

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k091845

B. Purpose for Submission:

New device

C. Measurand:

IgG and IgM Antibodies to cardiolipin

IgG and IgM Antibodies to β 2-glycoprotein I

D. Type of Test:

Semi-quantitative immunofluorescence assay

E. Applicant:

Phadia US Inc.

F. Proprietary and Established Names:

EliA™ Cardiolipin IgG Immunoassay

EliA™ Cardiolipin IgM Immunoassay

EliA™ β 2-Glycoprotein I IgG Immunoassay

EliA™ β 2-Glycoprotein I IgM Immunoassay

EliA™ APS Positive Control 100

EliA™ APS Positive Control 250

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.5660, Multiple Autoantibodies Immunological Test System

21 CFR § 866.5660, Multiple Autoantibodies Immunological Test System

21 CFR § 862.1660- Quality Control Material (assayed and unassayed)

2. Classification:

Class II (Assays)

Class I (Controls)

3. Product code:

MID, System Test, Anti-cardiolipin Immunological

MSV, Antibodies, β 2- Glycoprotein I (β 2-GPI)

JJY, A quality control material (assayed and unassayed)

4. Panel:

Immunology (82) (Assays)

Chemistry (75) (Controls)

H. Intended Use:

1. Intended use(s):

EliA Cardiolipin IgG is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to cardiolipin in human serum and plasma (Li-heparin, EDTA, citrate) to aid in the diagnosis of antiphospholipid syndrome (APS) as well as thrombotic disorders related to secondary antiphospholipid syndrome in conjunction with other laboratory and clinical findings. EliA Cardiolipin IgG uses the the EliA IgG method on the instrument Phadia100 and Phadia 250.

EliA Cardiolipin IgM is intended for the in vitro semi-quantitative measurement of IgM antibodies directed to cardiolipin in human serum and plasma (Li-heparin, EDTA, citrate) to aid in the diagnosis of antiphospholipid syndrome (APS) as well as thrombotic disorders related to secondary antiphospholipid syndrome in conjunction with other laboratory and clinical findings. EliA Cardiolipin IgM uses the the EliA IgM method on the instruments Phadia 100 and Phadia 250.

EliA β 2-Glycoprotein I IgG is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to β 2-Glycoprotein I in human serum and plasma (Li-heparin, EDTA, citrate) to aid in the diagnosis of antiphospholipid syndrome (APS) as well as thrombotic disorders related to secondary antiphospholipid syndrome in conjunction with other laboratory and clinical findings. EliA β 2-Glycoprotein I IgG uses the the EliA IgG method on the instruments Phadia® 100 and Phadia® 250.

EliA β 2-Glycoprotein I IgM is intended for the in vitro semi-quantitative measurement of IgM antibodies directed to β 2-Glycoprotein I in human serum and plasma (Li-heparin, EDTA, citrate) to aid in the diagnosis of antiphospholipid syndrome (APS) as well as thrombotic disorders related to secondary antiphospholipid syndrome in conjunction with other laboratory and clinical findings. EliA β 2-Glycoprotein I IgM uses the the EliA IgM method on the instruments Phadia 100 and Phadia 250.

EliA™ APS Positive Control 100 is intended for laboratory use in monitoring the performance of in vitro measurement of antibodies to cardiolipin and β 2-Glycoprotein I with Phadia 100 using the EliA IgG or IgM method.

EliA™ APS Positive Control 250 is intended for laboratory use in monitoring the performance of in vitro measurement of antibodies to cardiolipin and β 2-Glycoprotein I with Phadia 250 using the EliA IgG or IgM method.

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 (k061165)

I. Device Description:

The method specific reagents on ImmunoCAP 100 and ImmunoCAP 250 are identical; they are only filled in different containers.

Each device consists of: 1) antigen coated wells for 48 determinations (bovine cardiolipin antigen and bovine β 2-glycoprotein I as co-factor or β 2-glycoprotein I antigen) - 4 carriers (12 wells each), ready to use; 2) EliA method-specific sample diluent, PBS with 0.095% sodium azide – 6 vials, 9 mL each, ready to use; 3) Elia IgG/IgM conjugate, β -galactosidase labeled anti IgG/IgM (mouse monoclonal antibodies) – 2 or 6 vials, 4.8 mL

each, ready to use; 4) positive control: human monoclonal IgG and IgM antibodies specific to cardiolipin and monoclonal IgG and IgM antibodies specific to β 2-Glycoprotein I – 6 single use vials(0.3 mL each), ready to use; 5) negative control: containing normal human serum from healthy donors – 6 single-use vials, 0.3 mL each, ready to use; 6) EliA calibrators, human IgG/IgM 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 test specific, method specific and general reagents are packaged and purchased as separate units.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
 Varelisa Cardiolipin IgG Antibodies (k020752)
 Varelisa Cardiolipin IgM Antibodies (k020758)
 Varelisa β 2-Glycoprotein I IgG Antibodies (k040449)
 Varelisa β 2-Glycoprotein I IgM Antibodies (k040451)
2. Comparison with predicate:
 EliA™ Cardiolipin IgG vs. Varelisa Cardiolipin IgG Antibodies
 EliA™ Cardiolipin IgM vs. Varelisa Cardiolipin IgM Antibodies
 EliA β 2-GP I IgG Well vs. Varelisa β 2-Glycoprotein I IgG Antibodies
 EliA™ β 2-Glycoprotein I IgM vs. Varelisa β 2-Glycoprotein I IgM Antibodies

