510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k062787

B. Purpose for Submission:
New Device

C. Measurand:
Anti-tissue transglutaminase (tTG) IgA Antibodies

D. Type of Test:
Semi-quantitative fluoroenzyme immunoassay

E. Applicant:
Phadia US, Inc.

F. Proprietary and Established Names:
EliA™ Celikey IgA Immunoassay

G. Regulatory Information:
1. Regulation section:
   21 CFR §866.5660 Multiple autoantibodies immunological test system
2. Classification:
   Class II
3. Product code:
   MVM, Autoantibodies, endomysial (tissue transglutaminase)
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   EliA™ Celikey IgA is intended for the in vitro semi-quantitative measurement of IgA antibodies directed to tissue transglutaminase (tTG) in human serum and plasma (EDTA, citrate). EliA Celikey IgA is based on recombinant human tissue transglutaminase as antigen and is useful as an aid in the clinical diagnosis of patients with celiac disease in conjunction with other laboratory and clinical findings. EliA Celikey IgA uses the EliA IgA method on the instrument ImmunoCAP 100 and ImmunoCAP 250.
2. Indication(s) for use:
   Same as intended use.
3. Special conditions for use statement(s):
   For prescription use only.
4. Special instrument requirements:
   ImmunoCAP 100 and ImmunoCAP 250 (k061165)

I. Device Description:
The EliA reagents are available as modular packages, each purchased separately. The EliA Celikey IgA wells are coated with human recombinant tissue transglutaminase (tTG). These are packed in carriers which are stored in sealed aluminum foil bags containing a desiccant. The EliA Method-Specific reagents consists of: six levels of ready to use EliA IgA calibrators (0, 0.3, 1.5, 15, 80 μg/L); IgA calibrator well (coated with mouse monoclonal antibodies); ready to use positive and negative

controls; ready to use IgA curve control (5 µg/L); IgA conjugate (ß-Galactosidase anti-IgA mouse monoclonal antibodies) in PBS; sample diluent concentrate (PBS with BSA); ready to use development solution (0.1% 4-Methylumbelliferyl-ß-D-galactoside); and stop solution (4% Sodium Carbonate).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Celikey® Tissue Transglutaminase IgA Antibody Assay
2. Predicate 510(k) number(s):
   k041174
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Devices
	EliA™ Celikey IgA	Celikey® Tissue Transglutaminase IgA Antibody Assay
Antigen	Human recombinant tTG	Same
Assay Type	ELISA	Same
Solid phase	Microwells	Same

Differences		
Item	Device	Predicate
Intended use	For the semi-quantitative measurement of IgA antibodies directed to tissue transglutaminase (tTG) in human serum and plasma (EDTA, citrate). EliA Celikey IgA is based on recombinant human tissue transglutaminase as antigen and is useful as an aid in the clinical diagnosis of patients with celiac disease in conjunction with other laboratory and clinical findings.	For the semi-quantitative and qualitative measurement of IgA antibodies directed to tissue transglutaminase (tTG) in human serum and plasma. Celikey is based on recombinant human tissue transglutaminase as antigen and is useful as an aid in the clinical diagnosis of patients with celiac disease.
Assay Format	Semi-quantitative	Semi-quantitative and Qualitative
Assay Platform	48 well determinations	96 well determinations
Instrumentation	ImmunoCAP 100 and ImmunoCap 250(fully automated analyzers)	Microplate reader with 620 nm filter
Signal	Fluorescence	Optical Density

Differences		
Item	Device	Predicate
Reagent concept	Modular reagent concept (test-method specific and general reagents)	All reagents in a single kit
Internal Controls	Positive and negative control sera available separately	Positive and negative control sera included in the kit
Calibration	Total IgA calibrator (0, 0.3, 1.5, 15, 80 µg/L Option to store curve up to 28 days and run curve controls (provided in kit) in each assay for calibration	Analyte specific (tTG antibody concentrations of 0, 3, 7, 16, 40, 100 U/mL) in each assay.
Conjugate	Anti-human IgA ß-galactosidase (mouse monoclonal antibodies)	Anti-human IgA horseradish peroxidase (goat)
Sample type and dilution	Serum or plasma (EDTA, citrate) at 1:100 by the instrument	Serum or plasma at 1:101 dilution manually
Development solution	4-Methylumbelliferyl-ß-D-galactoside	TMB Chromogen
Stop solution	4% Sodium Carbonate	0.5 M H_2SO_4 ready to use
Reaction temperature	37ºC controlled	Room temperature, 18-25 ºC
Cut-off	Semi-quantitative: 10.0 U/mL	Semi-quantitative: 8.0 U/mL; Qualitative: Ratio 1.4

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

L. Test Principle:
The EliA Celikey IgA wells are coated with human recombinant tTG. If present in the patient's specimen, antibodies to tTG bind to their specific antigen in the wells. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgA (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 the patient samples is compared directly to the response for IgA calibrators.

