510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k112414

B. Purpose for Submission:
New device

C. Measurand:
IgA Antibodies to β2-Glycoprotein I

D. Type of Test:
Semi-quantitative immunofluorescence assay

E. Applicant:
Phadia US Inc.

F. Proprietary and Established Names:
EliA™ β2-Glycoprotein I IgA Immunoassay

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5660, Multiple Autoantibodies Immunological Test System

2. Classification:
   Class II

3. Product code:
   MSV, System, Test, Antibodies, β2-Glycoprotein I (β2-GPI)

4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   EliA™ ß2-Glycoprotein I IgA is intended for the in vitro semi-quantitative measurement of IgA antibodies directed to ß2-Glycoprotein I in human serum and plasma (heparin, EDTA, citrate) to aid in the diagnosis of antiphospholipid syndrome (APS) as well as thrombotic disorders related to systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA™ ß2-Glycoprotein I IgA uses the EliA IgA method on the instruments Phadia® 100 and Phadia® 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:
   Phadia® 100 and Phadia® 250 (formerly known as ImmunoCAP 100 and 250) (k061165)

I. Device Description:

The method specific reagents on Phadia® 100 and Phadia® 250 are identical; they are only filled in different containers.
Each device consists of:

- β2-glycoprotein I IgA wells are coated with human β2-glycoprotein I antigen – 2 carriers (12 wells each), ready to use;
- EliA method-specific sample diluent, PBS containing BSA, detergent and 0.095% sodium azide – 6 vials, 9 mL each, ready to use;
- EliA IgA conjugate, β-galactosidase labeled anti-IgA (mouse monoclonal antibodies) – 2 or 6 vials, 4.8 mL each, ready to use;
- Negative control: containing normal human serum from healthy donors – 6 single-use vials, 0.3 mL each, ready to use;
- EliA calibrators, human IgA in PBS (0, 0.3, 1.5, 5, 15, 80 µg/L) – 6 single-use vials, 0.3 mL each, ready to use;
- IgA curve control, human IgA (5µg/L) in PBS, 6 single-use vials, 0.3 mL each, ready to use.

The Phadia EliA™ Immunodiagnostic System is automated system for immunodiagnostic testing. The EliA™ reagents are available as modular packages, each purchased separately. All packages except the positive and negative controls are required to carry out an EliA™ β2-Glycoprotein I IgA Test.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
   Valerisa β2-Glycoprotein I IgA Antibodies, k040450

2. Comparison with predicate:

Similarities		
Item	New EliA™ Device	Predicate Varelisa IgA Device
Intended Use/Indications for Use	A semi-quantitative measurement of IgA antibodies directed to ß2-Glycoprotein I in human serum and plasma (heparin, EDTA, citrate) to aid in the diagnosis of antiphospholipid syndrome (APS) as well as thrombotic	Same

Similarities		
Item	New EliA™ Device	Predicate Varelisa IgA Device
	disorders related to systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings.	
Controls	Negative Control sera provided in a separate package	Same
Assay Type	ELISA	Same
Type of Test	Semi-quantitative	Semi-quantitative and qualitative
Antigen	Human purified ß2-Glycoprotein I	Same
Solid phase	Microwells	Same

Differences		
Item	New EliA™ Device	Predicate Varelisa IgA Device
Instrumentation	Phadia® 100 and 250 automated immunoassay analyzers	ELISA reader
Reaction Temperature	37°C controlled	Room temperature (18-25°C)
Detection Antibody (conjugate)	IgA Conjugate: anti-human IgA ß-Galactosidase (mouse monoclonal antibodies)	IgA Conjugate: anti-human IgA horse-radish peroxidase
Signal	Fluorescence	Optical density
Calibration	Total IgA calibration	Analyte-specific IgA calibration
Calibrators	0, 0.3, 1.5, 5, 15, 80 µg/L	0, 4, 8, 20, 40, 100 U/mL
Calibration Curve	Human IgA, 5 µg/L Option to store curve up to 28 days and run curve controls in each assay for calibration	Calibration curve in each assay
Measuring Range	0.3 – 183 U/mL	1 – 100 U/mL
IgA Cut-off	Negative <7 U/mL Equivocal 7 – 10 U/mL Positive >10 U/mL	Negative <10 U/mL Equivocal 10 – 15 U/mL Positive >15 U/mL
Concept	Modular reagents concept (test-method specific and general reagents)	All reagents in a single kit

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

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantification, Approved Guideline.

