510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:
K181329

B. Purpose for Submission:
Adding previously cleared assays on a new instrument platform (Phadia 2500/5000)

C. Measurand:
IgA antibodies to ß-2-glycoprotein I
IgA autoantibodies to Cardiolipin

D. Type of Test:
Automated semi-quantitative solid-phase measurement immunoassays

E. Applicant:
Phadia US, Inc

F. Proprietary and Established Names:
EliA B2-Glycoprotein I IgA Immunoassay,
EliA Cardiolipin 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)
   MID System, Test, Anti-Cardiolipin Immunological

4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended uses:
   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 (Li-heparin, EDTA) 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 instrument Phadia 2500/5000.

EliA Cardiolipin IgA is intended for the in vitro semi-quantitative measurement of IgA antibodies directed to cardiolipin in human serum and plasma (Li-heparin, EDTA) 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 Cardiolipin IgA uses the EliA IgA method on the instrument Phadia 2500/5000.

2. Indications for use:
   Same as intended uses

3. Special conditions for use statements:
   For prescription use only

4. Special instrument requirements:
   Phadia 2500/5000 instrument.

I. Device Description:

- EliA β2-Glycoprotein I IgA wells are coated with human β2-Glycoprotein I antigen, ready to use
- EliA Cardiolipin IgA wells are coated with bovine cardiolipin antigen and bovine β2-glycoprotein I as co-factor, ready to use

EliA Method-Specific Reagents (Phadia 2500/5000):

- EliA Sample Diluent (yellow colored): PBS containing BSA, detergent and 0.095% (w/v) sodium azide, ready to use
- EliA IgA Conjugate (blue colored): β-Galactosidase labeled anti-IgA (mouse monoclonal antibodies) in PBS containing BSA and 0.06% (w/v) sodium azide, ready to use
- EliA IgA Calibrator Strips: Human IgA (0, 0.3, 1.1, 5, 15, 80 µg/L) in PBS containing BSA, detergent and 0.095% (w/v) sodium azide, ready to use
- EliA IgA Curve Control Strips: Human IgA (20 µg/L) in PBS containing BSA, detergent and 0.095% (w/v) sodium azide, ready to use;
- EliA IgA Calibrator Well: Coated with mouse monoclonal antibodies, ready to use

Phadia 2500/5000 General Reagents:

- Development Solution (0.01 %4-Methylumbelliferyl-β-D-galacto-side, <0.0010% preservative), ready for use
- Stop Solution (4% Sodium Carbonate), ready for use
- Dilution Wells, high density polyethylene wells ready for use
- Pipette Tips in Racks, ready for use
- Washing Solution (information in separate Washing Solution package insert).

EliA uses a modular reagent system. The EliA reagents are available as modular packages, each purchased separately. All packages are required to carry out an EliA β2-Glycoprotein I IgA test and the EliA Cardiolipin IgA Test.

J. Substantial Equivalence Information:

1. Predicate device names and 510(k) numbers:
   K112414, EliA B2-Glycoprotein I IgA Immunoassay
   K131821, EliA Cardiolipin IgA Immunoassay

2. Comparison with predicate:

Similarities		
Item	Device *Phadia 2500/5000	Predicate Phadia 250
Intended Use EliA ß-2-Glycoprotein I IgA	EliA ß-2-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 (Li-heparin, EDTA) 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.	Same
Intended Use EliA Cardiolipin IgA	EliA Cardiolipin IgA is intended for the in vitro semi-quantitative measurement of IgA antibodies directed to cardiolipin in human serum and plasma (Li-heparin, EDTA) 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.	Same
Signal	Immuno-fluorescence measurement	Same
Assay type	ELISA	Same
Type of test	Semi-quantitative	Same
Antigen ß-2 Glycoprotein I Cardiolipin	 Human purified ß-2 Glycoprotein I Purifyed bovine cardiolipin and bovine ß-2 Glycoprotein I	 Same Same
Common, dedicated Phadia reagents	Same Introduction of new article numbers for Development Solution, Stop Solution and Washing Solution is	Same

Similarities		
Item	Device *Phadia 2500/5000	Predicate Phadia 250
	only due to larger filling volumes which are required for the bigger instruments Phadia 2500/5000	
Time to 1st result	About 2 hours	Same
Detection antibody (conjugate)	β-Galactosidase labeled anti-IgA (mouse anti human monoclonal antibodies)	Same
Incubation temperature	37°C	Same
Sample matrix	Serum or plasma (heparin, EDTA)	Serum or plasma (heparin, EDTA, citrate)
Calibrator strips	Human IgA (0, 0.3, 1.5, 5, 15, 80 µg/L)	Same
Control	EliA IgA curve control strips, 5µg/L	Same
Measuring range	ß-2 Glycoprotein I: 0.3-183 U/mL Cardiolipin: 0.3-181 APL-U/mL	Same
Cutt-off	ß-2 Glycoprotein I: Negative <7 U/mL Equivocal 7-10 U/mL Positive >10 U/mL Cardiolipin: Negative <14 APL-U/mL Equivocal 14-10 APL-U/mL Positive >20 U/mL APL-U/mL	Same
Assay set-up	Random access	Same

