510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
   k063775
B. Purpose for Submission:
   New device
C. Measurand:
   Anti-gliadin IgA antibody
   Anti-gliadin IgG antibody
D. Type of Test:
   Semi-quantitative fluoroenzyme immunoassay
E. Applicant:
   Phadia US, Inc.
F. Proprietary and Established Names:
   EliA™ Gliadin IgA Immunoassay
   EliA™ Gliadin IgG Immunoassay
   EliA™ Celiac Control
G. Regulatory Information:
   1. Regulation section:
      21 CFR§ 866.5750, Radioallergosorbent (RAST) Immunological Test System
      21 CFR§ 862.1660, Quality Control Material (Assayed and Unassayed)
   2. Classification:
      Device-Class II
      Quality control material-Class I
   3. Product code:
      MST, Antibodies, Gliadin
      JJY, Multi-Analyte Controls (Assayed and Unassayed)
   4. Panel:
      (82) Immunology
      (75) Chemistry
H. Intended Use:
   1. Intended use(s):
      EliA™ Gliadin IgA is intended for the in vitro semi-quantitative measurement of
      IgA antibodies directed to gliadin in serum and plasma to aid in the diagnosis of
      Celiac disease. EliA™ GliadinIgA uses the EliA IgA method on the instrument
      ImmunoCAP 100 and ImmunoCAP 250.

      EliA™ Gliadin IgG is intended for the in vitro semi-quantitative measurement of
      IgG antibodies directed to gliadin in serum and plasma to aid in the diagnosis of
      Celiac disease. EliA™ GliadinIgG uses the EliA IgG method on the instrument
      ImmunoCAP 100 and ImmunoCAP 250.

      EliA™ Celiac Control is intended for laboratory use in monitoring the
      performance of in vitro measurement of antibodies to tissue transglutaminase
      (tG) and gliadin with ImmunoCAP 100 or ImmunoCAP 250 using the EliA IgG

or IgA method.

2. <u>Indication(s) for use:</u>
   Same as above
3. <u>Special conditions for use statement(s):</u>
   The device is for prescription use only.
4. <u>Special instrument requirements:</u>
   ImmunoCAP 100 and ImmunoCAP 250 (k061165)

I. Device Description:

The EliA reagents are available as modular packages, each purchased separately. The EliA Gliadin IgA wells are coated with gliadin antigen. These are packed in carriers which are stored in sealed aluminum foil bags containing a desiccant. The EliA Method-Specific reagents consists of (1) sample diluent concentrate, (2) IgA Conjugate (blue colored) β-Galactosidase anti-IgA (mouse monoclonal antibodies) in PBS, (3) ready-to-use 6 level IgA calibrators (human IgA concentrations of 0,0.3,1.5,5,15 and 80 µg/L), (4) ready-to-use IgA Curve Control (5 µg/L), (5) IgA Calibrator well coated with mouse monoclonal antibody, (6) ready for use development solution containing 0.1% 4-methylumbelliferyl – β-D galactoside and (7) 4% sodium carbonate stop solution.

The EliA Gliadin IgG wells are coated with gliadin antigen. These are packed in carriers which are stored in sealed aluminum foil bags containing a desiccant. The EliA Method-Specific reagents consists of (1) sample diluent concentrate, (2) IgG Conjugate (blue colored) β-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS, (3) ready-to-use 6 level IgG calibrators (human IgG concentrations of 0,4,10,20,100 and 600 µg/L), (4) ready-to-use IgG Curve Control (20 µg/L), (5) IgG Calibrator well coated with mouse monoclonal antibody, (6) ready for use development solution containing 0.1% 4-methylumbelliferyl – β-D galactoside and (7) 4% sodium carbonate stop solution.

Curve Controls have defined ranges to check whether the stored calibration curve is still valid. Limits for the response of the Curve Controls are defined in the ImmunoCAP 100/250 Operator and Panel Software.

The EliA Celiac Control is a two- level control (negative and positive) containing IgG and IgA antibodies to tTG and gliadin. The EliA Celiac Control is prediluted and ready to use.