Similarities		
Item	New EliA™ Device	Predicate Varelisa Device
Intended Use	EliA™ Cardiolipin IgG/IgM and β 2-Glycoprotein I IgG/IgM are intended for the in vitro semi-quantitative measurement of IgG/IgM antibodies directed to cardiolipin or β 2-Glycoprotein I in serum and plasma (heparin, EDTA, citrate) to aid in the diagnosis of antiphospholipid syndrome (APS) as well as thrombotic disorders related to secondary antiphospholipid syndrome in conjunction with other laboratory and clinical findings. EliA β 2-Glycoprotein I IgG uses the EliA IgG method on the instruments Phadia 100 and Phadia 250.	The Varelisa Cardiolipin IgG/IgM and β 2-Glycoprotein I IgG/IgM Antibodies EIA kits are designed for the semi-quantitative and qualitative determination of IgG/IgM antibodies against cardiolipin or β 2-Glycoprotein I in serum or plasma to aid in the diagnosis of antiphospholipid syndrome (APS) and to evaluate the thrombotic risk in patients with systemic lupus erythematosus (SLE).
Controls	Positive and Negative Control Sera provided with the EliA APS	Same
Assay Type	ELISA	Same
Type of test	Semi-quantitative	Qualitative and semi-quantitative
Antigen used	Bovine cardiolipin antigen and bovine β 2-glycoprotein as co-factor or Human purified β 2-Glycoprotein I	Same

Similarities		
Item	New EliA™ Device	Predicate Varelisa Device
Solid Phase	Microwells	Same

Differences		
Item	Device	Predicate
Concept	Modular reagents concept (test-method specific and general reagents)	All reagents in a single kit
Instrumentation	Phadia 100 and Phadia 250 are fully automated immunoassay analyzers	ELISA-Reader
Reaction temperature	37°C controlled	Room temperature, 20-26°C
Detection antibody (conjugate)	mouse anti-human IgG/IgM β -Galactosidase labeled monoclonal antibodies	goat anti-human IgG/IgM horse-radish peroxidase labeled
Signal	Fluorescence	Optical density
Calibration	Total IgG and IgM Calibration	Analyte specific IgG Calibration
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	Calibration Curve in each assay
Calibrators: IgG Anti-Cardiolipin and anti- β 2-glycoprotein I	0, 4, 10, 20, 100, 600 μ g/L	0, 4, 8, 20, 50, 100 U/mL
IgM Anti-Cardiolipin and anti- β 2-glycoprotein I	0,10,35,80,500,1000 μ g/L	0, 4, 8, 20, 50, 100 U/mL
Cardiolipin Measuring range	IgG 4.4-418 GPL-U/mL IgM 2.5 - 466 MPL-U/mL	1-100 GPL-U/mL 1-100 MPL-U/mL
Cardiolipin IgG/IgM Cut-off	Negative - < 10 GPL-U/mL Weak positive - 10-40 GPL-U/mL Positive - > 40 GPL-U/mL	Negative - < 10 GPL-U/mL Equivocal - 10-15 GPL-U/mL Positive - > 15 GPL-U/mL
β 2-Glycoprotein I measuring range	IgG 3.8 - 532 U/mL IgM 3.9 - 500 U/mL	1.0 – 100 U/mL 1.0 – 100 U/mL
β 2-Glycoprotein I 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

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

Not applicable

L. Test Principle:

The EliA test wells are coated with bovine cardiolipin antigen and bovine β 2-glycoprotein I as co-factor or with human β 2-Glycoprotein I. If present in the patient's specimen, antibodies to cardiolipin or β 2-glycoprotein I bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG or IgM antibodies, are added to form an antibody-conjugate complex (EliA β -Galactosidase anti - IgG/IgM mouse monoclonal antibodies). 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, the more IgG or IgM is present in the specimen. To evaluate test results, the signal response for patient samples is compared directly to the signal response for calibrators of defined value.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

To determine the precision of the assays on the Phadia 100 and Phadia 250 instrument, the variability was assessed on 6 samples. Each sample was run on 3 instruments x 6 runs each over 6 days (equal to 108 replicate determinations per sample) with a calibration curve in each run. Additional 2-4 samples were run on the analytes that showed >10% CV. The statistical evaluation was performed by Analysis of Variance. The results are summarized in the tables below:

EliA Cardiolipin IgG IC100			
Mean value GPL - U/mL	Coefficients of variation (%)		
	Intra Run CV%	Inter Run (between days) CV%	Total Imprecision CV%
7.8	3.7	2.4	4.4
14.0*	5.5*	3.1*	6.4*
16.6	10.2	2.1	10.4
47.3	4.1	2.2	4.6
82.9	3.9	2.2	4.5
164.5	8.7	4.2	9.7
166.7*	5.1*	4.6*	6.8*
188.2*	4.3*	3.4*	5.5*
443.8	8.5	5.1	9.9

