

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

A. 510(k) Number:

k083052

B. Purpose for Submission:

New Device

C. Measurand:

Anti-gliadin IgA antibodies

D. Type of Test:

Semi-quantitative ELISA

E. Applicant:

EUROIMMUN US INC.

F. Proprietary and Established Names:

Anti-Gliadin (GAF-3X) ELISA (IgA) Kit

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):
The Anti-Gliadin (GAF-3X) ELISA (IgA) test kit is designed for the determination of IgA class antibodies against gliadin in human serum. 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.
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 plate reader capable of measuring OD at 450 nm, 620 nm and 650 nm.

I. Device Description:

Each Kit contains the following: a ninety-six (96) well (12 X 8) polystyrene microtiter plate strips coated with a synthetic form of a gliadin epitope, wash buffer, sample buffer, peroxidase-labeled, rabbit anti-human IgA, TMB chromogen substrate, stop solution, positive and negative controls, and three calibrators (prepared from human serum).

J. Substantial Equivalence Information:

1. Predicate device name(s):

QUANTA Lite™ Gliadin IgA II

2. Predicate K number(s):
k052143
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate
Intended Use	For the measurement of anti-gliadin IgA antibodies in human serum	Same
Method and format	ELISA; 96-well microtiter plates	Same
Sample type	Serum	Same
Sample volume	5 µl	Same
Substrate	TMB Chromogen	Same
Reagent preparation	Ready-to use	Same
Assay washing steps	Two steps	Same
Detection method	Colorimetric/Spectrophotometer	Same

Differences		
Item	New Device	Predicate
Indications for Use	As an aid in diagnosis of celiac disease and dermatitis herpetiformis Duhring	As an aid in diagnosis of celiac disease
Antigen	Deamidated gliadin epitope consisting of nine amino acids linked with an artificial gliadin homolog octapeptide	Synthetic, deamidated peptide
Measurement type	Semi-quantitative (using 3 calibrators) or qualitative (using one calibrator)	Semi-quantitative
Conjugate	Rabbit anti-human IgG labeled with horseradish peroxidase	Goat anti-human IgG labeled with horseradish peroxidase
Calibrator and Controls	Three calibrators prepared from human serum, pre-diluted: Calibrator 1: 200 RU/mL, Calibrator 2: 25 RU/mL, Calibrator 3: 2 RU/mL Two controls (positive and negative) ready-to-use, prediluted	Three levels (1 negative, 1 low positive, 1 high positive)

Differences		
Item	New Device	Predicate
Sample dilution	1:201	1:101
Stop Solution	0.5M sulphuric acid	0.344 MO sulphuric acid
Result Interpretation	Negative < 25 RU/mL or < Ratio 1.0 Positive ≥ 25 RU/mL or ≥ Ratio 1.0	Negative < 20 units Weak positive 20-30 units Moderate or strong positive > 30 units

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

None referenced

L. Test Principle:

Polystyrene microtiter plate strips are coated with an immunological dominant deamidated gliadin epitope consisting of nine amino acids linked with an artificial gliadin homolog octapeptide. Pre-diluted patient sample along with controls and calibrators are added individually to antigen coated wells. Anti-gliadin antibodies in the patient sample bind to the immobilized antigen. Unbound sample is washed away and the anti-human IgA enzyme conjugate reagent is added to each well to bind to the antibodies attached to the antigen. After a second rinse to wash away unbound enzyme conjugate, a chromogenic substrate is added. The ELISA plates are read at a wavelength of 450 nm and a reference wavelength of between 620 and 650 nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The intra- and inter-assay precision using the three calibrator method (results in RU/mL) were evaluated using 9 sera with values at spanning the measuring range (mean value range 18 – 162 RU/mL) including the clinical decision point of 25 RU/mL. Intra-assay precision was evaluated using 20 determinations in run on one day. Inter-assay precision testing was performed in 6 runs over 4 days, 4 replicates per run. Intra-assay %CV was ≤ 7.1%, mean 4.7%. Inter-assay %CV was ≤ 9.6%, mean 7.2%. The acceptance criteria of CV ≤ 12% were met. The following results were obtained:

	Intra-assay (n = 20)			Inter-assay (n = 24)		
	Mean (RU/mL)	SD	%CV	Mean (RU/mL)	SD	%CV
Sample 1	162	11.2	7.0	166	7.75	4.7
Sample 2	91	3.9	4.3	106	4.62	4.4
Sample 3	79	4.1	5.1	86	5.22	6.1
Sample 4	61	2.3	3.8	59	3.76	6.4
Sample 5	30	0.8	2.5	29	2.65	9.1
Sample 6	29	1.0	3.6	30	2.65	8.9
Sample 7	25	1.0	3.8	25	2.38	9.6
Sample 8	18	1.3	7.1	18	1.69	9.5
Sample 9	19	0.9	4.8	19	1.29	7.0