J. Substantial Equivalence Information:

1. <u>Predicate device name(s):</u>
   Varelisa Gliadin IgA Antibodies
   Varelisa Gliadin IgG Antibodies
2. <u>Predicate 510(k) number(s):</u>
   k041354
   k041357
3. <u>Comparison with predicate:</u>

Similarities		
Item	Device	Predicate
	EliA™ Gliadin IgA EliA™ Gliadin IgG	Varelisa Gliadin IgA Antibodies Varelisa Gliadin IgG Antibodies
Intended Use	For the semi-quantitative measurement of IgA and IgG antibodies directed to gliadin in serum and plasma to aid in the diagnosis of celiac disease.	Same
Sample type	Serum and plasma	Same
Solid phase	Microwells	Same
Assay type	Elisa	Same
Capture Antigens	Purified gliadin	Same

Differences		
Item	Device	Predicate
Instrumentation	ImmunoCAP 100 and 250 (fully automated)	Microplate reader with 620 nm filter
Assay format	Semi-quantitative	Semi-quantitative and qualitative
Internal Controls	Positive and Negative controls provided within the EliA Celiac control kit, sold separately.	Positive and Negative controls included in the kit
Calibration	Total IgA or IgG calibration Option to store curve for up to 28 days and run curve controls (provided in kit) in each assay for calibration	Analyte specific IgA or IgG calibration
Signal	Fluorescence	Optical density
Reaction temperature	37°C controlled	Room temperature, 18-25°C
Concept	Modular reagents concept (test-method specific and general reagents)	All reagents in a single kit
Conjugate	Anti-human IgA or IgG β-galactosidase (mouse monoclonal antibodies)	Anti-human IgA or IgG horseradish peroxidase (goat)
Result interpretation	Negative <7.0 EliA U/mL Equivocal 7-10 EliA U/mL Positive >10 EliA U/mL	Negative <11.0 U/mL Equivocal 11-17 U/mL Positive >17 U/mL

K. Standard/Guidance Document Referenced (if applicable):
     None referenced.
L. Test Principle:

The EliA Gliadin IgA wells are coated with gliadin antigen. If present in the patient's specimen, antibodies to gliadin will bind to their specific antigen in the wells. After washing away non-bound antibodies, enzyme- labeled antibodies against human IgA antibodies (EliA IgA conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with a development solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more specific IgA is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

The EliA Gliadin IgG wells are coated with gliadin antigen. If present in the patient's specimen, antibodies to gliadin will bind to their specific antigen in the wells. After washing away non-bound antibodies, enzyme- labeled antibodies against human IgG antibodies (EliA IgG conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with a development solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more specific IgG is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. *Precision/Reproducibility:*
      EliA™ Gliadin IgA
      For the ImmunoCAP 100, four samples were measured in duplicates on four instruments in 26 runs over 9 days with a calibration curve in each run. For the ImmunoCAP 250, four samples were measured in 3 replicates in 12 runs on two instruments over 6 weeks with a calibration curve in each run. The studies yielded the following:

ImmunoCAP 100

Sample	Mean (U/mL)	Intra-run (CV%)	Inter-run (CV%)
1	2.0	8.5	19.0
2	13.1	3.2	5.8
3	31.9	2.2	7.5
4	42.3	5.8	4.7

ImmunoCAP 250

Sample	Mean (U/mL)	Intra-run (CV%)	Inter-run (CV%)
1	4.8	4.2	12.2
2	9.3	4.5	2.6
3	93.2	4.6	4.0
4	142.3	4.0	3.4

EliA™ Gliadin IgG

For ImmunoCAP 100, four samples were measured in duplicates on three instruments in 26 runs over 9 days with a calibration curve in each run. For ImmunoCAP 250, four samples were measured in 3 replicates in 12 runs on two instruments over 6 weeks with a calibration curve in each run. The studies yielded the following:

ImmunoCAP 100

Sample	Mean (U/mL)	Intra-run (CV%)	Inter-run (CV%)
1	4.4	3.3	8.4
2	14.6	5.0	7.0
3	17.1	2.4	6.0
4	32.0	4.8	2.5

ImmunoCAP 250

Sample	Mean (U/mL)	Intra-run (CV%)	Inter-run (CV%)
1	5.7	5.0	4.4
2	10.1	4.6	2.3
3	31.9	3.7	2.8
4	93.7	3.8	3.8

b. *Linearity/assay reportable range:*
   Linearity was not claimed for this device.
   The measuring range for EliA Gliadin IgA and EliA Gliadin IgG are 0.2 to 213 EliA U/mL and 0.2 to 192 EliA U/mL respectively.