EliA Cardiolipin IgG IC250			
Mean value GPL - U/mL	Coefficients of variation (%)		
	Intra Run CV%	Inter Run (between days) CV%	Total Imprecision CV%
8.0	3.9	2.8	4.8
16.8	8.9	0.3	8.9
18.2*	3.4*	3.4*	4.8*
30.4*	6.5*	3.4*	7.3*
47.2	3.1	2.4	3.9
80.3	3.8	2.8	4.7
178.9	17.3	8.7	19.4
239.8*	3.3*	2.9*	4.4*
377.1*	5.6*	4.3*	7.0*
418.6	6.2	5.2	8.1

* Additional samples tested for analytes with >10% CV

EliA Cardiolipin IgM IC100			
Mean value MPL- U/mL	Coefficients of variation (%)		
	Intra Run CV%	Inter Run (between days) CV%	Total Imprecision CV%
3.3	9.3	9.6	13.4
21.9	4.9	5.6	7.5
52.5	4.1	4.7	6.2
105.3	3.7	5.2	6.4
184.7	6.8	5.7	8.9
420.2	4.5	2.1	5.0

EliA Cardiolipin IgM IC250			
Mean value MPL - U/mL	Coefficients of variation (%)		
	Intra Run CV%	Inter Run (between days) CV%	Total Imprecision CV% _v
2.0	12.0	8.9	15.0
24.0	3.3	5.4	6.3
53.0	3.7	3.2	4.9
105.1	3.5	3.2	4.7
210.1	5.0	3.7	6.2
490.8	3.1	1.6	3.5

EliA b2-Glycoprotein I IgG IC100			
Mean value U/mL	Coefficients of variation (%)		
	Intra Run CV%	Inter Run (between days) CV%	Total Imprecision CV%
4.6	6.1	6.0	8.6
8.7	7.2	15.5	17.1
9.0*	5*	4.4*	6.7*
10.9*	3.7*	3.9*	5.3*
13.9	5.1	3.7	6.3
39.8	3.8	2.3	4.5
113.7	4.4	4.8	6.5
271.1	4.7	3.5	5.9
501.0	7.2	4.9	8.7

EliA b2-Glycoprotein I IgG IC250			
Mean value U/mL	Coefficients of variation (%)		
	Intra Run CV%	Inter Run (between days) CV%	Total Imprecision CV%
4.4	3.4	4.3	5.5
9.1*	5.3*	3.8*	6.5*
10.7	7.8	12.9	15.1
14.1*	4.6*	3.9*	6.0*
15.5	4.7	3.8	6.0
43.0	3.7	3.0	4.8
112.1	4.5	3.1	5.5
231.0	3.3	2.8	4.3
449.3	4.4	3.8	5.8

* Additional samples tested for analytes with >10% CV

EliA b2-Glycoprotein I IgM IC100			
Mean value U/mL	Coefficients of variation (%)		
	Intra Run CV%	Inter Run (between days) CV%	Total Imprecision CV%
4.5	12.2	9.5	15.4
9.4	6.6	6.4	9.2
17.7	4.4	5.7	7.2
52.0	4.4	5.2	6.8
186.5	4.3	5.4	6.9
498.1	3.7	5.2	6.4

EliA b2-Glycoprotein I IgM IC250			
Mean value U/mL	Coefficients of variation (%)		
	Intra Run CV%	Inter Run (between days) CV%	Total Imprecision CV%
3.3	11.5	14.7	18.7
8.9	4.2	8.2	9.2
18.2	2.7	1.9	3.3
58.6	4.3	3.0	5.2
196.5	4.0	3.9	5.5
433.8	3.0	4.3	5.2

b. *Linearity/assay reportable range:*

Nine samples per analyte were diluted according to the following scheme after the method specific dilution (1:10 for EliA Cardiolipin IgG and IgM assays and the EliA β 2-Glycoprotein I IgG assay or 1:50 for the EliA β 2-Glycoprotein I IgM assay): 1/2, 1/4, 1/8, 1/16 and 1/32 using EliA™ Sample Diluent. Each sample was run in triplicates in two runs. For each analyte, 8 of the 9 sample sets were assayed on the IC100 and one sample set on the IC250. The sample concentrations tested for linearity were as follows: Cardiolipin IgG - 141.2 to 568.9 GPL-U/mL; Cardiolipin - IgM 87.5 to 466.4; β 2-Glycoprotein I IgG 128.9 to 806.4 U/mL; β 2-Glycoprotein I IgM – 172.7 to 500.5 U/mL. Representative results are summarized in the tables below.

EliA™ Cardiolipin IgG

Instrument	EliA Cardiolipin IgG Dilution Range (GPL-U/mL)	Slope (95% CI)	Y-intercept (95% CI)	R²	%CV Range
IC100	4.4 - 141.2	1.00 (0.93 to 1.07)	-2.92 (-7.76 to 1.93)	0.997	1.3 – 5.2
	6.5 – 517.4	1.03 (1.00 to 1.06)	-12.34 (-17.90 to -6.77)	0.999	0.1 – 2.2
IC250	6.0 – 568.9	1.00 (1.00 to 1.07)	-2.87 (-8.19 to 2.45)	0.998	0.3 – 2.7

Claimed linearity range is 4.4-418 GPL U/mL.

