510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k113377

B. Purpose for Submission:

New Device

C. Measurand:

IgA and IgG anti-Gliadin antibodies
IgA and IgG anti-deamidated gliadin peptide antibodies

D. Type of Test:

Semi-quantitative enzyme immunoassay

E. Applicant:

Grifols USA, LLC

F. Proprietary and Established Names:

α-Gliatest® IgA
α-Gliatest® IgG
α-GliaPep® IgA
α-GliaPep® IgG

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 α-Gliatest® IgA is intended for the semi-quantitative determination of IgA antibodies directed against gliadin in human serum. The assay is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.

   The α-Gliatest®IgG is intended for the semi-quantitative determination of IgG antibodies directed against gliadin in human serum. The assay is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.

   The α-GliaPep® IgA is intended for the semi-quantitative determination of IgA antibodies directed against deamidated gliadin peptide in human serum. The assay is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.

   The α-GliaPep® IgG is intended for the semi-quantitative determination of IgG antibodies directed against deamidated gliadin peptide in human serum. The assay is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests 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:

   Microtiter plate reader capable of measuring OD at 450 and 620 nm

I. Device Description:

α-Gliatest® IgA and α-Gliatest® IgG:

The α-Gliatest® IgA or α-Gliatest® IgG consists of one microtiter plate (12 strips, each with 8 wells coated with the purified α-gliadin), assay controls (positive and

negative), a ready-to-use set of five calibrators (0, 10, 20, 50, 100 (Absorption Units (AU)/mL for α-Gliatest® IgA, and 2, 10, 20, 50, 100 AU/mL for α-Gliatest® IgG), horseradish peroxidase (HRP) goat anti-human IgA or IgG conjugate, serum diluent, tetramethylbenzidine (TMB) enzyme substrate, stop solution (0.5M H_2SO4), and washing solution required for the assay.

α-GliaPep® IgA and α-GliaPep® IgG:

The α-GliaPep® IgA or α-GliaPep® IgG consists of one microtiter plate (12 strips, each with 8 wells coated with the deamidated gliadin peptide antigen), assay controls (positive and negative), a ready-to-use set of five calibrators (0, 10, 20, 50, 100 AU/mL), HRP goat anti-human IgA or IgG conjugate, serum diluent, TMB enzyme substrate, stop solution (0.5M H_2SO4), and washing solution required for the assay.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

   AESKULISA® Glia A and AESKULISA® Glia G (k052439)

2. Comparison with predicate:

Similarities		
Item	New Device α-Gliatest® IgA/IgG	Predicate AESKULISA® Glia A/G
Intended use	Determination of IgA/IgG antibodies directed against gliadin in human serum. The assay is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.	Same
Methodology	ELISA	Same
Analyte	IgA/IgG antibodies against gliadin	Same
Sample matrix	Human serum	Same
Capture antigen	Gliadin	Same
Detection antibody	HRP conjugated goat anti-human IgA/IgG	Same
Substrate	TMB	Same
OD reading	450 nm on spectrophotometer	Same
Sample volume required	100 µL	Same
Sample dilution	1:101	Same

Differences		
Item	New Device α-Gliatest® IgA/IgG	Predicate AESKULISA® Glia A/G
Type of assay	Semi-quantitative	Semi-quantitative and qualitative
Controls	2 controls (1 positive control, 1 negative control)	3 controls (1 positive, 1 negative, 1 cut-off control)
Calibrators	5 Calibrators for α-Gliatest® IgA: 0, 10, 20, 50, 100 AU/mL 5 Calibrators for α-Gliatest® IgG: 2, 10, 20, 50, 100 AU/mL	6 Calibrators: 0, 3, 10, 30, 100, 300 U/mL
Cut-off	8 AU/mL for α-Gliatest® IgA 50 AU/mL for α-Gliatest® IgG	15 U/mL

Similarities		
Item	New Device α-GliaPep® IgA/IgG	Predicate AESKULISA® Glia A/G
Intended use	The assay is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.	Same
Methodology	ELISA	Same
Sample matrix	Human serum	Same
Detection antibody	HRP conjugated goat anti-human IgA/IgG	Same
Substrate	TMB	Same
OD reading	450 nm on spectrophotometer	Same
Sample volume required	100 µL	Same
Sample dilution	1:101	Same