   High dose hook effect:
   For **EliA Gliadin IgA**, the possibility of antigen excess occurring when using the device was evaluated with serum sample above the assay measuring range. No hook effect was observed for concentrations up to 512 µg/mL, which is about 6 times above the value of the highest calibrator (80 µg/L).

   For **EliA Gliadin IgG**, the possibility of antigen excess occurring when using the device was evaluated with serum sample above the assay measuring range. No hook effect was observed for concentrations up to 8200 µg/mL, which is about 13 times above the value of the highest calibrator (600 µg/L).

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   The IgA and IgG calibrators are traceable to the International Reference Preparation (IRP) 67/86 of Human Immunoglobulins A, G, and M from WHO. New batches of calibrators are compared to a secondary standard (standardized with the IRP) or the IRP directly and adjusted accordingly to meet the correct concentration (6 levels). The instrument measures specific

IgA or IgG concentrations in µg/L. To obtain a test specific result, µg/L of IgA or IgG must be converted to EliA U/mL using a conversion factor.

EliA Celiac Control is prepared from selected pooled human sera. The controls are prediluted and ready for use. The acceptance ranges for the current control lot are stated on the Control Certificate included in the respective EliA Celiac Control kit. The mean values for every lot have been determined with 4 consecutive control assays, each in 6 replicates. Ranges are calculated as respective mean ± 3SD for the expected long term variation.

Open-vial stability is not tested as the EliA Celiac control is packaged in single vials. Closed-vial stability studies were performed with the following specifications: For the Positive Control, the quota (stressed/reference has to be equal to or in the range of 0.80 to 1.20. The negative Control should recover ≤2.8 U/mL. Specifications were met. Accelerated stability testing over a period of 6 weeks at 30°C equals 18 months at 2-8°C.

d. *Detection limit:*

EliA™ Gliadin IgA

The lower limit of the measuring range was determined by measuring dilutions (1:2, 1:4, and 1:8) of Calibrator 0.3 (0.3 µg/L) in the Calibrator Wells. The results in Response Units (RU) were compared with the result of the sample diluent on EliA Gliadin IgA Wells. The discrimination ability (D) of the assay should be >2. All samples were measured in triplicate.

Sample ID	Results on Calibrator Wells	
	Mean Response Units (RU)	SD
Calibrator 0.3 (1:2)	106	6.7
Calibrator 0.3 (1:4)	58	2.5
Calibrator 0.3 (1:8)	36	1.8

Sample ID	Results on Gliadin IgA Wells	
	Mean RU	SD
Sample Diluent	0	0.0

The 1/8 diluted calibrator 0.3 (0.0375 µg/L) still can be discriminated from background given by the signal of the diluent on Gliadin IgA wells. The lower limit of detection was set at 0.05 µg /L corresponding to 0.2 EliA U/mL.

EliA™ Gliadin IgG

The lower limit of the measuring range was determined by measuring dilutions (1:2, 1:4, and 1:8) of Calibrator 4.0 (4.0 µg/L) in the Calibrator Wells. The results in Response Units (RU) were compared with the result of the sample diluent on EliA Gliadin IgG Wells. The discrimination ability (D) of the assay should be >2. All samples were measured in triplicate.

	Results on Calibrator Wells	
Sample ID	Mean Response Units (RU)	SD
Calibrator 4.0 (1:2)	194	4.0
Calibrator 4.0 (1:4)	122	2.3
Calibrator 4.0 (1:8)	90	1.1

	Results on Gliadin IgGWells	
Sample ID	Mean RU	SD
Sample Diluent	0	0.0

The 1/8 diluted calibrator 4.0 (0.5 µg/L) still can be discriminated from background given by the signal of the diluent on Gliadin IgG wells. The lower limit of detection was set at 0.5 µg/L corresponding to 0.2 EliA U/mL.

e. *Analytical specificity:*
<u>Interfering substance</u>
The potential interferant and the corresponding blank were added to aliquots of two anti-Gliadin IgA and two Gliadin IgG positive sera. The spiked samples were tested in triplicate.