L. Test Principle:

The EliA™ ß2-glycoprotein I IgA wells are coated with purified human ß2-glycoprotein I. If present in the patient's specimen, antibodies bind to their specific antigen. 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 value of fluorescent signal detected by the instrument, the higher the amount of antibody bound and detected in the sample tested. 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:

To determine the precision of the assays on Phadia® 100 and Phadia® 250 instrument, the variability was assessed on 8 samples. Each sample was run in 4 replicates for 7 days on 3 instruments. One batch was used to determine the precision of the assays on Phadia® 100 (equal to 84 replicate determinations per sample). Three batches were used to determine the precision of the assays on Phadia® 250 (equal to 252 replicate determinations per sample). The results are summarized in the tables below:

EliA™ ß2-Glycoprotein I IgA on Phadia® 100 (n=84)

Mean value U/mL	Intra-Run CV%	Inter-Run CV%	Total Imprecision %CV
9.7	2.7	3.0	4.0
25.7	2.9	3.4	4.4
135.9	3.3	5.3	6.3
7.0	3.2	3.1	4.6
7.4	2.7	3.7	4.5
8.4	4.2	0.9	4.3
10.3	3.0	2.3	3.8
3.7	12.6	4.6	13.4

EliA™ ß2-Glycoprotein I IgA on Phadia® 250 (n = 252)

Mean value U/mL	Intra Run CV%	Inter Run CV%	Lot to Lot CV%	Total Imprecision %CV
10.4	3.9	1.4	3.0	4.1
27.7	3.9	1.8	4.0	4.3
146.6	5.2	3.7	4.6	6.3
7.0*	2.3	2.1	not done	3.1

7.4*	2.8	2.0	not done	3.6
8.8	4.9	1.6	1.6	5.2
10.8	3.9	1.4	4.2	4.1
3.9	9.7	6.9	9.4	11.9

* n = 84

b. *Linearity/assay reportable range:*
Eight patient serum samples were diluted in sample diluent and tested with one batch of EliA™ β2-Glycoprotein I IgA and one set of system reagents on Phadia® 100 or Phadia® 250. The ratios of observed/expected values were calculated. Depending on the lot specific EliA™ well factor, the upper limit of the measuring range may vary between different solid phase batches. The results are summarized below:

EliA™ β2-Glycoprotein I IgA on Phadia® 100

Dilution range (U/mL)	Slope	Intercept	R^2
57.5 – 0.9	0.99	-0.28	0.998
32.5 - 0.6	0.99	0.39	1.000
116.7 – 3.9	0.94	-1.63	0.999
39.0 – 1.2	1.02	-0.62	0.999
9.2 – 0.3	1.03	0.02	0.997
14.9 – 0.2	1.02	0.05	0.996
191.6 – 67.9	1.10	6.13	0.991

The claimed linear range for β2-Glycoprotein I IgA is 0.3 – 183 U/mL

EliA™ β2-Glycoprotein I IgA on Phadia® 250

Dilution range (APL-U/mL)	Slope	Intercept	R^2
68.0 – 1.0	1.01	-0.62	1.000
41.3 – 0.6	1.07	0.37	0.999
106.6 – 1.8	0.94	0.12	1.000
96.1 -1.6	0.93	0.58	0.999
9.7 – 0.5	1.03	0.04	0.998
9.2 – 0.6	1.04	-0.17	1.000
215.4 – 75.3	0.90	18.78	0.987

The claimed linear range for β2-Glycoprotein I IgA is 0.3 – 183 U/mL

Hook Effect/Over the Range Results:
Hook effect was investigated by using a serum sample above the highest calibration point. A high positive sample was diluted and the dilutions were measured in four replicates and compared to Cal-80 (80 µg/L about 180 U/mL).

No hook effect was observed for concentrations up to 2877.2 U/mL.

Results above the upper limit of the measuring range are reported as "above". No recommendations are made for dilution of samples outside measuring range in the

Package Insert.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability:
The IgA calibrators are traceable (via 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 IgA calibrators are compared to a secondary standard (standardized with the IRP) or the IRP directly and adjusted accordingly to meet the correct concentration.

The instrument measures specific IgA concentrations in µg/L. By using a conversion factor given by the lot-specific code of the EliA™ ß2-Glycoprotein I IgA Well, the results are automatically converted to U/mL.

Stability:
The EliA™ IgA system reagents, EliA™ APS Positive Control and EliA™ Negative Control are already FDA cleared under k063775, k091845 and k072393, respectively.

Shelf life:
Accelerated study was done to determine the shelf life EliA™ β2-Glycoprotein I IgA wells and was determined to be 24 months. Real time stability test is ongoing.

On board stability:
The onboard stability EliA™ β2-Glycoprotein I IgA carriers (containing the antigen coated wells) was tested over 6 weeks using 3 positive and 2 negative samples only on the Phadia® 250 instrument since for Phadia® 100 the reagents are stored off the instrument and are only loaded as needed for an assay. The on-board stability for the Phadia® 250 instrument was determined to be 28 days at 2-8°C.

