510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k061165

B. Purpose for Submission:
New device

C. Measurand:
Anti-cyclic citrullinated peptides (CCP)

D. Type of Test:
Semi-quantitative fluoroenzymeimmunoassay

E. Applicant:
Phadia US, Inc. (formerly Sweden Diagnostics (US), Inc.)

F. Proprietary and Established Names:
EliA CCP, EliA CCP Control

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5775 Rheumatoid Factor Immunological Test System
2. Classification:
   Class II
3. Product code:
   NHX, Antibodies, Anti-cyclic citrullinated peptide
4. Panel:
   Immunology 82

H. Intended Use:

1. Intended use(s):
   EliA CCP is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to (CCP) in human serum and plasma. The presence of anti-CCP antibodies can be used in conjunction with clinical findings and other laboratory tests as an aid in the clinical diagnosis of rheumatoid arthritis (RA). EliA CCP uses the EliA IgG method on the instrument ImmunoCAP 100 [or ImmunoCAP 250].
2. Indication(s) for use:
   Same as Intended Use.
3. Special conditions for use statement(s):
   For prescription use
4. Special instrument requirements:
   ImmunoCAP 100 or ImmunoCAP 250

I. Device Description:

The device components consist of: CCP Wells (coated with citrullinated synthetic peptides); CCP Controls: Negative, and Positive containing IgG antibodies to CCP; Sample Diluent; IgG Conjugate (β-Galactosidase anti-IgG mouse monoclonal antibodies; IgG Calibrators (0, 4, 10, 20, 100, 600 µg/L); IgG Curve Control; IgG Calibrator Well (coated with mouse monoclonal antibodies); Dummy Well; General Reagents (Development Solution of 1% 4-methylumbelliferyl-β-D-galactoside, Stop

Solution of 4% sodium carbonate, and ImmunoCAP Washing Solution).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Axis Shield Diagnostics, Ltd. DIASTAT Anti-CCP
2. Predicate 510(k) number(s):
   k023285
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	EliA CCP	**DIASTAT Anti-CCP**
Intended Use	Semi-quantitative measurement of IgG antibodies directed to CCP in human serum and plasma as an aid in the clinical diagnosis of RA	Semi-quantitative/ qualitative ELISA for the detection of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum and plasma as an aid in the diagnosis of RA
Antigen	Synthetic citrullinated peptide	Same
Controls	Positive and negative	Same
Solid phase	Microwells	Same

Differences		
Item	Device	Predicate
	EliA CCP	**DIASTAT Anti-CCP**
Method	Fluoroenzyme-immunoassay	Enzyme-linked immunosorbent assay
Instrumentation	ImmunoCAP 100 or ImmunoCAP 250	ELISA plate reader with 550 nm filter
Conjugated antibody	β-Galactosidase anti-IgG mouse monoclonal antibodies	Alkaline phosphatase-labeled murine monoclonal antibody to human IgG
Reference range	<7 U/mL = negative 7-10 U/mL = equivocal >10 U/mL = positive	Qualitative: absorbance ratio of <0.95 = negative; ≥0.95- ≤1.0 = borderline; > 1.0 = positive Semi-quantitative: ≤5 U/mL = negative >5 U/mL = positive
Substrate	4-methylumbelliferyl-β-D-galactoside	Phenolphthalein monophosphate
Calibration	Total IgG calibrators (0, 4, 10, 20, 100, 600 µg/L)	Anti-CCP standards (0, 2, 8, 30, 100 U/mL)

Differences		
Item	Device	Predicate
Signal	Fluorescence	Optical density
Sample diluent	PBS containing BSA, detergent and sodium azide – ready to use	Phosphate buffer, protein stabilizer with sodium azide – 5X, dilute before use
Stop solution	Sodium carbonate	Sodium hydroxide

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

"Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices"

L. Test Principle:

The EliA CCP Wells are coated with citrullinated synthetic peptides. If present in the patient's specimen, antibodies to CCP bind to their specific antigen. 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:

Three positive samples were measured in duplicate on one instrument in 6 runs on 3 different CCP well batches together with 3 different conjugate batches. The studies yielded the following:

Sample	Mean (U/mL)	Inter-run (CV %)	Intra-run (CV %)
1	18.3	7.0	2.7
2	90.7	5.1	2.6
3	419	6.6	5.4

b. Linearity/assay reportable range:

Linearity by dilution was tested by using 5 high positive anti-CCP samples. The samples were tested in duplicates for dilutions at 1:2, 1:4, 1:8, and 1:16. The results of the dilutions were compared with their expected values and the ratios of observed-to-expected values were calculated. The following ratios were obtained.

