100
Moderate Positive	51.7	80	80/0	100
Moderate to High Positive	64.7	80	80/0	100
High Positive	82.9	80	80/0	100

Lot-to-lot Reproducibility:

To evaluate lot-to-lot reproducibility, a panel of five samples with levels of gliadin antibodies spanning the assay range was tested in replicates of five on three different lots over the course of five days. All %CV values were within the manufacturer's pre-determined acceptance limit. The results are summarized in the table below for each assay:

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA:

Specimen	Mean (EU/mL)	Within Run		Between Lots		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
1	9.9	0.4	3.7	0.7	7.5	0.7	7.5
2	22.6	1.4	6.0	1.7	7.5	1.8	7.9
3	35.2	0.8	2.3	2.4	6.7	2.4	6.7
4	77.9	1.6	2.1	5.7	7.3	5.9	7.6
5	149.7	6.6	4.4	10.7	7.2	11.7	7.8

ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

Specimen	Mean (EU/mL)	Within Run		Between Lots		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
1	16.13	0.6	3.7	1.5	9.2	1.5	9.2
2	22.59	1.0	4.5	2.1	9.1	2.1	9.1
3	35.48	1.2	3.3	3.1	8.7	3.1	8.7
4	87.31	2.7	3.1	5.4	6.2	5.7	6.5
5	122.87	5.0	4.0	10	8.1	11.3	9.2

Site-to-site Reproducibility:

To evaluate site-to-site reproducibility, a panel of five samples with levels of gliadin antibodies spanning the assay range was tested in replicates of five at three different sites over the course of five days using one lot of reagent. All %CV values were within the manufacturer's pre-determined acceptance limit. The results are summarized in the table below for each assay:

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA**:**

Specimen	Mean (EU/mL)	Within Run		Between Sites		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
1	10.0	0.4	4.4	0.8	7.6	0.8	8.3
2	22.7	0.6	2.7	1.4	6.2	2.1	9.4
3	47.7	1.4	2.9	2.9	6.0	4.1	8.6
4	88.4	4.7	5.3	7.8	8.8	8.5	9.6
5	146.2	5.1	3.5	12.0	8.2	12.8	8.8

ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

Specimen	Mean (EU/mL)	Within Run		Between Sites		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
1	14.0	0.7	5.1	0.9	6.4	1.3	9.1
2	24.1	1.0	4.0	1.9	7.9	2.1	8.5
3	33.9	1.4	4.0	2.4	7.0	2.4	7.0
4	70.0	2.9	4.1	5.1	7.3	5.8	8.2
5	151.1	5.3	3.5	10.8	7.2	14.9	9.2

b. Linearity/assay reportable range:

Linearity and recovery were tested by diluting positive specimens across the measuring range in equidistant dilutions with negative patient sera. The observed values were graphed against the calculated values and a linear regression was performed. Results summarized in the table below were within the manufacturer's pre-determined acceptance criteria of recovery.

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA:

Specimen	Dilution Range (EU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	7.4 to 41.6	1.01 (0.92 to 1.10)	1.13 (-1.14 to 3.39)	0.99	92% to 94%
2	2.3 to 97.2	1.04 (0.95 to 1.12)	1.45 (-3.18 to 6.08)	0.99	90% to 96%
3	4.7 to 127.4	0.85 (0.79 to 0.91)	2.06 (-3.39 to 7.41)	1.0	102% to 117%

B2GPI IgG Qualitative Precision:

ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

Specimen	Dilution Range (EU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	9.0 to 47.0	0.98 (0.88 to 1.08)	0.87 (-2.16 to 3.90)	0.99	93% to 106%
2	12.7 to 116.8	0.98 (0.91 to 1.05)	-1.47 (-6.66 to 3.73)	1.0	102% to 110%
3	58.1 to 147.4	0.98 (0.87 to 1.08)	3.67 (-7.70 to 15.04)	0.99	98% to 106%

The linear range was determined to be 7.5–160 EU/mL for the gliadin IgA assay and 4.6–160 EU/mL for the gliadin IgG assay. For test results above 160 EU/mL, the package insert recommends dilution and retesting of the samples.