Differences		
Item	New Device α-GliaPep® IgA/IgG	Predicate AESKULISA® Glia A/IgG
Type of assay	Semi-quantitative	Semi-quantitative and qualitative
Analyte	IgA/IgG antibodies against deamidated gliadin peptide	IgA/IgG antibodies against gliadin in human serum
Capture antigen	Deamidated gliadin	Purified α-gliadin antigen

Controls	2 controls (1 positive control, 1 negative control)	3 controls (1 positive, 1 negative, and 1 cut-off control)
Calibrators	5 Calibrators for α-GliaPep® IgA/IgG: 0, 10, 20, 50, 100 AU/mL	6 Calibrators: 0, 3, 10, 30, 100, 300 U/mL
Cut-off	8 AU/mL for α-GliaPep® IgA 10 AU/mL for α-GliaPep® IgG	15 AU/mL

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

CLSI EP5-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition"

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

CLSI EP7-A2, "Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition"

CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline"

CLSI C28-A2, "Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory"

CLSI EP9-A2, "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition"

L. Test Principle:

The α-Gliatest® IgA and α-Gliatest® IgG tests are sandwich type enzyme immunoassays. Purified α-gliadin is attached to microwells, and antigen-specific antibodies in the patient serum bind to the antigens. Non-specific antibodies are removed by washing. Horseradish peroxidase labeled goat anti-human IgA or IgG are added and bind to human antibodies in the well. The excess conjugate is washed away; then a chromogenic substrate is added. After an appropriate incubation period, the OD value is measured using an ELISA microtiter plate reader capable of measuring OD at 450 nm.

The α-GliaPep® IgA and α-GliaPep® IgG tests are a sandwich type enzyme-immunoassay. Specific deamidated gliadin peptides are attached to microwells, and antigen-specific antibodies in the patient serum bind to the antigens. Non-specific antibodies are removed by washing. Horseradish peroxidase-labeled goat anti-human IgA is added to bind with human antibodies in the well. The excess conjugate is washed away; then a chromogenic substrate is added. After an appropriate incubation

period, the absorbance value is measured by means of an EIA reader (450 nm).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision studies:

The "Within-Laboratory Precision" studies, including "With-in Run" and "Between Run" precision, were performed by testing eight serum samples. For "With-in Run" precision, each sample was tested in 10 replicates in one run. Results are summarized in the following tables:

α-Gliatest® IgA:

Sample No.	1	2	3	4	5	6	7	8
Mean (AU/mL)	98.0	73.7	68.7	17.9	8.8	3.0	3.0	1.9
SD	3.50	2.62	2.08	0.49	0.30	0.20	0.08	0.15
CV% (<10%)	3.6	3.6	3.0	2.7	3.5	6.8	2.7	7.7

α-Gliatest® IgG:

Sample No.	1	2	3	4	5	6	7	8
Mean (AU/mL)	99.6	80.4	75.0	61.9	60.6	24.3	10.0	5.1
SD	2.38	3.35	2.53	2.35	2.39	0.86	0.36	0.28
CV% (<10%)	2.4	4.2	3.4	3.8	3.9	3.6	3.6	5.4

α-GliaPep® IgA:

Sample No.	1	2	3	4	5	6	7	8
Mean (AU/mL)	98.2	86.6	65.8	17.8	9.7	4.4	2.7	1.8
SD	6.61	2.83	4.43	0.95	0.94	0.25	0.19	0.15
CV% (<10%)	6.7	3.3	6.7	5.3	9.8	5.7	7.0	8.6

α-GliaPep® IgG:

Sample No.	1	2	3	4	5	6	7	8
Mean (AU/mL)	97.5	55.6	24.5	15.1	10.4	8.1	6.2	4.0
SD	5.69	2.73	1.29	0.95	0.62	0.30	0.44	0.19
CV% (<10%)	5.8	4.9	5.3	6.3	5.9	3.6	7.1	4.7

For "Between Run" precision, ten replicates/run were tested for 6 runs, using one lot of the assay kit. The results are summarized in the following tables:

α-Gliatest® IgA:

Sample No.	1	2	3	4	5	6	7	8
Mean (AU/mL)	95.9	77.8	69.0	17.8	9.0	3.3	3.1	1.9
SD	3.15	7.68	3.94	0.82	1.30	0.24	0.26	0.33

CV% (<15%)	3.3	9.9	5.7	4.6	14.4	7.2	8.3	17.3

α-Gliatest® IgG:

Sample No.	1	2	3	4	5	6	7	8
Mean (AU/mL)	97.9	82.4	75.5	64.4	60.2	26.1	10.6	5.8
SD	1.53	2.48	2.62	2.12	4.73	2.76	0.46	0.99
CV% (<15%)	1.6	3.0	3.5	3.3	7.9	11.0	4.3	16.9

α-GliaPep® IgA:

Sample No.	1	2	3	4	5	6	7	8
Mean (AU/mL)	96.6	84.4	61.1	18.1	9.6	3.8	2.8	1.5
SD	2.19	4.97	4.50	2.24	0.81	0.54	0.20	0.22
CV% (<15%)	2.3	5.9	7.4	12.4	8.4	14.2	7.2	14.9

α-GliaPep®IgG:

Sample No.	1	2	3	4	5	6	7	8
Mean (AU/mL)	97.5	49.3	25.4	15.9	10.0	7.9	5.9	4.1
SD	2.17	4.39	2.44	1.30	0.81	0.33	0.29	0.39
CV% (<15%)	2.2	8.9	9.6	8.2	8.1	4.2	4.9	9.4

In addition, Lot-to-Lot precision study was performed by testing five serum samples. Three lots of the assay kits were used. Each sample was tested in 5 replicate/lot of assay kit. The results are summarized in the following tables.

α-Gliatest® IgA

Sample No.	1	2	3	4	5
Mean (AU/mL)	87.3	43.8	22.7	11.6	7.1
SD	0.81	2.46	1.22	1.18	0.28
%CV	0.9	5.8	5.4	10.1	3.9

α-Gliatest® IgG

Sample No.	1	2	3	4	5
Mean (AU/mL)	84.9	50.3	42.7	24.4	11.1
SD	2.13	1.07	0.72	1.01	0.15
%CV	2.5	2.1	1.7	4.1	1.4

α-GliaPep® IgA

Sample No.	1	2	3	4	5
Mean (AU/mL)	85.4	42.6	22.6	14.3	7.3
SD	1.79	1.86	1.83	0.50	0.38
%CV	2.1	4.4	8.1	3.5	5.1

α-GliaPep® IgG

Sample No.	1	2	3	4	5
Mean (AU/mL)	83.1	45.6	26.3	13.1	4.1
SD	0.99	1.31	0.39	1.34	0.12
%CV	1.2	2.9	1.5	10.2	3.0

b. Linearity/assay reportable range:

The linearity studies were assessed according to CLSI EP6-A. Four positive serum samples (2 samples ~ 100 AU/mL and 2 samples ~ 50 AU/mL) for each of α-Gliatest® IgA, α-Gliatest® IgG. α-GliaPep® IgA and α-GliaPep® IgG were used in the study. Each sample was diluted with a low concentration serum sample (around limit of detection) and tested in duplicate. The observed values were graphed against the calculated values and linear regression was performed. The results are summarized in the table below:

Test Range (AU/mL)	Slope (95% CI)	Y-intercept (95% CI)	R^2
α-Gliatest® IgA			
3.3 – 99.0	1.017 (0.991 – 1.044)	-1.637 (-3.210 – -0.064)	0.999
3.4 – 103.5	1.016 (0.987 – 1.044)	-1.982 (-3.734 – -0.230)	0.999
3.5 – 53.3	1.014 (0.962 – 1.066)	-1.299 (-2.942 – 0.345)	0.996
1.1 – 49.8	1.000 (0.961 – 1.040)	-0.757 (-1.907 – 0.392)	0.997
α-Gliatest® IgG			
5.9 – 98.5	1.038 (1.000 – 1.077)	-3.014 (-5.324 – -0.704)	0.998
5.9 – 99.4	0.997 (0.931 – 1.064)	1.857 (-2.182 – 5.896)	0.992
5.9 – 51.8	1.039 (0.991 – 1.087)	-1.631 (-3.142 – -0.120)	0.996
2.5 – 50.7	0.999 (0.965 – 1.032)	-0.268 (-1.300 – 0.765)	0.998
α-GliaPep® IgA			
1.3 – 94.8	1.018 (0.994 – 1.042)	-1.697 (-3.075 – 0.320)	0.999
1.5 – 102.3	1.016 (0.997 – 1.036)	-0.561 (-1.755 – 0.633)	0.999
1.4 – 49.9	1.019 (0.977 – 1.062)	-0.805 (-2.079 – 0.470)	0.997