M. Performance Characteristics (if/when applicable):
  1. Analytical performance:
      a. *Precision/Reproducibility*:

The precision of the assay on ImmunoCAP 100 instrument was determined by testing four serum samples in duplicate on seven instruments in 23 runs on one EliA Celikey IgA well batch, together with one set of system reagent. Results showed that 2 samples positive with anti-tTG IgA (11.2-27.3U/mL) had ≤4.1 %CV and ≤6.8% CV for the intra-assay and inter-assay studies respectively; and 2 negative samples (1.0-7.9 U/mL) had ≤7.2 %CV and ≤13.3% CV for the intra-assay and inter-assay studies respectively (see below).

ImmunoCAP 100

Sample	Mean (U/mL)	Intra-assay (%CV)	Inter-assay (%CV)
1	1.0	7.2	13.3
2	7.9	4.4	4.6
3	11.2	4.1	6.0
4	27.3	2.9	6.8

The precision of the assay on ImmunoCAP 250 instrument was determined by testing four serum samples in duplicate on two instruments in 14 runs on one EliA Celikey IgA well batch, together with one set of system reagent. Results showed that 3 samples positive with anti-tTG IgA (11.0-126.1 U/mL) had ≤5.1 %CV and ≤4.4% CV for the intra-assay and inter-assay studies respectively; and one negative sample (0.4 U/mL) had 10.4 %CV and 8.1% CV for the intra-assay and inter-assay studies respectively (see below).

ImmunoCAP 250

Sample	Mean (U/mL)	Intra-assay (%CV)	Inter-assay (%CV)
1	0.4	10.4	8.1
2	11.0	4.8	2.8
3	15.6	4.1	1.6
4	126.1	5.1	4.4

b. *Linearity/assay reportable range:*
Linearity by dilution was tested using 5 anti-tTG IgA positive samples (20.5-72.8 µg/L). These samples were tested in duplicates for dilutions 1/100, 1/200, 1/400, 1/800 and 1/1600. The results of the dilutions were compared with their expected value. The ratio observed/expected values were calculated. Specifications for the ratio (O/E) were 0.8 and 1.2 within all sample dilutions. Samples 1 and 5 had results of 1.2-1.4, which were slightly above 1.2. The following sentence had been included in package insert: "Please note that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the measuring range".

Hook Effect:
The possibility of antigen excess occurring when using the device was evaluated with serum sample above the assay measuring range. The measuring range for EliA Celikey IgA is from 0.1 to ≥128 EliA U/mL. No

hook effect was observed for concentrations up to 654 µg/L, which is 8 fold above the measuring range.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   The EliA IgA calibrators are traceable (via unbroken of calibrations) to the International Reference Preparation (IRP) 67/86 of the Human Serum Immunoglobulins A, G, M from WHO. New batches of IgA 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). There are no international standards for tTG antibodies. The positive and negative controls are prepared in house and arbitrary EliA Units/mL are assigned during the development process.

d. *Detection limit:*
   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 EliA IgA Calibrator wells. The results in Response Units (RU) were compared with the result of the sample diluent on EliA Celikey IgA wells. All the samples were measured in triplicates. The discrimination ability 'D' of the assay should be >2 for calibrator 0.3 diluted 1:4 (i.e. 0.075 µg/L IgA/L). 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 Celikey IgA wells (as shown in the table below). The lower limit of detection was set at 0.05 µg/L corresponding to 0.1 EliA U/mL.