The intra- and inter-assay precision using calibrator 2 alone (results presented as a ratio) were evaluated using 9 sera with values at spanning the measuring range (mean value range 0.61 – 4.97) including the clinical decision point of 1.0. Intra-assay precision was evaluated using 20 determinations in run on one day. Inter-assay precision testing was performed in 6 runs over 4 days, 4 replicates per run. Intra-assay %CV was $\leq 6.8\%$, mean 4.1%. Inter-assay %CV $\leq 9.0\%$, mean 7.0%. The acceptance criteria of CV $\leq 12\%$ were met. The following results were obtained:

	Intra-assay (n = 20)			Inter-assay (n = 24)		
	Mean (RU/mL)	SD	%CV	Mean (RU/mL)	SD	%CV
Sample 1	5.14	0.340	6.6	5.029	0.273	5.2
Sample 2	3.01	0.118	3.9	3.46	0.147	4.2
Sample 3	2.64	0.123	4.6	2.85	0.176	6.2
Sample 4	2.10	0.070	3.3	2.04	0.113	5.6
Sample 5	1.19	0.025	2.1	1.12	0.084	7.5
Sample 6	1.15	0.034	3.0	1.15	0.084	7.3
Sample 7	1.04	0.032	3.1	0.99	0.078	7.9
Sample 8	0.76	0.043	5.6	0.76	0.055	7.3
Sample 9	0.78	0.030	3.9	0.78	0.044	5.6

Precision was calculated for the positive and negative controls. The mean for the positive control was 116 RU/mL and the %CV for the positive control was $\leq 5.7\%$. The mean for the negative control was 0.963 RU/mL and standard deviation was 0.67 RU/mL.

Lot-to-lot reproducibility was evaluated using 3 different kit lots and 3 serum samples. The %CV was less than 10%.

b. Linearity/assay reportable range:

The linearity of the test was determined using serial two-fold dilutions of four (4) positive patient samples with high anti-gliadin IgG titers. The dilutions were measured in singlicate and ordinary least squares regression was used to analyze the results. The acceptance criteria for the coefficient r^2 had to be ≥ 0.95 . Results showed the assay was linear for the specified assay range when using the 3 point calibrator system (RU/mL) or the single calibrator (ratio).

Sample	Concentration range of dilutions (RU/mL)	(R^2)	Range of Ratios	(R^2)
1	9 – 105	0.9999	0.5 – 3.5	0.9999
2	17 – 171	0.9994	0.7 – 5.6	0.9994
3	19 – 195	0.9987	0.8 – 6.4	0.9987
4	15 – 127	0.9995	0.7 – 4.2	0.9995

The assay is linear in the range 9-195 RU/mL.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
No reference standard for gliadin or gliadin peptides exists. The results are expressed as either relative units (RU/mL) using the three point calibrator, or as a ratio using the single calibrator 2.

Stability studies (accelerated, real time sealed, real time opened, and real time reconstituted as applicable) were conducted using three lots. The results support a shelf life claim of 12 months. The opened reagents are stable for 3 to 6 months. The Wash buffer is stable for up to 28 days.

The kit includes a positive control and negative control, and three calibrators (~ 2, 25, and 200 RU/mL).

- d. *Detection limit:*
Limit of blank (LoB) is defined as the mean value of sample buffer run 20 times plus 3 standard deviations. The LoB is 0.5 RU/mL.
- e. *Analytical specificity:*
Cross reactivity: Potential cross-reactive disease panels were evaluated using the Anti-Gliadin (GAF-3X) ELISA (IgA) test using a total of 828 frozen, clinically characterized, retrospective sera and 23 serologically characterized sera. Samples included Rheumatoid arthritis (300), Sjogren's syndrome (200), Systemic lupus erythematosus (150), Progressive systemic sclerosis (126), Bullous pemphigoid (30), Linear IgA dermatosis (22), Anti-Saccharomyces cerevisiae antibodies (10), anti-intestinal globed cell antibodies positive samples and/or anti-pancreas antibodies (13) samples. The results are shown in the table below:

Panel	Anti-Gliadin (GAF-3X) ELISA (IgG)	
	N	positive (%)
Rheumatoid arthritis	300	12 (4.0%)
Sjögren's syndrome	200	9 (4.5%)
Systemic lupus erythematosus	150	7 (4.7%)
Progressive systemic sclerosis	126	6 (4.8%)
Bullous pemphigoid	30	0 (0.0%)
Linear IgA dermatosis (LAD)	22	0 (0.0%)
Anti-Saccharomyces cerevisiae antibodies (ASCA)	10	0 (0.0%)
Anti-Intestinal globed cell antibodies (GAB) and/or anti-Pancreas antibodies (PAB)	13	2 (15.4%)