Test Range (AU/mL)	Slope (95% CI)	Y-intercept (95% CI)	R^2
1.1 – 50.7	1.015 (0.964 – 1.065)	0.116 (-1.410 – 1.643)	0.996
α-GliaPep® IgG			
1.5 – 102.1	1.014 (0.987 – 1.041)	-1.705 (-3.354 – -0.056)	0.999
1.5 – 98.4	1.023 (0.995 – 1.052)	-1.790 (-3.467 – -0.113)	0.999
1.5 – 52.4	1.063 (1.017 – 1.109)	-1.784 (-3.177 – -0.392)	0.997
1.1 – 51.0	1.000 (0.965 – 1.035)	-0.296 (-1.356 – 0.764)	0.998

The linear range is 1.1 – 100 AU/mL for α-Gliatest® IgA, 2.5 – 99.4 AU/mL for α-Gliatest® IgG, 1.1 – 100 AU/mL for α-GliaPep® IgA, and 1.1 – 100 AU/mL for α-GliaPep® IgG.

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

Traceability: There is no recognized standard for this analyte.

Stability:

Kit stability: Stability studies were performed on three lots each of the α-Gliatest® IgA, α-Gliatest® IgG, α-GliaPep® IgA, and α-GliaPep® IgG test kits after stored at 2-8°C for 2, 6, 12, and 15 months. Five (5) calibrators were tested in the study. Testing was done at t=0 (batch release), t=2, t=6, t=12, and t=15 months. Based on these studies, the claimed shelf life for the test kits is 12 months when stored at 2-8°C.

Open vial stability: A study was performed on three lots each of α-Gliatest® IgA, α-Gliatest® IgG, α-GliaPep® IgA, and α-GliaPep® IgG test kits after open and after being stored at 2 – 8°C. Five (5) calibrators (S1-S5) were also tested in the study. Testing was done at t=0 and t=2 months. The results showed that kits are stable up to 2 months after first open and stored at 2 – 8°C.

Sample stability: Sample stability was performed and results support the claim that specimens can be stored at 2-8°C for five days.

Calibrators:

Calibrators are prepared in-house from dilutions of the pooled serum of anti-gliadin antibody (AGA) from patients with celiac disease. The new calibrator

is formulated from an array of AGA antibody positive sera obtained from various commercial plasma centers. As new lots of calibrators are developed, studies are performed to calibrate values against the previously approved lot of the 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	α-Gliatest® IgA (AU/mL)	α-Gliatest® IgG (AU/mL)	α-GliaPep® IgA (AU/mL)	α-GliaPep® IgG (AU/mL)
Cal S5	100	100	100	100
Cal S4	50	50	50	50
Cal S3	20	20	20	20
Cal S2	10	10	10	10
Cal S1	0	2	0	0

Controls:

One positive and one negative control serum sample are included in each of the α-Gliatest® IgA, α-Gliatest® IgG, α-GliaPep® IgA, and α-GliaPep® IgG assay kit. The positive and negative controls are prepared in-house and with an assigned range of concentrations. The assigned ranges of concentrations for the positive and negative controls are summarized in the following table:

Controls	Assigned range of concentrations (AU/mL)			
	α-Gliatest® IgA	α-Gliatest® IgG	α-GliaPep® IgA	α-GliaPep® IgG
Positive Control	30 – 75	50 – 100	30 – 75	30 – 75
Negative Control	<5	<10	<5	<5

d. Detection limit:

The analytical sensitivity (detection limit) of the α-Gliatest® IgA/IgG and α-GliaPep® IgA/IgG were evaluated in accordance with CLSI Standard EP17-A. The limit of blank (LoB) was determined by running six serum diluent samples (buffer solution provided with the kit) in ten replicates for a total of 60 measurements. The limit of detection (LoD) was determined and calculated by running six low concentration samples in the range from LoB to approximately 4 xLoB, according to CLSI EP17-A. The samples were tested in ten replicates each, for a total of 60 measurements. The results for LoB and LoD are summarized in the following table.