Additives	Concentration in raw sample	Final concentration in diluted sample (1:100)	Normal Values
Bilirubin F	18.8 mg/dL	0.188	<1.0
Bilirubin C	20 mg/dL	0.2	<1.0
Chyle	236,000 Units/dL	2360	No data
Hemoglobin	453 mg/dL	4.5	<2.0
Rheumatoid Factor IgM	550 IU/mL	5.5	<40.0

The specification was set such that the ratio of the result of the sample spiked with the interfering substance and the sample spiked with a buffer blank should be between 0.8 and 1.2. The tables below show the results of the study.

EliA™ Gliadin IgA

		Sample 1			Sample 2		
Additive	Blank/spiked sample	Conc. U/mL	CV %	Ratio	Conc. (U/mL)	CV %	Ratio
Bilirubin F	Blank	13.0	8.8	0.97	46.6	2.1	0.96
	Sample	12.7	3.3		45.0	4.4	
Bilirubin C	Blank	12.7	4.9	0.97	46.0	7.4	1.03
	Sample	12.3	3.5		47.3	3.3	
Hemoglobin	Blank	13.7	0.9	0.98	44.3	8.5	1.08

Additive	Blank/spiked sample	Sample 1			Sample 2		
		Conc. U/mL	CV %	**Ratio**	Conc. (U/mL)	CV %	**Ratio**
	Sample	13.3	5.7		48.0	1.3	
Chyle	Blank	13.0	4.7	1.05	49.3	0.6	1.00
	Sample	13.7	3.6		49.3	2.4	
RF	Blank	13.7	2.3	0.95	50.9	2.8	0.94
	Sample	13.0	1.7		47.6	4.3	

EliA™ Gliadin IgG

Additive	Blank/spiked sample	Sample 1			Sample 2		
		Conc. U/mL	CV %	**Ratio**	Conc. (U/mL)	CV %	**Ratio**
Bilirubin F	Blank	5.1	2.1	0.96	30.0	2.1	0.96
	Sample	4.9	3.5		29.4	3.5	
Bilirubin C	Blank	5.0	3.2	0.98	29.6	3.2	0.98
	Sample	5.0	4.9		30.5	4.9	
Hemoglobin	Blank	5.4	5.3	0.99	30.7	5.3	0.99
	Sample	5.3	2.3		30.7	2.3	
Chyle	Blank	5.3	3.8	1.04	28.2	3.8	1.04
	Sample	5.5	3.4		28.2	3.4	
RF	Blank	5.6	2.8	0.94	28.5	2.8	0.94
	Sample	5.2	1.4		28.5	1.4	

The interfering substances listed did not appear to adversely affect the results of the new devices.

f. *Assay cut-off:*
The purpose of the normal sera studies was to evaluate expected values in the normal population and to confirm the defined cut-off. Samples from 400 apparently healthy Caucasian adult blood donors were measured. The individuals were equally distributed by sex and age. Results were tabulated in the tables below:

	EliA Gliadin IgA (EliA U/mL)	EliA Gliadin IgG (EliA U/mL)
Median	1.9	0.9
Mean	2.1	1.7
Mean +2SD	5.9	9.7
Mean +3SD	7.7	13.7
90th Percentile	3.3	3.3
95th Percentile	4.1	5.0
99th Percentile	5.8	12.6

The results appeared to be equally distributed and not dependent on age or gender. The 95th percentile lies below the lower limit of the equivocal range of 7-10 EliA U/mL.

2. Comparison studies:
   a. *Method comparison with predicate device:*
   Clinical samples:
   Two hundred forty nine (249) patient samples covering the measuring range were tested with the new devices, EliA Gliadin IgA and EliA Gliadin IgG and the predicate devices, Varelisa Gliadin IgA and Varelisa Gliadin IgG respectively. These samples included 98 patients with a diagnosis of Celiac disease. One hundred fifty one (151) samples were from disease controls:

   101 non-CD, normal biopsy
   10 ulcerative colitis
   10 morbus crohn
   20 rheumatoid arthritis
   10 rheumatoid factor positive samples

   In the assessment of the method comparison studies, the following values were considered:

	EliA Assays	Varelisa Assays
Negative	<7.0 U/mL	<11.0 U/mL
Equivocal	7-10 U/mL	11-17 U/mL
Positive	>10 U/mL	>17 U/mL

   Equivocal results were excluded from calculation. Results showed the following:

		Varelisa™ Gliadin IgA			
		positive	equivocal	negative	Total
EliA Gliadin IgA	positive	67	6	0	73
	equivocal	2	4	6	12
	negative	2	2	160	164
	Total	71	12	166	249