Results on Calibrator Wells			
Sample ID	Mean Response Units RU	SD	D*
Calibrator 0.3 1/2	109	1.9	37.3
Calibrator 0.3 1/4	67	8.9	7.2
Calibrator 0.3 1/8 (0.0375µg/L)	43	4.2	8.8
Results on Celikey IgA Wells			
Sample ID	Mean RU	SD	
Sample Diluent	1	2.2	

e. *Analytical specificity:*
   <u>Interfering substances</u>

   The potential interferent and the corresponding blanks were added to two anti-tTG positive sera from patients with Celiac disease. The spiked samples were tested in triplicate. The ratio of the result of the sample spiked with the interfering substances and the sample spiked with a buffer blank was determined as shown in the table below:

Additive	blank/spiked sample	Positive Sample			Low Positive Sample		
		Conc. [µg/l]	CV %	Ratio	Conc. [µg/l]	CV %	Ratio
Bilirubin F	blank	29.8	2.0	1.04	6.9	1.3	1.00
	sample	30.9	3.1		6.9	0.9	
Bilirubin C	blank	28.7	3.6	1.04	6.8	1.7	1.01
	sample	29.8	5.6		6.9	2.4	
Haemoglobin	blank	31.7	3.6	0.93	7.1	4.6	0.97
	sample	29.3	2.4		6.9	2.9	
Chyle	blank	30.4	6.3	1.03	7.0	4.5	1.00
	sample	31.4	5.9		7.0	0.4	
Rheumatoid factor	blank	33.5	4.6	0.98	7.1	3.2	1.01
	sample	32.7	3.9		7.2	4.7	

The interfering substances Bilirubin C, Bilirubin F, Chyle, Hemoglobin and Rheumatoid Factor did not adversely affect the results of the new device.

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 age and gender.
The results were equally distributed and not dependent on age or gender. The 99th percentile (7.4 U/mL) lies below the lower limit of the equivocal range (7-10 U/mL).

2. Comparison studies:
a. *Method comparison with predicate:*
Testing was performed on 244 samples from clinically defined patients (93 celiac, 101 non-celiac normal biopsy, 10 Ulcerative Colitis, 10 Morbus Crohn, 20 Rheumatoid Arthritis, 10 Rheumatoid factor positive samples. Equivocals were excluded from the percent agreement calculations. The Positive Percent Agreement was 100% (89/89); the Negative Percent Agreement was 100% (144/144); and the Overall Agreement was 100% (233/233) (see table below).

		Celikey IgA on Varelisa™			
		Positive	Equivocal*	Negative	Total
EliA Celikey IgA	Positive	89	6	0	95
	Equivocal*	0	3	1	4
	Negative	0	1	144	145
	Total	89	10	145	244

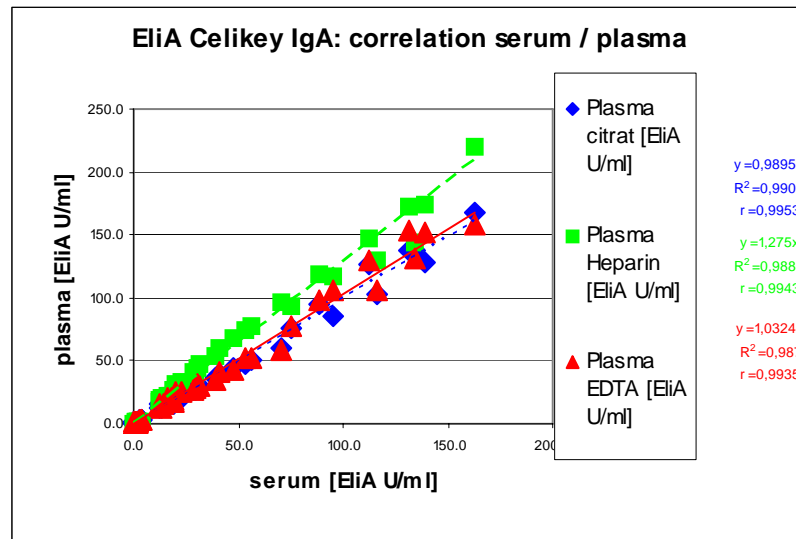*Equivocal results were excluded from the analysis