	LoB (AU/mL)	LoD (AU/mL)	
		Tested	Claimed
α-Gliatest® IgA	0.59	0.92	1.1
α-Gliatest® IgG	1.66	2.36	2.5

α-GliaPep® IgA	0.13	0.55	1.1
α-GliaPep® IgG	0.18	0.61	1.1

e. Analytical specificity:

Interference by endogenous substances: Study was done to evaluate the analytical specificity of α-Gliatest® IgA, α-Gliatest® IgG, α-GliaPep® IgA, and α-GliaPep® IgG tests in the presence of endogenous interfering substances. For each assay, five serum samples including one negative, two around the cut-off and two strong positives were tested by spiking with the interference substance: Hemoglobin, Bilirubin, Lipid, and Rheumatoid Factor (RF). For each test sample, the samples without spiking with interference substance were used as control. The recovery of test sample was calculated as percent recovery compared to the control sample. No significant interference was noted for samples containing interference substance at concentration listed below:

Interfering substance	α-Gliatest® IgA	α-Gliatest® IgG	α-GliaPep® IgA	α-GliaPep® IgG
Hemoglobin (mg/L)	2000	2000	1500	1500
Bilirubin (μmol/L)	342	256	256	342
Lipid (mg/L)	950	950	950	1300
RF (IU/mL)	100	100	100	100

f. Assay cut-off:

The cut-off of each assay was established by testing serum samples from healthy subjects and non-celiac controls (inflammatory Bowel Disease) patients. For α-Gliatest® IgA and IgG assays, 248 samples were tested with α-Gliatest® IgA while 258 samples were tested with Gliatest® IgG. The cut-off was determined as the mean plus 3 standard deviations of the detection levels of samples. For α-GliaPep® IgA and α-GliaPep®IgG assays, 169 samples were tested and the cut-off was determined as the mean plus 2 standard deviations of the detection levels for the tested samples.

The assay cut-off value for each assay was determined as follows:

	Negative	Positive
α-Gliatest® IgA	< 8 AU/mL	≥ 8 AU/mL
α-Gliatest® IgG	< 50 AU/mL	≥ 50 AU/mL
α-GliaPep® IgA	< 8 AU/mL	≥ 8 AU/mL
α-GliaPep® IgG	<10 AU/mL	≥ 10 AU/mL

2. Comparison studies:

a. Method comparison with predicate device:

Samples within the reportable range of the assay were tested with each of α-Gliatest® IgA/IgG and α-GliaPep® IgA/IgG and their corresponding predicate Aeskulisa® Glia A/G. The samples consist of clinical diagnosed celiac positives (biopsy or clinically proven diagnosis), IgA deficient celiac patient samples, and negative samples from patients with other defined diseases, including non-celiac autoimmune disease, Inflammatory Bowel Syndrome (IBS), Inflammatory Bowel Disease (IBD), infectious disease, food intolerance and Type 1 diabetes. The study results are summarized in the tables below:

α-Gliatest® IgA:

IgA deficient patients were tested with both methods, as expected; all were negative and are not included in the analysis:

		Aeskulisa® Glia A (Cut-off: 15 AU/mL)		
		Positive	Negative	Total
α-Gliatest® IgA (Cuf-off: 8 AU/mL)	Positive	52	18	70
	Negative	6	102	108
	Total	58	120	178

Positive Agreement: 89.7% (95% CI: 78.8% – 96.1%)
Negative Agreement: 85.0% (95% CI: 77.3 – 90.9%)
Overall Agreement: 86.5% (95% CI: 80.6% – 91.2%)

Discrepant Result Analyses – 42 out of 45 (93.3%) samples from celiac disease patients were identified by α-Gliatest® IgA, while 35 out of 45 (77.8%) were identified by the predicate device. Out of 18 samples tested positive with α-Gliatest® IgA but negative with the predicate, 7 were celiac disease samples, and 11 were non-celiac disease control samples. For negative agreement, all 6 samples tested negative with the α-Gliatest® IgA but positive with the predicate were from non-celiac disease controls.