High dose hook effect: Four high concentrations specimens were serially diluted 1:100 to assess the presence of any artefactual decrease in assay signal associated with gliadin antibody excess (hook effect). No hook effect was demonstrated in dilution sample levels up to 5515.8 EU/mL of gliadin IgA and 1686.3 EU/mL of gliadin IgG.

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

i. *Traceability*:

There are currently no recognized international standards for the measurement of gliadin antibodies. Calibrator and Control values are directly traceable to in-house standards.

ii. *Value Assignment:*

Calibrators and positive controls are dilutions of pooled gliadin antibody positive sera obtained from various commercial plasma centers. The calibrators and controls are taken from different pooled sera. In order to have traceability with newly made calibrators, each new lot of calibrators is assayed on an already cleared kit and compared to the existing calibrators as reference. Each lot of calibrator is also tested in comparison with normal human sera, clinical samples and internal standards. Five calibrator levels (Cal A-E) with assigned values from 0–160 EU/mL are included to provide semi-quantitation and must be used with each run. The cut-off calibrator for the qualitative analysis is derived from Cal D. Test results greater than or equal to Cal D are considered positive.

For semi-quantitative determinations the positive controls must give values in the range stated on the vial. The negative control contains negative human serum in buffer. The negative control must be <10 EU/mL.

iii. *Stability:*

Shelf-life Stability:

Accelerated and real-time stability studies for each ImmuLisa™ Enhanced Gliadin Antibody ELISA were conducted on three lots of components/reagents. The accelerated stability study was conducted with materials incubated at 37°C. Data from the accelerated stability study set the shelf-life stability at 18 months. A real-time stability study is on-going and currently supports an 18-month shelf-life stability claim.

Open Kit Stability:

For the open kit stability study, materials in each ImmuLisa™ Enhanced Gliadin Antibody ELISA are opened and stored in a dark environment as required for bench-top usage, then assayed at 15, 45 and 90 day intervals. Data from the open kit stability study demonstrate that

opened reagents are stable at 45 days, but the sponsor chose a more restrictive one month open kit stability claim.

Sample Stability and Storage:

The package insert recommends that sera be assayed soon after separation or stored in aliquots at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples. It is recommended that frozen specimens be tested within one year.

d. Detection limit:

The analytical sensitivity was determined in accordance with CLSI EP17-A2. The Limit of Blank (LoB) was determined by assaying a blank sample in 60 replicates. The limit of detection (LoD) was determined by assaying six low negative sera in ten replicates over three days using two reagent lots of each ImmuLisa™ Enhanced Gliadin Antibody ELISA. The Limit of Quantitation was determined by testing four low level samples in replicates of four over three days using two reagent lots. The claimed LoB, LoD and LoQ are summarized in the table below:

ImmuLisa™ Gliadin Antibody ELISAs	**LoB** (EU/mL)	**LoD** (EU/mL)	**LoQ** (EU/mL)
Gliadin IgA	2.2	3.6	7.5
Gliadin IgG	1.6	2.8	4.6

e. Analytical specificity:

i. Endogenous Interference:

Interference studies were performed according to CLSI EP07-A2 by testing five serum samples with gliadin antibody levels corresponding to the negative range, near the assay cutoff and in the low, moderate and high positive range. Each sample was mixed with known quantities of potentially interfering substances and analyzed in one assay run with two replicates on one kit lot of each assay. The recovery was calculated by comparing to control samples spiked with the same volume of diluents. No significant interference was demonstrated in the ImmuLisa™ Enhanced Gliadin Antibody ELISAs with the following substances up to the concentrations listed in the table below:

Potential Interfering Compound	**Test Concentration**
Hemoglobin	2 g/L
Bilirubin	342 µmol/L
Triglycerides	37 mmol/L
Rheumatoid Factor	100 EU/mL
Total cholesterol	13 mmol/L

The 'Limitations of Procedure' section of the Package Insert states "*This test should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples*".

ii. Cross-reactivity:

Numerous potentially cross-reactive autoimmune and infectious disease sera were tested for gliadin antibody levels. Refer to test results for 456 serum samples from patients in the Non-Target Disease Group in the table presented in the section below on Clinical studies. The number of serum samples tested positive is 12 (2.4%) for gliadin IgA and 21 (4.6%) for gliadin IgG. The '*Limitations of Procedure'* section of the Package Insert states: "*The results obtained serve only as an aid in the diagnosis and should not be interpreted as diagnostic in themselves*."

f. Assay cut-off:

The assay cut-offs for the ImmuLisa™ Enhanced Gliadin Antibody ELISAs were determined by testing specimens from 126 healthy blood donors. Using the 97.5th percentile value of the results obtained, an arbitrary value of 20 EU/mL was assigned for the cut-off.