EliA™ Cardioliipin IgM

Instrument	EliA Cardiolipin IgM Dilution Range (MPL-U/mL)	Slope (95% CI)	Y-intercept (95% CI)	R²	%CV range
IC100	2.5 - 88.9	1.00 (0.99 to 1.01)	0.32 (-0.10 to 0.73)	1.000	1.3 – 6.0
	7.2 – 399.0	1.01 (0.97 to 1.05)	-13.93 (-20.11 to -7.75)	0.998	1.2 – 7.5
IC250	9.2 – 466.4	1.01 (0.98 to 1.05)	-17.90 (-24.86 to 10.94)	0.998	0.4 – 3.1

Claimed linearity range is 2.5-466 MPL-U/mL.

EliA™ β2-Glycoprotein I IgG

Instrument	EliA β2-GPI IgG Dilution Range (U/mL)	Slope (95% CI)	Y-intercept (95% CI)	R²	%CV range
IC100	3.8 - 128.9	0.98 (0.80 to 1.15)	-4.10 (-15.79 to 7.58)	0.983	0.5 – 5.7
	9.4 – 742.3	1.04 (1.01 to 1.07)	-14.85 (-23.62 to -6.08)	0.998	0.3 – 4.4
IC250	8.1 – 806.4	1.04 (0.99 to 1.09)	-4.50 (-20.34 to 11.35)	0.995	0.3 – 4.4

Claimed linearity range is 3.8-532 U/mL.

EliA™ β2-Glycoprotein I IgM

Instrument	EliA β2-GPI IgM Dilution Range (U/mL)	Slope (95% CI)	Y-intercept (95% CI)	R²	%CV Range
IC100	3.9 to 176.8	0.99 (0.94 to 1.04)	3.34 (-0.82 to 7.51)	0.999	0.7 – 2.9
	5.5 – 500.5	1.00 (0.97 to 1.04)	-14.04 (-21.14 to -6.93)	0.998	1.2 – 14.1
	5.5 – 172.7	0.98 (0.96 to 1.00)	2.16 (0.37 to 3.95)	0.999	1.2 – 14.1*
IC250	6.3 – 466.3	1.01 (0.97 to 1.04)	-14.25 (-20.52 to -7.97)	0.998	0.8 – 5.6

Claimed linearity range is 3.9 - 500 U/mL.

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

Values below detection limit are reported as < x.x GPL-, MPL or EliA U/mL depending on the analyte.

High Dose Hook Effect:

IgG method: High dose hook effect was analyzed by using dilutions from high positive serum samples with an estimated concentration higher than the calibrator 600 (containing 600 µg/L of IgG). The sample dilutions were measured in 4 replicates.

EliA Cardiolipin IgG: A hook effect was not observed when analyzing a high positive sample that had a concentration up to 6.5 times above the upper limit of the measuring range. The upper limit of the measuring range was set to 418 GPL-U/mL.

EliA β2-Glycoprotein I Ig: A hook effect was not observed when analyzing a high positive sample that had a concentration up to 6.4 times above the upper limit of the measuring range. The upper limit of the measuring range was set to 532 U/mL.

IgM method: High dose hook effect was analyzed by using dilutions from high positive serum samples with an estimated concentration higher than the calibrator 1000 (containing 1000 µg/L of IgM). The sample dilutions were measured in 4 replicates.

EliA Cardiolipin IgM: A hook effect was not observed when analyzing a high positive sample that had a concentration up to 5.85 times above the upper limit of the measuring range. The upper limit of the measuring range was set to 466 MPL-U/mL.

EliA β2-Glycoprotein I IgM: A hook effect was not observed when analyzing a high positive sample that had a concentration up to 8.1 times above the upper limit of the measuring range. The upper limit of the measuring range was set to 500 U/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The EliA IgG system reagents and EliA Negative Control are cleared under the k061165 for the EliA IgG system; k072149/k072393 for EliA Negative Control.

The IgG and IgM 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 IgG and IgM 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 IgG/IgM concentrations in µg/L. By using a conversion factor given by the lot-specific code of the EliA Cardiolipin IgG and IgM Well, the results are automatically converted to GPL-U/mL or MPL-U/mL, respectively.

Shelf Life of test components:

Three batches of EliA Cardiolipin IgG/IgM or β 2-Glycoprotein I IgG/IgM wells were tested in real time stability studies to support the claimed shelf life at 2-8°C up to 18 months.

EliA APS Positive Control stability:

Shelf of the EliA APS positive control is summarized below:

Open stability:

Open stability is not tested as the EliA APS Positive Control and Negative Control are packaged in single use vials.

Closed stability:

A study was done to investigate the accelerated stability of the EliA APS Positive Control. A preliminary shelf life of 18 months was given for the EliA APS Positive Control. Real time studies are being done alongside the accelerated studies.

d. *Detection limit:*

The purpose of this study was to verify the lower limit of the measuring ranges for EliA Cardiolipin IgG and EliA β 2-Glycoprotein I IgG (detection limit) by providing the ability of the new device to differentiate between the background sample diluent) and a 1/8 dilution of the lowest calibrator point.

Detection limit (LoD) IgG method:

Calibrator 4.0 (containing 4 μ g/L of IgG) was diluted 1:2 (i.e. 2 μ g/L), 1:4 (i.e. 1 μ g/L) and 1:8 (i.e. 0.5 μ g/L) in EliA sample diluent and run on EliA IgG Calibrator wells. EliA Sample diluent was run on the different EliA Cardiolipin IgG and EliA β 2-Glycoprotein I IgG wells. EliA sample diluent results were compared with the Calibrator results.