	Mean concentration (U/mL)	O/E (ratio)
Sample 1	283.9	1.00
1:2	166.7	1.17
1:4	79.9	1.13
1:8	40.7	1.15
1:16	21.1	1.19

	Mean concentration (U/mL)	O/E (ratio)
Sample 2	287.8	1.00
1:2	155.2	1.08
1:4	73.3	1.02
1:8	40.6	1.13
1:16	20.4	1.14
Sample 3	238.1	1.00
1:2	129.2	1.09
1:4	69.3	1.16
1:8	36.4	1.22
1:16	17.4	1.17
Sample 4	74.6	1.00
1:2	39.5	1.06
1:4	20.0	1.07
1:8	10.7	1.14
1:16	5.7	1.22
Sample 5	130.2	1.00
1:2	68.8	1.06
1:4	36.4	1.12
1:8	19.0	1.17
1:16	9.6	1.18

Two of the calculated ratios were above the specifications for this study. The package insert includes a statement that not all sera can be diluted linearly within the measuring range of the assay due to differing binding characteristics of the antibodies in the patient samples. The assay measuring range for the EliA CCP is from 0.4 to ≥ 340 EliA U/mL/

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The IgG calibrators are traceable (via an unbroken chain of calibrations) to the International Reference Preparation (IRP) 67/86 of Human Serum 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).

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

Results on Calibrator Wells		
Sample ID	Mean Response Units (RU)	SD
Sample Diluent	31	-
Calibrator 4.0	316	-
Calibrator 4.0 at 1:2	127	-

Results on Calibrator Wells		
Calibrator 4.0 at 1:4	89	-
Calibrator 4.0 at 1:8	61	1.47
Results on CCP Wells		
Sample ID	Mean RU	SD
Sample Diluent	20	10.6

The discrimination ability was 3.8 and the 1:8 dilution of the 4 µg/L calibrator could be discriminated from background. The lower limit of the measuring range was set at 0.5 µg/L, corresponding to 0.4 EliA U/mL.

e. Hook effect:

Hook effect was analyzed by using dilutions from a highly positive anti-CCP serum sample with an estimated anti-CCP IgG concentration around 4400 U/mL. The sample was measured in triplicate.

Results on Calibrator Wells					
Sample ID	Mean RU	CV%	Mean conc. µg/L	CV%	
Cal-600	17533	0.2	600.9	0.6	
Results on CCP Wells					
Sample	Mean RU	CV%	Mean conc. µg/L	CV%	Estimated conc. U/mL
1:25	22236	2.5	Over		4438
1:100	20882	2.1	Over		1109
1:200	17766	7.5	Over		555
1:400	13858	1.1	251.8	2.0	277

Dilutions showed that no hook effect occurred for this sample. The discrimination ability was 8.4 for the sample compared to the response received for Calibrator 600.

f. Analytical specificity:

The potential interferant and the corresponding blanks were added to two anti-CCP positive sera from patients with RA. 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 ratio of the result of the sample spiked with the interfering substance and

the sample spiked with a buffer blank was determined:

		Positive Sample			Low Positive Sample		
Additive	Blank/spiked sample	Conc. (U/mL)	CV %	**Ratio**	Conc. (U/mL)	CV %	**Ratio**
Bilirubin C	Blank	67.1	8.4	0.98	13.4	6.2	1.01
	Sample	65.6	0.4		13.5	8.3	
Bilirubin F	Blank	61.0	2.9	1.03	12.4	4.2	0.95
	Sample	62.9	2.6		11.8	13.5	
Hemoglobin	Blank	63.6	1.6	0.92	12.6	0.7	0.93
	Sample	58.5	12.0		11.7	6.7	
Chyle	Blank	64.3	4.3	1.01	13.0	8.5	1.02
	Sample	64.7	3.7		13.2	10.0	
RF	Blank	62.5	4.8	0.96	13.5	9.5	0.90
	sample	59.8	4.9		12.2	2.1	

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

g. 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.

	EliA U/mL
Mean	2.6
Median	2.3
Mean + 2 SD	7.6
Mean + 3 SD	10.1
95th percentile	4.3
99th percentile	6.2

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

2. Comparison studies:

a. Method comparison with predicate device:

Samples from 55 anti-CCP positive RA patients and 25 non-RA sample were tested.