   Total agreement = 99.1%
   Positive % agreement = 97.1% (95%CI 89.9-99.6)
   Negative% agreement = 100.0% (95%CI 97.7-100.0)

| | | Varelisa™ Gliadin IgG | | | |
		positive	equivocal	negative	Total
EliA Gliadin IgG	positive	70	5	15	90
	equivocal	4	9	12	25
	negative	1	3	130	134
	Total	75	17	157	249

Total agreement = 92.6%
Positive % agreement = 98.6% (95%CI 92.4-100.0)
Negative% agreement = 89.7% (95%CI 83.5-94.1)

b. *Matrix comparison:*
    Fifty sets of samples from different donors were tested in double determinations. Sample demographics were not provided. Each set contained serum, EDTA, heparin and citrate plasma samples. For positive and equivocal serum samples quotas between serum and each type of plasma were calculated. Mean quota of plasma to serum concentration should be 0.8-1.2 for positive sera. Negative samples should not switch to positive in all serum and plasma samples. Linear regression comparing the quotas between serum and each type of plasma for the positive samples was performed and showed:

EliA Gliadin IgA: correlation serum and plasma

	n	Slope	Intercept	Correlation Coefficient
Serum vs. Plasma Citrate	50	0.9895	-0.0844	0.9976
Serum vs. Plasma Heparin	50	0.9974	0.1385	0.9955
Serum vs. PlasmaEDTA	50	0.9934	0.1268	0.9957

EliA Gliadin IgG: correlation serum and plasma

	n	Slope	Intercept	Correlation Coefficient
Serum vs. Plasma Citrate	50	1.0077	-0.1954	0.9966
Serum vs. Plasma Heparin	50	0.9537	0.4186	0.9988
Serum vs. PlasmaEDTA	50	0.9647	0.2595	0.9983

The specifications for this study are fulfilled for serum, heparin, EDTA and citrate plasma samples. This information is specified on the Specimen Collection section of the Package Insert.

c. *Instrument Platform comparison:*
The purpose of this study was to demonstrate that the performance of EliA Gliadin IgA and EliA Gliadin IgG are equivalent on the ImmunoCAP 100 and the ImmunoCAP 250.

For this comparison study, a total of 36 samples distributed over the measuring range were assayed: 4 negative samples, and 32 positive samples. All samples were run on three ImmunoCAP 100 instruments and two ImmunoCAP 250 instruments in two runs and in single replicates. Results of the comparison study showed y = 0.993x and y = 1.0313x for Gliadin IgA and IgG respectively where y was ImmunoCAP 250 and x was ImmunoCAP 100. The correlation coefficient (r) for both Gliadin IgA and IgG was 0.99.

3. Clinical studies:
   a. *Clinical Sensitivity and Clinical Specificity:*
   Ninety eight (98) patients with known diagnosis of Celiac disease and 101 non-CD patients (suspected CD, negative by biopsy) were tested for this study. See table below for results:

	EliA Gliadin IgA		
n = 199	>10 U/mL	≤10 U/mL	
CD (98)	69	29	**Sensitivity (95%CI)** 70.4 (60.3-79.2%)
Disease Controls (101)	3	98	**Specificity (95%CI)** 97.0 (91.6-99.4%)

	EliA Gliadin IgG		
n = 199	>10 U/mL	≤10 U/mL	
CD (98)	69	29	**Sensitivity (95%CI)** 70.4 (60.3-79.2%)
Disease Controls (101)	20	81	**Specificity (95%CI)** 80.2 (71.1-87.5%)

Clinical sensitivity of EliA Gliadin IgA and EliA Gliadin IgG were comparable to the sensitivities of predicate devices which were 62.7% (95%CI 51.1-74.3%) and 44.8% (95%CI 32.9-56.7%) for the Varelisa Gliadin IgA and IgG respectively.

Clinical specificity of EliA Gliadin IgA and EliA Gliadin IgG were

comparable to the specificities of predicate devices which were 100.0% for and 77.9% (95%CI 68.6-87.2%) for the Varelisa Gliadin IgA and IgG respectively.

    b. *Other clinical supportive data (when a. is not applicable)*:
      Not applicable.
4. <u>Clinical cut-off</u>**:**
   See assay cut-off.
5. <u>Expected values/Reference range:</u>
   Expected value in the normal population is negative.

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.