α-Gliatest® IgG:

		Aeskulisa® Glia G (Cut-off: 15 AU/mL)		
		Positive	Negative	Total
α-Gliatest® IgG (Cuf-off: 50 AU/mL)	Positive	52	15	67
	Negative	14	117	131
	Total	66	132	198

Positive Agreement: 78.8% (95% CI: 67.0% – 87.9%)
Negative Agreement: 88.6% (95% CI: 82.0 – 93.5%)
Overall Agreement: 85.4% (95% CI: 79.6% – 90.0%)

Discrepant Result Analyses – Out of 15 samples which were tested positive with the α-Gliatest® IgG but negative with the predicate, 8 samples were from celiac positive samples, 1 from IgA-deficient celiac patient sample, and 6 from other disease controls. Out of 14 samples tested negative with the α-Gliatest® IgG but positive with the predicate, 4 were from celiac positive patients, 3 were from IgA-deficient celiac patients, and 7 were from other disease controls.

α-GliaPep® IgA:

10 IgA deficient patient samples were tested with both methods, as expected; all were negative and are not included in the analysis:

		Aeskulisa® Glia A (Cut-off: 15 AU/mL)		
		Positive	Negative	Total
α-GliaPep® IgA (Cuf-off: 8 AU/mL)	Positive	36	11	47
	Negative	14	118	132
	Total	50	129	179

Positive Agreement: 72.0% (95% CI: 57.5% – 83.8%)
Negative Agreement: 91.5% (95% CI: 85.3 – 95.7%)
Overall Agreement: 86.0% (95% CI: 80.1% – 90.8%)

Discrepant Result Analyses – Eleven samples were tested positive with α-GliaPep® IgA but negative with the predicate. Out of these 11 samples, 6 were celiac-positive samples, 5 were other disease control samples. Fourteen (14) samples were tested negative with α-GliaPep® IgA but positive with the predicate. Four (4) out of these 14 samples were celiac positive patient samples and 10 were other disease control samples.

α-GliaPep® IgG:

		Aeskulisa® Glia G (Cut-off: 15 AU/mL)		
		Positive	Negative	Total
α-GliaPep® IgG (Cuf-off: 10 AU/mL)	Positive	50	16	66
	Negative	13	122	135
	Total	63	138	201

Positive Agreement: 79.4% (95% CI: 67.3% – 88.5%)
Negative Agreement: 88.4% (95% CI: 81.9% – 93.2%)
Overall Agreement: 85.6% (95% CI: 79.9% – 90.1%)

Discrepant Result Analyses – Sixteen samples were tested positive with α-GliaPep® IgG but negative with the predicate. Out of these 16 samples, 7 were celiac-positive patient samples, 1 were IgA-deficient celiac patient sample, and 8 were celiac negative samples. Out of 13 samples which were tested negative with the α-GliaPep® IgG but positive with the predicate, 5 were celiac positive samples and 8 were celiac negative samples.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Clinical Specificity:

The clinical validation study for each of α-Gliatest® IgA, α-Gliatest® IgG, α-GliaPep® IgA, and α-GliaPep® IgG was performed in four sites – three external and one in-house. The study included samples from patients who had been clinical defined as suffering from celiac disease (biopsy or clinically proven diagnosis), and samples from non-celiac disease controls. 148 disease control samples included 15 samples of rheumatoid arthritis, 30 samples of systemic lupus erythematosus, 4 samples of systemic sclerosis, 16 samples of Hashimoto's thyroiditis, 9 samples Graves' disease, 12 samples of autoimmune hepatitis, 2 samples of Sjögren's syndrome, 13 samples of Crhon's disease, 9 samples of ulcerative colitis, 17 samples of IBS, 11 samples of Type 1 Diabetes and 10 samples of infectious disease (H. pylori). The results for each of α-Gliatest® IgA, α-Gliatest® IgG, α-GliaPep® IgA and α-GliaPep® IgG were compared to the clinical diagnosis.

α-Gliatest® IgA:

The study included 262 clinical samples, of which 114 samples were from celiac disease patients and 148 samples were disease control samples.