Detection limit (LoD) IgM method:

Calibrator 10.0 (containing 10 μ g/L of IgM) was diluted 1:2 (i.e. 5 μ g/L), 1:4 (i.e. 2.5 μ g/L) and 1:8 (i.e. 1.25 μ g/L) in EliA sample diluent and run on EliA IgM Calibrator wells. EliA Sample diluent was run on the different EliA Cardiolipin IgM and EliA β 2-Glycoprotein I IgM wells. EliA sample diluent results were compared with the Calibrator results.

Results are summarized below:

EliA Cardiolipin IgG: LoD was set to 0.5 GPL-U/mL.

EliA β 2-Glycoprotein I IgG: LoD was set to 0.6 U/mL.

EliA Cardiolipin IgM: LoD was set to 0.8 MPL-U/mL.

EliA β 2-Glycoprotein I IgM: LoD was set to 0.9 U/mL.

f. *Analytical specificity/Interference:*

The purpose of this study was to investigate whether high concentrations of potentially interfering substances in serum, like bilirubin, hemoglobin, chyle and rheumatoid factor adversely affect the results of EliA assays. Two positive serum samples with concentration levels around the cut-off and one high positive serum sample with concentration level around calibrator 5 were spiked with the interfering substances bilirubin C, bilirubin F, hemoglobin, chyle or rheumatoid

factor. The same samples were also spiked with substance specific blanks. The samples were tested in 3 replicates. A calibration curve in two replicates was run in each assay. The runs were repeated twice. The table below shows the end concentration of the interference substances spiked into the 1:10 diluted serum samples:

Substance	Concentration in the 1:10 Diluted Serum sample
Bilirubin C	20.6 µg/mL
Bilirubin F	21.1 µg/mL
Hemoglobin	519 µg/mL
Chyle	1570 Units/dl
Rheumatoid factor	55 IU/mL*

Bilirubin C, bilirubin F, chyle and hemoglobin did not adversely affect the results.

*Increased levels of rheumatoid factor may have an effect on the response on *EliA Cardiolipin IgM Well* and can influence the β 2-Glycoprotein I IgM test result when the patient sample contains β 2-Glycoprotein I IgG antibodies.

Generally, the use of sera containing lipemic, hemolized or microbial contaminations is not recommended as stated in the package inserts.

Carry over study:

A sample with a specific anti-Cardiolipin IgM titer above the measuring range was first pipetted on EliA Dummy wells (wells which are not coated with Cardiolipin antigen). The pipette then was washed by the ordinary washing routine of the instrument. Thereafter pure diluent was pipetted on EliA Cardiolipin IgM wells. If there remained any of the sample with high specific anti-Cardiolipin IgM titer on the pipette surface it would increase the signal compared to the basic signal normally obtained when measuring pure diluent on EliA Cardiolipin IgM wells. This was repeated five times. The study has been performed on three instruments. Signal level for pure diluent on EliA Cardiolipin well IgM was around 18 relative fluorescence units (RU). The carry over could hardly be expressed in U/mL since the lowest calibration point 0 U/mL is normally found around 19 RU.

A difference between instrument dilution 1:2 and manual dilution 1:2 could hardly be detected. Therefore after pipetting the sample with the high titer of specific anti-Cardiolipin antibodies, only a few RUs difference compared to the prediluted sample pipetting could be seen, which is too low to be expressed in U/mL.

g. Assay cut-off:

A study was done on 400 apparently healthy Blood Donor samples from Caucasian individuals equally distributed by sex and age. The 95th percentile should be calculated and should be taken into account for setting of the cut-off. The results are summarized below:

Cardiolipin IgG/IgM:

Test	Unit	No. of samples	Mean value	95%-percentile	99%-percentile
EliA Cardiolipin IgG	GPL-U/ml	400	4.6	5.7	23.8

Cut-off:

< 10 GPL-U/mL	Negative
10 – 40 GPL-U/mL	Weak positive
> 40 GPL-U/mL	Positive

Test	Unit	No. of samples	Mean value	95%-percentile	99%-percentile
EliA Cardiolipin IgM	MPL-U/ml	400	4.1	11.7	29.9

Cut-off:

< 10 MPL-U/mL	Negative
10 – 40 MPL-U/mL	Weak positive
> 40 MPL-U/mL	Positive

β2-Glycoprotein I IgG/IgM:

The expected value in the normal population is negative. However, up to 3% of apparently healthy, asymptomatic individuals may have increased levels of β2-glycoprotein I antibodies. Expected values may vary depending on the population tested.

Test	Unit	No. of samples	Mean value	95%-percentile	99%-percentile
EliA β2-Glycoprotein I IgG	U/ml	400	3.3	8.2	17.7

Cut-off:

< 7 U/mL	Negative
7 – 10 U/mL	Equivocal
> 10 U/mL	Positive

Test	Unit	No. of samples	Mean value	95%-percentile	99%-percentile
EliA β2-Glycoprotein I IgM	U/ml	400	1.0	3.2	5.7

Cut-off:

< 7 U/mL	Negative
7 – 10 U/mL	Equivocal
> 10 U/mL	Positive

2. Comparison studies:

a. Method comparison with predicate device:

289 serum samples were collected from the serum bank at Phadia GmbH. In this study 64 samples from patients who had been clinically defined as suffering from Antiphospholipid Syndrome (APS) were included. Each sample was analyzed in duplicates in EliA Cardioliipin IgG kits. The study was evaluated by excluding the clinical samples below the limit of detection and above the measuring range. All method comparison studies were run on ImmunoCAP 250 instruments. The results are summarized below:

EliA Cardioliipin tests, in contrast to the Varelisa Cardioliipin tests, do not yield equivocal, but weak positive results. When evaluating the method comparison the area between 10 and 40 GPL- / MPL-U/ml were considered only as positive (> 10 GPL-U/mL or MPL-U/mL).