Differences		
Item	Device	Predicate
Instrument	Instrument Phadia 2500/5000	Instrument Phadia 250
Sample dilution, pipets	Disposable Pipette Tips in Racks for pipetting samples in Dilution Well	Steel pipette to dilute the samples in Dilution Plates
Loading of EliA Carriers	The Phadia 2500/5000 instruments do not have Loading Tray. The EliA carriers are loaded into racks which are directly transferred to the cooled storage compartment	EliA carriers are loaded manually on the Loading Tray from where they can be processed directly or transferred to the cooled storage compartment.
Barcode reader	On the Phadia 2500/5000	The Phadia 250 instrument has a

Differences		
Item	Device	Predicate
	the reagents are on a moving belt which conveys them past the barcode reader. The lot-specific information will be read automatically by the instrument during loading.	built-in barcode reader at the front of the instrument, but the operator needs to scan the barcodes manually by showing the reagents to the barcode reader. Alternatively, the operator can also enter the characters below the barcode manually.
Process time / Time to patient result	Phadia 2500/5000 instruments process two Wells in parallel in 48 seconds. Phadia 2500/5000 provides the results at a 24 seconds interval.	Phadia 250 needs one minute to process one Well. Phadia 250 provides the results at a one minute interval.
Daily throughput	About 2500/5000 tests	About 250 tests

*Phadia 2500/5000 – Phadia 2500 and Phadia 5000 are identical instruments except for sample throughput. The difference is that Phadia 2500 consists of 1 process module (2 process lines) and Phadia 5000 consists of 2 process modules (2x2 process lines). For that reason Phadia uses the Phadia 2500 and Phadia 5000 instrument names interchangeably.

K. Standard/Guidance Document Referenced:

- EP17-A Protocols for Determination of Limits of Detection and Limits of Quantification
- EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures
- EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

L. Test Principle:

The EliA wells are molded cups comparable to excised wells from a microtiter plate. They are made of polystyrene and are coated with the respective antigen. The wells are at the same time a holder of the coupled antigen for convenient automation and a reaction chamber with reaction/washing solution handling based on pipetting to add and aspiration to remove liquids.

The EliA wells are coated with human β2-Glycoprotein I antigen or with bovine cardiolipin antigen. If present in the patient's specimen, antibodies to these proteins bind to the 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 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.

M. Performance Characteristics:

1. Analytical performance:
   All results presented below met the manufacturer's predetermined acceptance criteria

   a. Precision/Reproducibility:
   The precision of the assay was assessed in a study with a total of 21 runs, one run/day over a period of seven days. Each sample was tested in four replicates/run giving a total of 84 replicates per sample (3 instruments × 7 runs x 4 replicates = 84). The data was calculated against the calibration curve from Day 1. Four serum native patient serum samples were tested, the same samples were tested on all instruments.

   One lot of EliA β2-Glycoprotein I IgA and EliA Cardiolipin IgA was used in these studies because inter-lot-variation was deteremined in the predicate devices K112414 and K131821. The results for all three instruments are summarized in the tables below:

EliA β2-Glycoprotein I IgA on Phadia 2500/5000:

Mean (EliA U/mL)	Within-Run		Between-Run		Between-Instrument		Total Imprecision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1.6	0.1	8.0	0.2	9.8	0.4	23.0	0.4	26.2
8.0	0.3	3.7	0.3	3.5	0.3	4.2	0.5	6.6
10.4	0.4	3.7	0.5	5.0	0.4	3.4	0.7	7.1
42.8	1.4	3.3	2.2	5.2	1.2	2.8	2.9	6.7
136.6	6.6	4.8	10.3	7.5	5.1	3.7	13.2	9.7

EliA Cardiolipin IgA on Phadia 2500/5000:

Mean (APL-U/mL)	Within-Run		Between-Run		Between-Instrument		Total Imprecision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
2.1	0.2	7.2	0.2	7.9	0.3	15.1	0.4	18.5
13.5	0.8	5.6	0.3	2.3	0.2	1.4	0.8	6.2
19.4	0.7	3.9	1.1	5.5	0.6	3.0	1.4	7.4
93.0	5.4	5.8	1.4	1.5	4.8	5.2	7.4	7.9
153.0	10.7	7.0	12.0	7.9	8.5	5.5	18.2	11.9

   b. Linearity/assay reportable range:
   Four patient serum samples were diluted in sample diluent and tested in triplicates with one lot of EliA β2-Glycoprotein I IgA and EliA Cardiolipin IgA reagents and one set of system reagents on Phadia 2500/5000. The results of the dilutions were compared with their expected values. The ratio observed/expected (O/E) was calculated. Mean observed value was used in the calculation. The results of the linear regression analysis are summarized below:

EliA β2-Glycoprotein I IgA on Phadia 2500/5000

Dilution range (EliA U/mL)	Slope	Intercept	R^2
0.4 – 66.6	1.00	0.24	1.00
5.2 – 205.2	1.03	-0.12	1.00
6.4 – 242.8	1.02	-0.46	1.00
5.5 – 198.8	1.02	1.80	1.00

The reportable range (Limit of Detection to upper limit) for EliA ß2-Glycoprotein I IgA is from 0.3 to 183 U/mL. The measuring range (Limit of Quantitation, to upper limit) is from 1.1 to 183 U/mL.

EliA Cardiolipin IgA on Phadia 2500/5000

Dilution range (APL-U/mL)	Slope	Intercept	R^2
0.5 – 49.5	1.01	0.40	1.00
1.8 – 91.3	0.98	-0.25	1.00
2.4 – 147.0	0.98	-1.45	1.00
4.4 – 221.6	1.00	3.76	1.00

The reportable range (Limit of Detection to upper limit) for EliA Cardiolipin IgA is from 0.3 to 181 APL-U/mL. The measuring range (Limit of Quantitation to upper limit is from 1.0 to 181 APL-U/mL.

The following statements is included in the package inserts: "*Please note that concentration values between LoD and LoQ may show a higher uncertainty... 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*."

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
Traceability, stability, Expected values (controls, calibrators, or methods) for the EliA IgA method was previously reviewed in K062787.

Controls:
Controls are sold separately from the assay.

Traceability:
The IgA calibrators are traceable via unbroken ochain off calibrations to the International Reference Preparation (IRP) 67/86 of the Human Serum Immunoglobulins A, G, M from World Health Organization (WHO).

There are no international standards for ß2-Glycoprotein I antibodies. Results are given in arbitrary EliA Units/mL. The instrument measures specific IgA concentration in µg/mL. By using a conversion factor given by the lot-specific code of the EliA ß2-Glycoprotein I IgA Well, thte results are automatically converted to U/mL.

The standardization of the Cardiolipin IgA assay is adjusted to a set of established standard sera (Harris et al., 1987). Results are expressed in APL-U/ml (1 APL-Unit corresponds to the binding activity of 1 µg/ml of cardiolipin IgA antibody that was purified from the standard serum by affinity chromatography).

d. Detection limit:
The limit of blank (LoB) is based on on 66 blank replicates. The limit of detection is based on 66 low level replicates. The limit of quantitation is based on 66 determinations and a target uncertainty goal of 20%. The results are summarized in the table below:

Phadia 2500/5000	Units	LoB	LoD	LoQ
EliA β2-Glycoprotein I IgA	EliA U/mL	0.1	0.3	1.1
EliA Cardiolipin IgA	APL-U/mL	0.1	0.3	1.0

e. Analytical specificity:

Interference: Interference studies were previously reviewed in K112414 for ß2-Glycoprotein I IgA and in K131821 for Cardiolipin IgA.

Carry-over: Phadia 2500/5000 intruments use disposable tips for pipetting samples and a separate pipete for the conjugate, therefore carry-over from samples to conjugate was not evaluated.

f. Assay cut-off:
The assays' cut-offs are the same as in the predicate devices, K112414 for ß2-Glycoprotein I IgA and in K131821 for Cardiolipin IgA and are summarized in the table below:

	EliA™ β2-Glycoprotein I IgA	EliA Cardiolipin IgA
Negative	<7 U/mL	< 14 APL-U/mL
Equivical	7-10 U/mL	14-20 APL-U/mL
Positive	>10 U/mL	> 20 APL-U/mL

2. Comparison studies:
   a. Method comparison with predicate device:
   Instrument comparison was performed with the predicate device, see 2c below.

   b. Matrix comparison:
   Matrix comparison between serum and plasma (Li-Heparin, and EDTA) was reviewed in the predicate K112414 for ß2-Glycoprotein I IgA and in K131821 for Cardiolipin IgA.

   c. Instrument comparison
   The purpose of this study is to evaluate conformance and show comparability of ß2-Glycoprotein I IgA and Cardiolipin IgA assays on the instruments Phadia 250

(PH250) versus Phadia 2500/5000 (PH2500/5000). The samples were run in single replicates on one PH 250 instrument and three PH2500/5000 (A, B, C) instruments. Only samples inside the measuring range were included in the calculations. Results were analyzed by Passing-Bablock regression.