	N = 80	**Diastat anti-CCP**		
		Positive	Negative	Total
EliA CCP	Positive	51	3	54
	Negative	1	25	26
	Total	52	28	80

Positive percent agreement 51/52 x 100 = 98.1%
Negative percent agreement 25/28 x 100 = 89.3%
Overall percent agreement 76/80 x 100 = 95%

b. Matrix comparison:

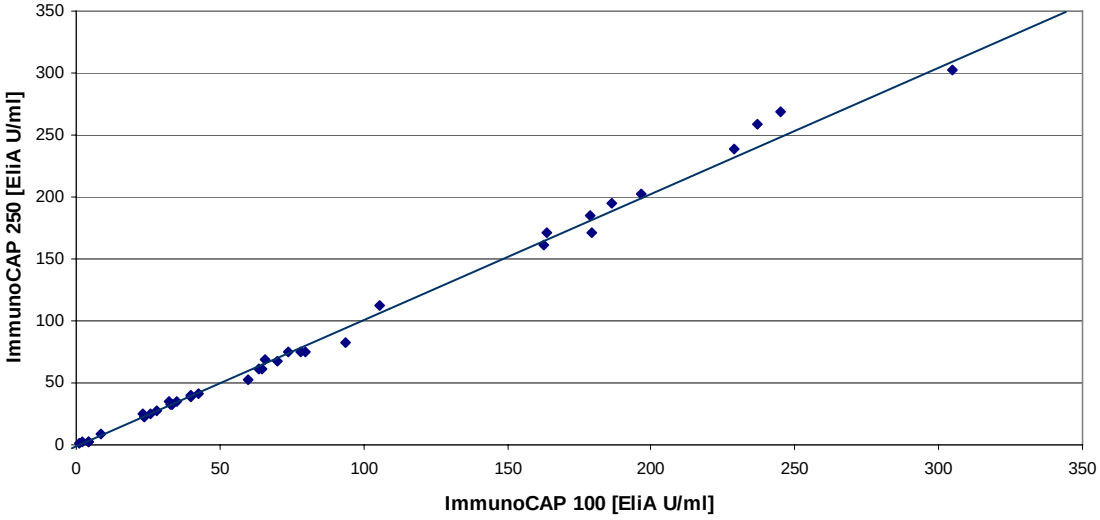
Fifty sets of samples from different donors were tested in double determinations. Each set contained serum, EDTA, heparin and citrate plasma samples taken at the same time from a single donor. All samples were tested and found to be negative for anti-CCP antibodies in all 4 sample types. Antibodies were spiked into 25 of the sample sets to create 25 positive sample sets. Linear regression comparing the quotas between serum and each type of plasma for the positive samples was performed and showed:

Plasma	**r value**	**Slope**
Heparin	0.9966	0.9861
EDTA	0.9975	0.9749
Citrate	0.9987	0.9912

The data show acceptable results for plasma versus serum in positive and negative samples.

c. Instrument platform comparison

For this comparison study, a total of 36 samples distributed over the measuring range were assayed: 4 negative samples, 1 equivocal sample and 31 positive samples. All samples were run on three ImmunoCAP 100 instruments and three ImmunoCAP 250 instruments in two runs and in single replicates. Results of the conformance study are summarized in the figure below:



Correlation Result: y=0.99x+1.0275 where x is ImmunoCAP 100 and y is ImmunoCAP 250

3. Clinical studies:
   a. *Clinical sensitivity and specificity:*
      The clinical studies were performed with 534 sera from subjects with clinically confirmed RA (n=82) and 452 other diseases (listed below).

Other disease groups	**No. of patients**	**No. of positives**
CTD		
UCTD	11	3
SLE	30	0
Scleroderma	39	3
Sjögren's syndrome	20	3
MCTD	10	0
Myositis	10	0
Total	120	9
Infectious diseases		
CMV	25	0
EBV	30	1
HBV	10	0
HCV	10	0
HIV	15	0
Other infectious diseases	95	2
Total	185	3
Other		
Osteoarthritis	35	0
Vasculitis	21	0
Autoimmune thyroid diseases	20	0
Crohn's disease	20	0
Ulcerative colitis	20	0
Other misc.	31	3
Total	147	3

Study results:

		Rheumatoid Arthritis		
		Positive	Negative	Total
EliA CCP result	Positive	72	15	87
	Negative	10	437	447
	Total	82	452	534

Clinical sensitivity: 87.8% (72/82)
Clinical specificity: 96.7% (437/452)
Overall agreement: 95.3% (509/534)

b. Other clinical supportive data (when a. is not applicable):
Not applicable

4. Clinical cut-off**:**
   See assay cut-off.
5. Expected values/Reference range:
   The expected value in the normal population is negative. The proportion of sera found positive for anti-CCP antibodies using the EliA CCP assay in the adult normal population tested was less than 1%.

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.