EliA Cardioliipin IgG Well - Equivocal Varelisa results considered as negative:

n=275		Varelisa Cardioliipin IgG Abs.		
		>15 GPL-U/mL	<15 GPL-U/mL	Total
EliA Cardioliipin IgG	>10 GPL-U/mL	37	13	50
	<10 GPL-U/mL	6	219	225
	Total	43	232	275

Positive Percent Agreement 86% (37/43) (95% CI: 72.1% – 94.7%)

Negative Percent Agreement 94.4% (219/232) (95%CI: 90.6% – 97.0%)

Total Agreement 93.1% [(37+219)/275] (95%CI: 89.4% – 95.8%)

EliA Cardioliipin IgG Well - Equivocal Varelisa results considered as positive

n=275		Varelisa Cardioliipin IgG Abs.		
		>10 GPL-U/mL	<10 GPL-U/mL	Total
EliA Cardioliipin IgG	>10 GPL-U/mL	41	9	50
	<10 GPL-U/mL	20	205	225
	Total	61	214	275

Positive Percent Agreement 67.2% (41/61) (95% CI: 54.0% – 78.7%)

Negative Percent Agreement 95.8% (205/214) (95%CI: 92.2% – 98.1%)

Total Agreement 89.5% [(41+205)/275] (95%CI: 85.2% – 92.8%)

EliA Cardiolipin IgM Well: Equivocal Varelisa results considered as negative:

n=230		Varelisa Cardiolipin IgM Abs.		
		>15 MPL-U/mL	<15 MPL-U/mL	Total
EliA Cardiolipin IgM	>10 MPL-U/mL	17	25	42
	<10 MPL-U/mL	3	185	188
	Total	20	210	230

Positive Percent Agreement 85% (17/20) (95% CI: 62.1% – 96.8%)

Negative Percent Agreement 88.1% (185/210) (95%CI: 82.9% – 92.1%)

Total Agreement 87.8% [(17+185)/230] (95%CI: 82.9% – 91.8%)

EliA Cardiolipin IgM Well with equivocal Varelisa results considered as positive:

n=230		Varelisa Cardiolipin IgM Abs.		
		>10 MPL-U/mL	<10 MPL-U/mL	Total
EliA Cardiolipin IgM	>10 MPL-U/mL	23	19	42
	<10 MPL-U/mL	8	180	188
	Total	31	199	230

Positive Percent Agreement 74.2% (23/31) (95% CI: 55.4% – 88.1%)

Negative Percent Agreement 90.5% (180/199) (95%CI: 85.5% – 94.2%)

Total Agreement 88.3% [(23+180)/230] (95%CI: 83.4% – 92.1%)

EliA β 2-Glycoprotein I IgG

Equivocals EliA 7-10 U/mL - 7.7% Varelisa 7-15 U/mL – 12.1%

EliA β 2-Glycoprotein I IgG Well: equivocal samples considered as negative:

n=297		Varelisa β 2-GP I IgG Abs.		
		>15 MPL-U/mL	<15 MPL-U/mL	Total
EliA β 2- GP I IgG	>10 MPL-U/mL	36	22	58
	<10 MPL-U/mL	7	232	239
	Total	43	254	297

Positive Percent Agreement 83.7% (36/43) (95% CI: 69.3% – 93.2%)

Negative Percent Agreement 91.3% (232/254) (95%CI: 87.2% – 94.5%)

Total Agreement 90.2% [(36+232)/297] (95%CI: 86.3% – 93.4%)

EliA β 2-Glycoprotein I IgG Well with equivocal samples considered as positive:

n=297		Varelisa β 2-GP I IgG Abs.		
		>10 MPL-U/mL	<10 MPL-U/mL	Total
EliA β 2-GP I IgG	>10 MPL-U/mL	61	20	81
	<10 MPL-U/mL	10	206	216
	Total	71	226	297

Positive Percent Agreement 85.9% (61/71) (95% CI: 75.6% – 93.0%)

Negative Percent Agreement 91.2% (206/226) (95%CI: 86.7% – 94.5%)

Total Agreement 89.9% [(61+206)/297] (95%CI: 85.9% – 93.1%)

EliA β 2-Glycoprotein I IgM

Equivocals EliA 7-10 U/mL - 9.4% Varelisa 7-15 U/mL 21.9%

EliA β 2-Glycoprotein I IgM Well with equivocal samples considered as negative:

n=128		Varelisa β 2-GP I IgM Abs.		
		>15 MPL-U/mL	<15 MPL-U/mL	Total
EliA β 2-GP I IgM	>10 MPL-U/mL	24	19	43
	<10 MPL-U/mL	0	85	85
	Total	24	104	128