Interference: Three (3) different samples were spiked with potential interfering substances in 3 different concentrations and incubated with the test

system. The recovery when compared to the original, unspiked sample was calculated. Mean recovery was within the acceptance criteria of 85-115%. No interference was observed with hemolytic, lipemic, or icteric samples for concentrations of up to 1000 mg/dL for hemoglobin (recovery 96-102%), 2000 mg/dL for triglyceride (recovery 88-98%) and 40 mg/dL for bilirubin (recovery 96-112%).

HAMA and Rheumatoid Factor: Five (5) different samples were spiked with heterophilic antibodies (HAMA) (975 ng/mL), or Rheumatoid Factor (RF) (500 IU/mL). Sample 16-278 RU/mL; % recovery between 94-118% for HAMA and between 86-114% for RF.

Hook Effect: Serial dilutions of high positive samples were tests. No Hook effect is seen with the highest sample tested 443 RU/mL.

f. Assay cut-off:

Negative < 25 RU/mL or < Ratio 1.0

Positive ≥ 25 RU/mL or ≥ Ratio 1.0

2. Comparison studies:

a. Method comparison with predicate device:

A total of 293 samples from patients positive or negative for celiac disease were tested with the EUROIMMUN Anti-gliadin (GAF-3X) ELISA (IgA) and with the predicate, the Inova QUANTA Lite™ Gliadin IgA II test kit, using the cut-offs for each as recommended in the test instructions.

N = 293		Predicate			
		Positive	Weak positive	Negative	Total
EUROIMMUN Anti-Gliadin (GAF-3X) ELISA (IgG)	Positive	111	9	6	126
	Negative	0	6	161	167
	Total	111	15	167	293

Positive % agreement 120/126 = 95.2% 95% CI (89.9% – 98.2%)

Negative % agreement 161/167 = 96.4% 95% CI (92.3% – 98.7%)

Overall % agreement 281/293 = 95.9% 95% CI (93.0% – 97.9%)

b. Matrix comparison:

Serum is the only claimed matrix.

3. Clinical Studies:

Clinical Sensitivity and Specificity:

The clinical sensitivity and specificity were evaluated in an external clinical laboratory studies using 515 frozen clinically characterized retrospective sera. The samples included 180 biopsy confirmed celiac disease samples from children without IgA deficiency, 59 celiac disease (age 1-54) biopsy-proven

samples and 36 samples from adults with Duhring's dermatitis herpetiformis patients, and 241 samples from biopsy negative patients with other gastroenteropathies, and chronic inflammatory bowel disease. The range of results evaluated for celiac disease ranged from 3 to > 700 RU/mL and for Duhring from 2 to 200 RU/mL. The sensitivity was 82.4% (95% CI: 76.9 - 87.0%) for celiac disease and 83.3% (95% CI: 67.2- 93.6%) for Duhring's dermatitis herpetiformis. The specificity of the device for disease controls with negative biopsy was 96.3% (95% CI: 93.0 – 98.3%).

Panel	Anti-Gliadin (GAF-3X) ELISA (IgG)	
	n	Positive (%)
Celiac disease (0-18 years, without IgA deficiency, at no diet, biopsy-proven)	180	147 (81.7%)
Celiac disease (1-54 years, biopsy-proven)	58	49 (84.5%)
Sensitivity for celiac disease	238	196 (82.4%)
Duhring's dermatitis herpetiformis patients (adults, diet unknown)	36	30 (83.3%)
Sensitivity for Duhring's dermatitis herpetiformis	36	30 (83.3%)
Panel	Anti-Gliadin (GAF-3X) ELISA (IgG)	
	n	Negative (%)
Gastroenteropathies, negative biopsy for celiac disease	192	183 (95.3%)
Chronic-inflammatory bowel disease, negative biopsy for celiac disease	49	49 (100%)
Specificity	241	234 (97.1%)

4. Clinical cut-off:

Same as assay cut-off

5. Expected values/Reference range:

The levels of anti-gliadin (GAF-3X) antibodies (IgA) were analyzed with the EUROIMMUN ELISA in a panel of 400 apparently healthy blood donors (176 women and 224 men aged 18 to 67 years with an average age of 39 years). With a cut-off of 25 RU/mL, 9 (2.3 %) of the samples were anti-gliadin (GAF-3X) positive. The mean concentration was 4.9 RU/mL (\pm 14.4 RU/mL of standard deviation) and the values ranged from 0.4 to 200 RU/mL.

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.