		Celiac Disease		Total
		Positive	Negative	
α-Gliatest® IgA	Positive	79	36	115
	Negative	35	112	147
	Total	114	148	262

Sensitivity: 69.3% (95% CI: 60.0% – 77.6%)
Specificity: 75.7% (95% CI: 95.8% – 99.5%)

α-Gliatest® IgG:

The study included 285 samples, of which 137 samples were celiac positive samples (including 10 samples from IgA deficient celiac patients) and 148

samples were disease controls samples.

		Celiac Disease		Total
		Positive	Negative	
α-Gliatest® IgG	Positive	109	17	126
	Negative	28	131	159
	Total	137	148	285

Sensitivity: 79.6% (95% CI: 71.8% – 86.0%)
Specificity: 88.5% (95% CI: 82.2% – 93.2%)

α-GliaPep® IgA:

The study included 241 clinical samples, of which 93 samples were from patients diagnosed with celiac disease and 148 samples were disease control samples.

		Celiac Disease		Total
		Positive	Negative	
α-GliaPep® IgA	Positive	74	10	84
	Negative	19	138	159
	Total	93	148	241

Sensitivity: 79.6% (95% CI: 69.9% – 87.2%)
Specificity: 93.2% (95% CI: 87.9% – 96.7%)

α-GliaPep® IgG:

The study included 253 clinical samples, of which 105 samples were from celiac disease patients (including 10 samples from IgA deficient celiac patients) and 148 samples were disease control samples. All samples from IgA deficient celias patients were tested positive with α-GliaPep® IgG

		Celiac Disease		Total
		Positive	Negative	
α-GliaPep® IgG	Positive	95	22	117
	Negative	10	126	136
	Total	105	148	253

Sensitivity: 90.5% (95% CI: 83.2% – 95.3%)
Specificity: 85.1% (95% CI: 78.4% – 90.4%)

The clinical cross reactivity of α-Gliatest® IgA, α-Gliatest® IgA, α-Gliatest® IgA, and α-Gliatest® IgA in patients with non-celiac control diseases was summarized in the following table.

Sample Type	n	Positive 'n' (%)			
		α-Gliatest® IgA	α-Gliatest® IgG	α-GliaPep® IgA	α-GliaPep® IgG
Rheumatoid Arthritis	15	1 (6.7%)	1 (6.7%)	0 (0%)	2 (13.3%)
Systemic Lupus Erythematosus	30	11 (36.7%)	2 (6.7%)	0 (0%)	2 (6.7%)
Hashimoto's Thyroiditis	16	7 (43.8%)	0 (0%)	4 (25%)	3 (18.8%)
Graves' Disease	9	6 (66.7%)	1 (11.1%)	1 (11.1%)	4 (44.4%)
Autoimmune Hepatitis	12	1 (8.3%)	0 (0%)	1 (8.3%)	1 (8.3%)
Sjogren's syndrome	2	0 (0%)	1 (50%)	0 (0%)	0 (0%)
Systemic Sclerosis	4	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Crohn's Disease	13	1 (7.7%)	2 (15.4%)	0 (0%)	2 (15.4%)
UlcerativeColitis (UC)	9	0 (0%)	2 (22.2%)	0 (0%)	1 (11.1%)
IBS	17	5 (29.4%)	5 (29.4%)	2 (11.8%)	2 (11.8%)
Type 1 Diabetes	11	4 (36.4%)	3 (27.3%)	1 (9.1%)	2 (18.2%)
H. pylori	10	0 (0%)	0 (0%)	1 (10%)	3 (30%)

4. Clinical cut-off**:**

   Same as assay cut-off

5. Expected values/Reference range:

   The study was done to test 196 healthy population with each of α-Gliatest® IgA and α-Gliatest® IgG. Out of these, 190 (96.94%) were negative with α-Gliatest® IgA assay and 186 (94.9%) were negative with α-Gliatest® IgG assay, respectively. For α-GliaPep® IgA and α-GliaPep® IgG, 145 healthy population were tested. The results showed that 140 (96.55%) was negative with α-GliaPep® IgA and 132 (91.03%) was negative with α-GliaPep® IgG.

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.