Positive Percent Agreement 100% (24/24) (95% CI: 85.8% – 100%)

Negative Percent Agreement 81.7% (85/104) (95%CI: 72.9% – 88.6%)

Total Agreement 85.2% [(24+85)/128] (95%CI: 77.8% – 90.8%)

EliA β 2-Glycoprotein I IgM Well with equivocal samples considered as positive:

n=128		Varelisa β 2-GP I IgM Abs.		
		>10 MPL-U/mL	<10 MPL-U/mL	Total
EliA β 2-GP I IgM	>10 MPL-U/mL	40	15	55
	<10 MPL-U/mL	1	72	73
	Total	41	87	128

Positive Percent Agreement 97.6% (40/41) (95% CI: 87.1% – 99.9%)

Negative Percent Agreement 82.8% (72/87) (95%CI: 73.2% – 90.0%)

Total Agreement 87.5% [(40+72)/128] (95%CI: 80.5% – 92.7%)

Summary:

Equivocal results were considered as negative.

Product	Predicate Device	Number of samples analyzed	Total agreement [%]	95% CI
EliA Cardioliipin IgG	Varelisa Cardioliipin IgG Abs.	275	93.1	89.4 – 95.8
EliA Cardioliipin IgM	Varelisa Cardioliipin IgM Abs.	230	87.8	82.9 – 91.8
EliA β 2-Glycoprotein I IgG	Varelisa β 2-GP I IgG Abs.	297	90.2	86.3 – 93.4
EliA β 2-Glycoprotein I IgM	Varelisa β 2-GP I IgM Abs.	128	85.2	77.8 – 90.8

Equivocal results were considered as positive.

Product	Predicate Device	Number of samples analyzed	Total agreement [%]	95% CI
EliA Cardioliipin IgG	Varelisa Cardioliipin IgG Abs.	275	89.5	85.2 – 92.8
EliA Cardioliipin IgM	Varelisa Cardioliipin IgM Abs.	230	88.3	83.4 – 92.1
EliA β 2-Glycoprotein I IgG	Varelisa β 2-GP I IgG Abs.	297	89.9	85.9 – 93.1
EliA β 2-Glycoprotein I IgM	Varelisa β 2-GP I IgM Abs.	128	87.5	80.5 – 92.7

b. Instrument comparison:

A study was done to demonstrate that the performance of EliA™ Cardioliipin IgG, EliA™ Cardioliipin IgM, EliA™ β 2-Glycoprotein I IgG and EliA™ β 2-Glycoprotein I IgM is equivalent on the Instrument Phadia 100 and Phadia 250.

The results for 36 samples (4 negative samples and 32 positive samples) distributed over the measuring range were determined. All samples were run on three different instruments in two runs and single replicates (6 independent values for Phadia 250, 6 independent values for Phadia 100). Results are summarized below:

	Slope (95% CI)	Y-intercept(95% CI)
EliA Cardioliipin IgG	1.016 (0.969 to 1.67)	-0.984 (-2.464 to 0.194)
EliA Cardioliipin IgM	0.901 (0.877 to 0.928)	-0.607 (-1.023 to -0.217)
EliA β 2-Glycoprotein I IgG	1.008 (0.963 to 1.093)	-0.626 (-4.188 to 1.539)
EliA β 2-Glycoprotein I IgM	0.996 (0.964 to 1.038)	0.425 (-1.435 to 0.368)

- b. *Matrix comparison:* The purpose of this study was to demonstrate that the new devices give the same results for Serum, Heparin plasma, Citrate plasma and EDTA plasma collected from the same patient. The study was done on 50 samples, 25 negative and 25 positive, spread across the assay range. Samples were run in single replicates. All the method comparison studies were run on ImmunoCAP 250 instruments. The results are summarized below:

test	No of. Samples	Range tested	Slope	Y.intercept	R ²
EliA Cardiolipin IgG					
		GPL-U/ml	95% CI	95% CI	
Serum v plasma citrate	24 pos/ 26 neg., 8 low pos*	1 - 522	0,99 (0,98 to 1,01)	0,93 (-1,12 to 2,98)	1
Serum v plasma EDTA	24 pos/ 26 neg., 8 low pos*	1,1 - 484	1,01 (0,99 to 1,02)	-0,43 (-2,04 to 1,20)	1
Serum v plasma heparin	24 pos/ 26 neg., 8 low pos*	1,4 - 490	1,00 (0,98 to 1,02)	-0,74 (-3,09 to 1,60)	1
EliA Cardiolipin IgM					
		MPL-U/ml	95% CI	95% CI	
Serum v plasma citrate	24 pos/ 26 neg., 8 low pos*	1 - 522	0,99 (0,98 to 1,01)	0,93 (-1,12 to 2,98)	1
Serum v plasma EDTA	24 pos/ 26 neg., 8 low pos*	1,1 - 484	1,01 (0,99 to 1,02)	-0,43 (-2,04 to 1,20)	1
Serum v plasma heparin	24 pos/ 26 neg., 8 low pos*	1,4 - 490	1,00 (0,98 to 1,02)	-0,74 (-3,09 to 1,60)	1
EliA β2-GPI IgG					
		U/ml	95% CI	95% CI	
Serum v plasma citrate	24 pos/ 26 neg., 0 equiv.	0,1 - 488	0,93 (0,91 to 0,95)	1,02 (-2,28 to 4,31)	0,99
Serum v plasma EDTA	25 pos/ 26 neg., 0 equiv.	0,4 - 441	1,04 (1,01 to 1,07)	-0,69 (-4,72 to 3,35)	0,99
Serum v plasma heparin	26 pos/ 26 neg., 0 equiv.	0,5 - 525	0,90 (0,88 to 0,91)	3,5 (0,85 to 6,14)	1
EliA β2-GPI IgM					
		U/ml	95% CI	95% CI	
Serum v plasma citrate	24 pos/ 26 neg., 0 equiv.	0,0 - 469	0,90 (0,89 to 0,92)	1,9 (-0,46 to 4,3)	1
Serum v plasma EDTA	25 pos/ 26 neg., 0 equiv.	0,0 - 446	1,00 (0,99 to 1,02)	0,14 (-2,41 to 2,69)	1
Serum v plasma heparin	26 pos/ 26 neg., 0 equiv.	0,0 - 463	0,92 (0,91 to 0,94)	-148 (-3,79 to 0,83)	1
* low pos sample are included within the amount of pos samples					