Gliadin antibody value	Result interpretation
<20 EU/mL	Negative
20–25 EU/mL	Indeterminate (Borderline)
>25 EU/mL	Positive

Utilizing the 20 EU/mL cut-off value, 4.2% (5/120) and 1.7% (2/120) of the samples from healthy blood donors and patients in the Non-Target Disease Group were positive for gliadin IgA and gliadin IgG, respectively

2. Comparison studies:

a. Method comparison with predicate devices:

For analysis of agreement with a predicate device, clinically defined serum samples from the target disease group and non-target disease group were assayed for each gliadin antibody on both ImmuLisa™ Enhanced Gliadin Antibody ELISA and Anti-Gliadin (AGA) ELISA. The diagnosed conditions in each group and the numbers of specimens tested for each gliadin antibody are presented in the table below:

	Gliadin IgA	**Gliadin IgG**
Target Disease Group		
Celiac Disease (CD)	135	136
IgA Deficient CD (Adult)	23	24
IgA Deficient CD (Pedriatic)	11	22
Pediatric Celiac Disease	35	37
Dermatitis Herpetiformis	26	27
Total	230	246

Non-Target Disease Group		
Systemic Lupus Erythematosus	21	23
Rheumatoid Arthritis	30	30
Systemic Sclerosis	12	15
Hashimoto's Thyroiditis	4	7
Graves's Disease	6	5
Crohn's Disease	14	13
Ulcerative Colitis	8	15
Toxoplasmosis	7	13
Cytomegalovirus	11	11
Herpes Simplex Virus 1	8	12
Herpes Simplex Virus 2	8	13
Rubella	8	15
Lyme Disease	9	13
Helicobacter pylori	24	28
Total	170	213

Only specimens in the linear range of the assays were included in the method comparison. Greater than 15% of the samples fell near the cutoff of both assays. The results for each assay are summarized below:

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA:

		AGA IgA ELISA (EU/mL)		
		Positive: >20	Negative: ≤20	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA (EU/mL)	Positive: >25	96	17	113
	Equivocal: 20–25	10	12	22
	Negative: <20	3	262	265
Total		109	291	400*
* target disease group (n=230) and non-target disease group (n=170)				

Agreements were calculated by grouping ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA indeterminate results (20-25 EU/mL) with its test negative results, and then agreements were calculated again by grouping Indeterminate results with the test positive results:

Indeterminate gliadin IgA results considered as negative:

		AGA IgA ELISA (EU/mL)		
		Positive: >20	Negative: ≤20	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA (EU/mL)	Positive: >25	96	17	113
	Negative: ≤25	13	274	287
	Total	109	291	400
Positive percent agreement: 88.1% (96/109)		95% CI: 80.1%–93.2%		
Negative percent agreement: 94.2% (274/291)		95% CI: 90.6%–96.5%		
Total percent agreement: 92.5% (370/400)		95% CI : 89.5%–94.7%		

Indeterminate gliadin IgA results considered as positive:

		AGA IgA ELISA (EU/mL)		
		Positive: >20	Negative: ≤20	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA (EU/mL)	Positive: ≥20	106	29	135
	Negative: <20	3	262	265
	Total	109	291	400
Positive percent agreement: 97.2% (106/109)			95% CI: 91.6%–99.3%	
Negative percent agreement: 90.0% (262/291)			95% CI: 85.9%–93.1%	
Total percent agreement: 92.0% (368/400)			95% CI: 88.9%–94.3%	

ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

		AGA IgG ELISA (EU/mL)		
		Positive: >20	Negative: ≤20	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA (EU/mL)	Positive: >25	190	10	200
	Equivocal: 20–25	9	4	13
	Negative: <20	6	240	246
Total		205	254	459*
* target disease group (n=246) and non-target disease group (n=213)				