Open Stability:
Stability of the foilbag containing the EliA™ wells after first opening was tested using k082759 tTG test and determined to be 9 months at 2-8°C.

d. *Detection limit:*
The limit of blank (LoB) and limit of detection (LoD) studies were done on both Phadia® 100 and Phadia® 250. One negative and five blood donors with low antibody concentration were measured in twelve replicates in each of six runs on six different days (12 replicates x 6 runs x 6 days = 432 replicates per sample). On Phadia® 250 the runs are spread on three instruments, with two runs per instrument. On Phadia® 100 there is one run per instrument. The results are summarized in the table below:

EliA™ β2-Glycoprotein I IgA (U/mL)	LoB	LoD
Phadia® 100	0.17	0.25
Phadia® 250	0.14	0.19

It was decided to use a single LoD of 0.3 U/mL for the study.

e. Analytical specificity:

Endogenous Interference: A study was run to investigate whether high concentrations of potentially interfering substances in serum, like bilirubin, hemoglobin, lipemic factor and rheumatoid factor adversely affect the results of the new devices. Three serum samples were prediluted in EliA™ Sample Diluent and spiked with the different interfering substances or their respective blank solutions, and analyzed in triplicates. A calibration curve was run in duplicate. The runs were repeated twice. One batch of EliA™ antigen wells and one batch of system reagents were used throughout the studies.

Samples with concentrations around 6.5, 10 and 47 U/mL were tested. The ratio of blank/spiked sample was 0.89 – 1.04. No interference was observed up to the concentrations listed in the table below:

Potential Interfering Compound	Concentration
Bilirubin F	20.6 mg/dL
Bilirubin C	21.1 mg/dL
Hemoglobin	519 mg/dL
Lipemic factor	1%
Rheumatoid factor	500 IU/mL

Carry-over: A study was carried out on two Phadia® 250 instruments using the test EliA Celikey IgA, cleared under k062787 (for tTG). A serum sample was diluted 1:100 using the instrument dilution and manual dilution. No carry over effect was observed.

f. Assay cut-off:

A study was done on 400 apparently healthy blood donor samples from Caucasian individuals equally distributed by sex and age in order to evaluate expected values for each of the antigens in the submission in the normal population and to confirm the defined cut-off. The samples were measured on the Phadia® 250 instrument. The 99th percentile of the 400 samples were calculated and taken into account for setting of the cut-off. The following values were selected for the cut-off:

EliA™ β2-Glycoprotein I IgA	
<7 U/mL	Negative
7 – 10 U/mL	Equivical
>10 U/mL	Positive

2. Comparison studies:

a. Method comparison with predicate device:

A total of 424 serum samples were collected in a laboratory specialized in APS diagnosis from patients with APS (n = 124), Systemic Lupus Erythematosus (SLE; n = 100), viral infections (n = 60), mixed connective tissue diseases (MCTD; n = 50),

rheumatoid arthritis (n = 40), non-viral infections (n = 20), syphilis (n = 20) or cancer (n = 10). Samples were analyzed with the EliA™ ß2-Glycoprotein I IgA and Varelisa ß2-Glycoprotein I IgA assays. Additional 88 samples with known antibody status and unconfirmed clinical diagnosis were analyzed with ß2-Glycoprotein I IgA. The test was run in single determination and values outside the measuring range were excluded from statistical analyses. The results are summarized in the tables below:

EliA™ β2-Glycoprotein I – Equivocal results evaluated as negative:

		Valerisa β2-Glycoprotein I IgA (U/mL)		
		Positive >15	Negative <15	Total
EliA™ β2-Glycoprotein I IgA (U/mL)	Positive >10	85	33	118
	Negative ≤10	17	351	368
	Total	102	384	486

Positive percent agreement: 83.3% (85/102) (95% CI: 74.7% – 90.0%)
Negative percent agreement: 91.4% (351/384) (95% CI: 88.1% – 94.0%)
Total percent agreement: 89.7% [(85+351)/486] (95% CI: 86.7% – 92.3%)

EliA™ β2-Glycoprotein I – Equivocal results evaluated as positive:

		Valerisa β2-Glycoprotein I IgA (U/mL)		
		Positive >10	Negative <10	Total
EliA™ β2-Glycoprotein I IgA (U/mL)	Positive ≥7	118	37	155
	Negative <7	17	314	331
	Total	135	351	486