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

The samples that were used for the method comparison were used to calculate the sensitivity/specificity of the assay. SLE samples with unknown APS status were also evaluated for their sensitivity/specificity.

Clinical Evaluation of EliA Cardiolipin IgG

EliA Cardiolipin IgG	Diagnostic Group APS		
	+	-	Total
n=224			
Positive test > 10 GPL U/mL	23	4	27
Negative test ≤ 10 GPL U/mL	26	171	197
Total	49	175	224

Sensitivity:	46.9%	95% CI: 32.5 – 61.7 %
Specificity:	97.7%	95% CI: 94.3 – 99.4 %

Clinical Evaluation of EliA Cardiolipin IgM

EliA Cardiolipin IgM	Diagnostic Group APS		
	+	-	Total
n=188			
Positive test > 10 MPL U/ml	14	10	24
Negative test ≤ 10 MPL U/ml	45	119	164
Total	59	129	188

Sensitivity:	23.7%	95% CI: 13.6 – 36.6 %
Specificity:	92.2%	95% CI: 86.2 – 96.2 %

Clinical Evaluation of EliA β2-Glycoprotein I IgG

EliA β2-GPI IgG	Diagnostic Group APS		
	+	-	Total
n=220			
Positive test > 10 U/ml	24	2	26
Negative test ≤ 10 U/ml	30	164	194
Total	54	166	220

Sensitivity:	44.4%	95% CI: 30.9 – 58.6 %
Specificity:	98.8%	95% CI: 95.7 – 99.9 %

Clinical Evaluation of EliA β 2-Glycoprotein I IgM

EliA β 2-GPI IgM	Diagnostic Group APS		
n=77	+	-	Total
Positive test > 10 U/ml	8	4	12
Negative test \leq 10 U/ml	25	40	65
Total	33	44	77

Sensitivity:	24.2%	95% CI: 11.1 – 42.3 %
Specificity:	90.9%	95% CI: 78.3 – 97.5 %

The table below shows the results for each clinical subgroup:

Condition	β -2 Glycoprotein I IgG % positive (>10 U/mL)	β -2 Glycoprotein I IgG % positive (>10 U/mL)	Cardiolipin IgG % positive (>10 U/mL)	Cardiolipin IgM % positive (>10 U/mL)
Anti-Phospholipid Syndrome (APS) (37 samples from primary APS)	34/64=53%	8/64=12.5%	23/49=46.9%	14/60=23.3%
Systemic lupus Erythematosus (SLE)	7/41=17.0%	9/41=22.0%	10/37=27.0%	5/29=17.2%
Unknown	27/37=73.0%	27/37=73.0%	13/14=92.9%	13/13=100%
¹ Infectious diseases	1/112=0.89%	2/112=1.8%	2/11=11.8%	7/84=8.3%
² Other	1/72=1.4%	2/72=2.8%	2/64=3.1%	3/47=6.4%

¹Infectious diseases include: Hepatitis C Virus (HCV), HIV, Epstein Barr Virus (EBV), Helicobacter pylori infection (HBP), Syphilis, Toxoplasmosis (TP), Varicella Zoster Virus (VZV), Yersinia Pestis (YP)

²Other include: Marine-Lenhart Syndrome (MLS), Secondary Sjögren's Syndrome (SSS), Chronic lymphocytic Thyroiditis, atropic (clThy), Hashimoto's disease (HM), Hypothyreosis (Hypthy), Morbus Basedow (MB), Morbus Basedow endocrine ophthalmopathy (MBeO), Mixed Connective Tissue Disease (MCTD), Primary Sjögren's Syndrome (PSS), Rheumatoid Arthritis (RA), Rheum factor (RF), CM

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

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range: :

Cardiolipin IgG/IgM:

The expected value in the normal population is negative. The sponsor states that a low percentage (up to 5 %) of apparently healthy, asymptomatic individuals have been reported to be positive for Anti-Cardiolipin Antibody. Their proportion may increase with age. Expected values may vary depending on the population tested.

β 2-Glycoprotein I IgG/IgM:

The expected value in the normal population is negative. The sponsor states that up to 3% of apparently healthy, asymptomatic individuals may have increased levels of β 2-glycoprotein I antibodies. 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: SE

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