Agreements were calculated by grouping ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA indeterminate results (20−25 EU/mL) with its test negative results, and then agreements were calculated again by grouping indeterminate results with the test positive results:

Indeterminate gliadin IgG results considered as negative:

		AGA IgG ELISA (EU/mL)		
		Positive: >20	Negative: ≤20	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA (EU/mL)	Positive: >25	190	10	200
	Negative: ≤25	15	244	259
	Total	205	254	459
Positive percent agreement: 92.7% (190/205)			95% CI: 88.0%–95.7%	
Negative percent agreement: 96.1% (244/254)			95% CI: 92.7%–98.0%	
Total percent agreement: 94.6% (444/459)			95% CI : 92.1%–96.3%	

Indeterminate gliadin IgG results considered as positive:

		AGA IgG ELISA (EU/mL)		
		Positive: >20	Negative: ≤20	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA (EU/mL)	Positive: >20	199	14	203
	Negative: ≤20	6	240	246
	Total	205	254	459
Positive percent agreement: 97.1% (199/205)			95% CI: 93.4%–98.8%	
Negative percent agreement: 94.5% (240/254)			95% CI: 90.7%–96.8%	
Total percent agreement: 95.6% (439/459)			95% CI: 93.4%–97.2%	

b. Matrix comparison:

Not applicable since human serum is the only claimed specimen matrix. The '*Specimen Collection and Handling'* section in the Package Insert states "*Only serum specimens should be used in this procedure*".

3. Clinical studies:

a. Clinical sensitivity and specificity:

A total of 751 serum samples were included in the clinical validation for the ImmuLisa™ Enhanced Gliadin Antibody ELISAs. The validation set of samples includes a target disease group (250 celiac disease and 45 dermatitis herpetiformis) and a non-target disease group (456 samples from patients with autoimmune and infectious conditions expected to be found in the intended use population). Test results for each disease group are shown below:

		Gliadin IgA		**Gliadin IgG**	
		# Pos*	% Pos	# Pos*	% Pos
Target Disease Group					
Celiac Disease	200	130	65	154	77
Pediatric Celiac Disease	50	34	68	37	74
Dermatitis Herpetiformis	45	16	35.6	26	57.8
Total	295	180	50.1	217	72.5
Non-Target Disease Group					
Systemic Lupus Erythematosus	30	2	6.7	3	10
Granulomatosis with Polyangiitis	30	0	0	1	0
Rheumatoid Arthritis	30	1	3.3	0	0
Systemic Sclerosis	30	0	0	0	0
Hashimoto's Thyroiditis	15	0	0	1	6.7
Graves's Disease	15	1	6.7	0	0
Crohn's Disease	25	1	4	2	8
Ulcerative Colitis	25	0	0	1	4
Autoimmune Hepatitis	30	0	0	1	3.3
Primary Biliary Liver Cirrhosis	50	4	8	3	6
Toxoplasmosis	20	0	0	0	10
Cytomegalovirus	20	0	0	0	0
Herpes Simplex Virus 1	20	0	0	0	0
Herpes Simplex Virus 2	20	0	0	3	15
Lyme's Disease	36	0	0	3	8.3
Rubella	20	0	0	0	0
Helicobacter pylori	40	3	7.5	3	7.5
Total	456	12	2.4	21	4.6

Pos: positive
* Indeterminate samples were considered positive

The performance of each ImmuLisa™ Enhanced Gliadin Antibody ELISA was evaluated against the clinical diagnosis of celiac disease and dermatitis herpetiformis separately. In both evaluations, the clinical sensitivity and specificity were calculated by grouping the assay's indeterminate results with its test negative results, and then sensitivity and specificity were calculated again by grouping the assay's equivocal results with its test positive results.