Positive percent agreement: 87.4% (118/135) (95% CI: 80.6% – 92.5%)
Negative percent agreement: 89.5% (314/351) (95% CI: 85.8% – 92.5%)
Total percent agreement : 88.9% [(118+314)/486] (95% CI: 85.8% – 91.5%)

b. Matrix comparison:

Serum, lithium heparin plasma, citrate plasma and EDTA plasma were collected from the same patients (n = 50) to demonstrate that the plasma results do not deviate from the corresponding serum results and are within the pre-defined specifications. Samples were spiked with a serum sample of high antibody titer to cover the measuring range. Samples were tested in duplicates. Passing & Bablok regression plots were generated using the first replicate only and by plotting the concentration observed from the control tube (serum) versus the concentration for each test collection tube. The corresponding slopes of regression and coefficient determination are summarized in the tables below:

	Range tested (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
Serum vs. citrate plasma	1.1 – 201.2	1.05 (0.99 – 1.07)	0.10 (-0.28 – 0.20)	1.00
Serum vs EDTA plasma	1.3 – 193.8	1.03 (0.99 – 1.07)	0.06 (-0.08 – 0.30)	1.00
Serum vs heparin plasma	1.5 – 196.8	1.04 (1.01 – 1.06)	-0.01 (-0.25 – 0.18)	1.00

c. *Instrument comparison*

Performance of EliA™ β2-Glycoprotein I IgA was evaluated on the Phadia® 100 and Phadia® 250 instruments using 32 positive and 4 negative samples. The samples were analyzed in six runs in single replicates on three Phadia®100 and three Phadia® 250 instruments, with 2 runs on each instrument. The regression analysis results are summarized as follows:

	Intercept	Slope
Estimate	-0.538	1.045
95% CI	-1.076 to 0.160	0.986 to 1.081

3. Clinical studies:

a. Clinical Sensitivity and specificity:

The clinical samples that were used for the method comparison were used to determine sensitivity and specificity of each assay. SLE samples with unknown APS status were also evaluated for their sensitivity/specificity. The results are summarized in the tables below:

EliA™ β2-Glycoprotein I IgA – diagnostic group APS - equivocal results evaluated as negative:

	Diagnostic Group – APS		
	+	-	total
Positive test >10 U/mL	55	7	62
Negative test ≤10 U/mL	69	193	262
Total	124	200	324

Sensitivity (95% CI): 44.4% (35.4% – 53.5%)
Specificity (95% CI): 96.5% (92.9% – 98.6%)

EliA™ β2-Glycoprotein I IgA – diagnostic group APS - equivocal results evaluated as positive:

	Diagnostic Group – APS		
	+	-	total
Positive test >7 U/mL	66	11	77
Negative test ≤7 U/mL	58	189	247
Total	124	200	324

Sensitivity (95% CI): 53.2% (44.1% – 62.2%)
Specificity (95% CI): 94.5% (90.4% – 97.2%)

EliA™ β2-Glycoprotein I IgA – diagnostic group SLE - equivocal results evaluated as negative:

	Diagnostic Group – SLE		
	+	-	total
Positive test > 10 U/mL	13	7	20
Negative test ≤10 U/mL	87	193	280
Total	100	200	300

Sensitivity (95% CI): 13.0% (7.1% – 21.2%)
Specificity (95% CI): 96.5% (92.9% – 98.6%)

EliA™ β2-Glycoprotein I IgA – diagnostic group SLE - Equivocal results evaluated as positive:

	Diagnostic Group – SLE		
	+	-	total
Positive test ≥7 U/mL	30	11	41
Negative test <7 U/mL	70	189	259
Total	100	200	300

Sensitivity (95% CI): 30.0% (21.2% – 40.0%)
Specificity (95% CI): 94.5% (90.4% – 97.2%)

The table below shows the results for each clinical subgroup:

Condition	Number of samples	No (%) pos on EliA™	No (%) pos. on predicate
APS	124	55 (44.4%)	37 (29.8%)
SLE	100	13 (13%)	16 (16%)
MCTD	50	1 (2%)	3 (6%)
Syphilis	20	1 (5%)	2 (10%)
Viral infection	60	3 (5%)	3 (5%)
Bacterial infections	20	1 (5%)	0 (0%)
RA	40	1 (2.5%)	0 (0%)
Cancer	10	0 (0%)	0 (0%)

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable

4. Clinical cut-off**:**
Same as assay cut-off

5. Expected values/Reference range:
The expected value in the normal population is negative. The sponsor states that the proportion of sera from a normal population found positive by the EliA™ ß2-Glycoprotein I IgA test is up to 3%. The proportions of positive sera in apparently healthy and asymptomatic individuals increase with age, and men tend to show higher values. Expected values may vary depending on the population tested.

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.