EliA ß2-Glycoprotein I IgA: 100 samples were used in the study, 63 positive, 25 negative and 12 equivocal samples. The results are summarised below:

Instrument	Intercept	95% CI	Slope	95% CI
PH2500/5000 A	0.85	0.58 to 1.26	0.98	0.95 to 1.01
PH2500/5000 B	0.44	-0.20 to 0.76	1.02	0.99 to 1.04
PH2500/5000 C	0.22	-0.06 to 0.53	1.01	0.98 to 1.05

Positive percent agreement (PPA), negative percent agreement (NPA), and total percent agreement (TPA) were evaluated with equivocal results considered positive in the table below:

	PH2500/5000 A	PH2500/5000 B	PH2500/5000 C
PPA	98.7%	97.3%	98.7%
95% CI	92.8% – 100%	90.6% – 99.7%	92.8% – 100%
NPA	80.0%	88.0%	84.0%
95% CI	59.3% – 93.2%	68.8% – 97.5%	63.9% – 95.5%
TPA	94.0%	94.9%	95.0%
95% CI	87.4% – 97.8%	88.6% – 98.3%	88.7% – 98.4%

Results when equivocal results are considered negative are presented in the table below:

	PH2500/5000 A	PH2500/5000 B	PH2500/5000 C
PPA	100.0%	100.0%	100.0%
95% CI	94.3% – 100%	94.2% – 100%	94.3% – 100%
NPA	94.6%	97.3%	100.0%
95% CI	81.8% – 99.3%	85.8% – 99.9%	90.5% – 100%
TPA	98.0%	99.0%	100.0%
95% CI	93.0% – 99.8%	94.5% – 100%	96.4% – 100%

EliA Cardiolipin IgA**:** 101 samples were used in the study, 45 positive, 43 negative and 13 equivocal samples. The results are summarised below:

Instrument	Intercept	95% CI	Slope	95% CI
PH2500/5000 A	0.30	-0.01 to 0.56	0.98	0.96 to 1.01
PH2500/5000 B	-0.27	-0.64 to 0.01	0.99	0.95 to 1.02
PH2500/5000 C	-0.40	-0.90 to -0.15	1.06	1.04 to 1.10

Positive percent agreement (PPA), negative percent agreement (NPA), and total percent agreement (TPA) were evaluated with equivocal results considered positive in the table below:

criteria	PH2500/5000 A	PH2500/5000 B	PH2500/5000 C
PPA	100.0%	100.0%	100.0%
95% CI	93.8% – 100%	93.8% – 100%	93.8% – 100%
NPA	88.4%	97.7%	95.3%
95% CI	74.9% – 96.1%	87.7% – 99.9%	84.2% – 99.4%
TPA	95.0%	99.0%	98.0%
95% CI	88.8% – 98.4%	94.6% – 100%	93.0% – 99.8%

Results when equivocal results are considered negative are presented in the table below:

criteria	PH2500/5000 A	PH2500/5000 B	PH2500/5000 C
PPA	95.6%	93.3%	97.8%
95% CI	84.9% – 99.5%	81.7% – 98.6%	88.2% – 99.9%
NPA	94.6%	94.6%	94.6%
95% CI	85.1% – 98.9%	85.1% – 98.9%	85.1% – 98.9%
TPA	95.0%	94.1%	96.0%
95% CI	88.8% – 98.4%	87.5% – 97.8%	90.2% – 98.9%

3. Clinical studies:
   Clinical performance was previously reviewed in K112414 for ß2-Glycoprotein I IgA and in K131821 for Cardiolipin IgA.

   a. Clinical Sensitivity:
   Not applicable

   b. Clinical specificity:
   Not applicable

4. Clinical cut-off**:**
   See assay cut-off

5. Expected values/Reference range:
   A total of 400 apparantly healthy Caucasian blood donors matched by age and gender were tested on the Phadia 2500/5000 instrument to evaluate expected values in the normal population. The results are summarized below:

n=400	ß2-Glycoprotein I IgA		Cardiolipin IgA	
	EliA U/mL		APL-U/mL	
	PH250	PH2500/500	PH250	PH2500/500
Mean	1.8	2.1	3.6	3.6
Median	1.4	1.8	2.8	2.8
95^{th} percentile	4.3	4.1	7.1	6.6
99^{th} percentile	8.7	9.5	17.1	16.5

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.