The clinical sensitivity and specificity of the ImmuLisa™ Enhanced Gliadin Antibody ELISAs in this sample cohort are summarized in the following tables for celiac disease:

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA:

		Celiac Disease Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA	Positive	139	7	146
	Equivocal	25	5	30
	Negative	86	444	530
	Total	250	456	706*
* celiac disease (n=200), pedriatic celiac disease (n=50) and non-target disease group (n=456)				

Indeterminate gliadin IgA results considered as negative:

		Celiac Disease Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA	Positive	139	7	146
	Negative	111	449	560
	Total	250	456	706
Sensitivity: 55.6% (139/250) 95% CI: 49.2%–61.8% Specificity: 98.5% (449/456) 95% CI: 96.7%–99.3%				

Indeterminate gliadin IgA results considered as positive:

		Celiac Disease Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA	Positive	164	12	176
	Negative	86	444	530
	Total	250	456	706
Sensitivity: 65.6% (164/250) 95% CI: 59.3%–71.4% Specificity: 97.4% (444/456) 95% CI: 95.3%–98.6%				

ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

		Celiac Disease Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA	Positive	171	15	186
	Equivocal	20	6	26
	Negative	59	435	494
	Total	250	456	706

Indeterminate gliadin IgG results considered as negative:

		Celiac Disease Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA	Positive	171	15	186
	Negative	79	441	520
	Total	250	456	706
Sensitivity: 68.4% (171/250) 95% CI: 62.2%–74.0% Specificity: 96.7% (441/456) 95% CI: 94.5%–98.1%				

Indeterminate gliadin IgG results considered as positive:

		Celiac Disease Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA	Positive	191	21	176
	Negative	59	435	494
	Total	250	456	706
Sensitivity: 76.4% (191/250) 95% CI: 70.5%–81.4% Specificity: 95.4% (435/456) 95% CI: 92.9%–97.1%				

The clinical sensitivity and specificity of the ImmuLisa™ Enhanced Gliadin Antibody ELISAs in this sample cohort are summarized in the following tables for dermatitis herpetiformis:

ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA:

		Dermatitis Herpetiformis Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA	Positive	15	7	22
	Equivocal	1	5	6
	Negative	29	444	473
	Total	45	456	501
* dermatitis herpetiformis (n=45) and non-target disease group (n=456)				

Indeterminate gliadin IgA results considered as negative:

		Dermatitis Herpetiformis Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA	Positive	15	7	22
	Negative	30	449	479
	Total	45	456	501
Sensitivity: 33.3% (15/45) 95% CI: 20.4%–49.1% Specificity: 98.5% (449/456) 95% CI: 96.7%–99.3%				

Indeterminate gliadin IgA results considered as positive:

		Dermatitis Herpetiformis Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgA Antibody ELISA	Positive	16	12	28
	Negative	29	444	473
	Total	45	456	501
Sensitivity: 35.6% (16/45) 95% CI: 22.3%–51.3% Specificity: 97.4% (444/456) 95% CI: 95.3%–98.6%				

ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA:

		Dermatitis Herpetiformis Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA	Positive	25	15	40
	Equivocal	1	6	7
	Negative	19	435	454
	Total	45	456	501

Indeterminate gliadin IgG results considered as negative:

		Dermatitis Herpetiformis Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA	Positive	25	15	40
	Negative	20	441	461
	Total	45	456	501
Sensitivity: 55.6% (25/45) 95% CI: 40.1%–70.0% Specificity: 96.7% (441/456) 95% CI: 94.5%–98.1%				

Indeterminate gliadin IgG results considered as positive:

		Dermatitis Herpetiformis Diagnosis		
		Positive	Negative	**Total**
ImmuLisa™ Enhanced Gliadin IgG Antibody ELISA	Positive	26	21	47
	Negative	19	435	454
	Total	45	456	501
Sensitivity: 57.8% (26/45) 95% CI: 42.2%–72.0% Specificity: 95.4% (435/456) 95% CI: 92.9%–97.1%				

4. Clinical cut-off**:**

   Refer to assay cut-off.

5. Expected values/Reference range:

   Test results in a normal population are expected to be negative. However, it has been determined that some apparently healthy, asymptomatic individuals may test positive for gliadin IgA or gliadin IgG (Rashtak S. et al. *Clin Gastroenterol Hepatol*. 2008; 6:426-432).

   A total of 269 normal human sera (130 pediatrics and 139 adults) tested with the ImmuLisa™ Enhanced Gliadin Antibody ELISAs yielded 8 (3%) positive results for gliadin IgA (all adults) and 10 (3.7%) positive for gliadin IgG (4 pediatrics and 6 adults).

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.