Positive percent agreement =   100% (89/89)
Negative Percent Agreement = 100% (144/144)
Overall Agreement =   100% (233/233)
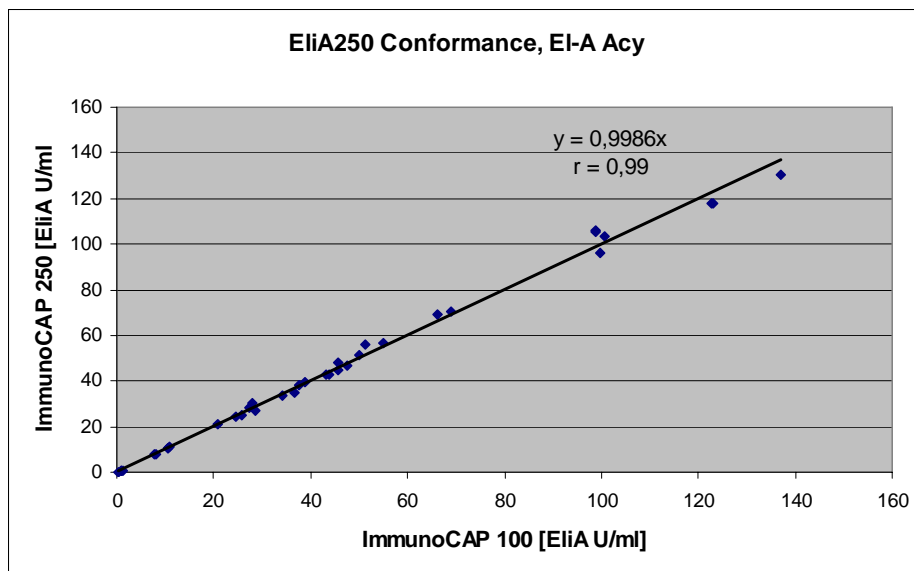
b. *Matrix comparison:*

The assay uses serum and plasma (EDTA, citrate) as sample matrices. Sixty sets of samples from different donors were tested. Each set contained serum, EDTA, heparin, and citrate plasma samples. Specifications were as follows: mean quota of plasma to serum concentration should be 0.8-1.2 for positive sera and negative samples should not switch to positive in all serum and plasma sample. Linear regression comparing the quota between serum and each type of plasma for the samples was performed as shown below:

EliA Celikey IgA: correlation serum / plasma

Legend:
- Plasma citrat [EliA U/ml] — $y = 0{,}9895x$, $R^2 = 0{,}9907$, $r = 0{,}9953$
- Plasma Heparin [EliA U/ml] — $y = 1{,}275x$, $R^2 = 0{,}9886$, $r = 0{,}9943$
- Plasma EDTA [EliA U/ml] — $y = 1{,}0324x$, $R^2 = 0{,}987$, $r = 0{,}9935$

The specification for the positive samples in this study was fulfilled for EDTA (1.04) and citrate (0.98), but not for heparin (1.37). All negative samples remain negative in all serum and plasma samples. The following sentence was included in package insert Specimen Collection section: "the use of plasma preparations with heparin is not recommended because heparin interferes with the measurement of tTG antibodies".

c. Instrument Platform comparison:

For this comparison study, a total of 36 samples distributed over the measuring range were assayed as follows: 4 negative samples, 2 equivocal samples and 30 positive samples. All samples were run on three ImmunoCAP250 instruments and three ImmunoCAP100 instruments in two runs and in single replicates. Specification for correlation was fulfilled (r >0.9). Results of the conformance study are summarized in the figure below:

EliA250 Conformance, El-A Acy

$y = 0,9986x$
$r = 0,99$

X-axis: ImmunoCAP 100 [EliA U/ml]
Y-axis: ImmunoCAP 250 [EliA U/ml]

3. Clinical studies:
   a. *Clinical Sensitivity and Specificity*:
   Ninety three (93) patients with known diagnosis of Celiac Disease (CD) and 101 non-CD patients with normal biopsy were tested for this study. Results showed clinical sensitivity of 95.7% (89/93); (95% CI 89.4-98.8%) and clinical specificity of 95% (96/101); (95% CI 88.8-98.4%) as shown in the table below:

		Diagnosis		
n=194		Positive (Celiac disease)	Negative (Disease Controls)	Total
EliA Celikey IgA	Positive	89	5	94
	Negative	4	96	100
	Total	93	101	194

Sensitivity: 95.7% (89/93); 95% CI 89.4-98.8%
Specificity: 95.0% (96/101); (95% CI 88.8-98.4%)

   b. *Other clinical supportive data (when a. is not applicable)*:
   Not applicable.
4. Clinical cut-off**:**
   Same as assay cut-off.
5. Expected values/Reference range:
   Expected values in the normal population should be 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.