510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k090755

B. Purpose for Submission:
New Device

C. Measurand:
IgM and IgG class antibodies to phosphatidylserine/prothrombin complex (PS/PT)

D. Type of Test:
Semi-quantitative ELISA

E. Applicant:
INOVA Diagnostics, Inc.

F. Proprietary and Established Names:
Quanta Lite ™ aPS/PT IgG ELISA
Quanta Lite ™ aPS/PT IgM ELISA

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5660 Multiple auto-antibodies immunological test system
2. Classification:
   Class II
3. Product code:
   MSV, system, test, antibodies, β2-glycoprotein 1 (β2-GP1)
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   QUANTA Lite™ aPS/PT IgG and QUANTA Lite™ aPS/PT IgM kits are semi-quantitative and qualitative enzyme-linked immunosorbent assays (ELISA) for the detection of IgG and IgM class antibodies to phosphatidylserine/prothrombin complex (PS/PT) in serum or plasma. For use as an aid in the diagnosis of certain autoimmune thrombotic disorders, such as anti-phospholipid syndrome (APS) and those secondary to systemic lupus erythematosus or other lupus-like diseases, in conjunction with other laboratory and clinical findings.
2. Indication(s) for use:
   Same as intended use
3. Special conditions for use statement(s):
   For prescription only
4. Special instrument requirements:
   Microplate reader capable of measuring OD at 450 nm (or 620 for dual wavelength readings)

I. Device Description:
QUANTA Lite™ aPS/PT IgG and IgM ELISA consists of a polystyrene microwell ELISA plate coated with purified non-recombinant PS/PT complex; high positive, low positive, and negative controls; sample diluent; wash concentrate; goat anti-

human IgG or IgM, horseradish peroxidase conjugate; TMB chromogen; and stop solution. The PS/PT antigen consists of naturally occurring lipid phosphatidylserine complexed with human prothrombin.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   QUANTA Lite™ β2-GPI IgG and IgM ELISA
2. Predicate 510(k) number(s):
   k970551 and k973014
3. Comparison with predicate:

SIMILARITIES		
	QUANTA Lite™ aPS/PT IgG and IgM ELISAs	QUANTA Lite™ β2-GPI IgG and IgM ELISAs
Intended Use	QUANTA Lite™ aPS/PT IgG and QUANTA Lite™ aPS/PT IgM kits are semi-quantitative and qualitative enzyme-linked immunosorbent assays (ELISA) for the detection of IgG and IgM class antibodies to phosphatidylserine/ prothrombin complex (PS/PT) in serum or plasma. For use as an aid in the diagnosis of certain autoimmune thrombotic disorders, such as anti-phospholipid syndrome (APS) and those secondary to systemic lupus erythematosus or other lupus-like diseases in conjunction with other laboratory and clinical findings.	QUANTA Lite™ ß2 GPI IgG and IgM ELISA kits are enzyme-linked immunosorbent assays (ELISA) for the semi-quantitative detection of ß2 GPI IgG and IgM antibodies in human serum. The presence of ß2 GPI IgG and IgM antibodies can be used in conjunction with other clinical findings and laboratory tests to aid in the diagnosis of certain autoimmune disorders, such as those secondary to systemic lupus erythematosus (SLE) or other lupus-like thrombotic diseases
Methodology	ELISA	Same
Assay format	Semi-quantitative	Same
Conjugate	Anti-human IgG HRP Anti-human IgM HRP	Same
Substrate/ Chromogen	TMB Chromogen	Same
Screening dilution	1:101	Same
Reading	450 nm (620 nm for dual wavelength readings)	Same

DIFFERENCES		
	Device	**Predicate**
Analyte	Anti-phosphatidylserine/prothrombin complex (aPS/PT)	β2-glycoprotein 1 (β2-GP1)
Sample type	Serum or plasma	Serum
Cutoff	30 Units	20 Units
Positive Control	PS/PT IgG or IgM ELISA control	ß2 GPI IgG ELISA control ß2 GPI IgM ELISA control
Calibrators	PS/PT IgG or IgM Calibrators (A-E)	ß2 GPI IgG or IgM Calibrators (A-E)
Linear Range	PS/PT IgG: 5.1-150 units PS/PT IgM: 3.6-150 units	Not given
Limit of Detection	PS/PT IgG: 5.1 units PS/PT IgM: 3.6 units	Not given

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

Evaluation of the Linearity of Quantitative Analytical Methods (EP6-P2)

L. Test Principle:

QUANTA Lite™ aPS/PT IgG and IgM ELISAs employ the sandwich ELISA technique. Plastic microwell plate wells are coated with purified PS/PT complex and then stabilized. Upon incubation, sample containing PS/PT IgG or IgM antibodies bind to the PS/PT complexes. Unbound protein is removed by washing and polyclonal goat anti-human IgG or IgM horseradish peroxidase (HRP) labeled conjugate is added to the wells. After incubation, the unbound conjugate is removed by washing. A peroxidase substrate is added, which undergoes a color change in the presence of the conjugated enzyme. After stopping the enzymatic production of colored product, the presence or absence of phosphatidylserine/prothrombin antibody is determined spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with that of a five point calibration curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Eight clinical samples were tested spanning a range of values from low negative (1-3 units) to high positive (139-150 units). Each sample was run 6 times on 6 different days on the PS/PT IgG ELISA by the same operator for a total of 36 runs. The unit value results are summarized below:

IgG units value (Units)	1.4	1.7	8.3	22.2	36.8	53.8	79.3	139.3
Intra-assay Sdev	0.1	0.1	0.8	1.7	2.5	3.4	4.9	6.8
%CV	8.1	7.1	9.9	7.8	6.7	6.4	6.2	4.9
Inter-assay Sdev	0.2	0.1	0.4	1.2	1.6	2.3	3.3	4.2
%CV	12.0	8.3	5.2	5.3	4.5	4.2	4.2	3.0

Another eight samples were tested as six replicates on the PS/PT IgM ELISA on the same six different days (total n= 36). The results are summarized below.

IgM units value (Units)	3.1	3.2	9.8	18.2	36.0	58.2	75.8	153.2
Intra-assay Sdev	0.2	0.2	0.6	0.7	1.7	2.6	3.3	5.9
%CV	5.8	6.4	6.3	3.8	4.6	4.5	4.4	3.8
Inter assay Sdev	0.3	0.2	0.4	0.5	1.1	2.1	2.4	4.3
%CV	9.9	7.4	3.8	2.6	3.0	3.6	3.2	2.8

For each assay, 2 additional clinical samples were run, one near the clinical decision point (30 units) and one near the top of the measuring range of each assay. These samples were run in duplicate over 3 days for a total of 6 runs. These results are summarized below:

PS/PT IgG Units

Sample	Avg Units	SD	%CV
1	40.4	4.16	10.3
2	159.15	17.53	11.0

PS/PT IgM Units

Sample	Avg Units	SD	%CV
1	31.5	3.38	10.7
2	255.9	16.55	6.5

b. Linearity/assay reportable range:

To assess the linearity of the PS/PT IgG and PS/PT IgM assays, 3 high positive samples at or near the upper limit of the assay range (150 units) and one sample in the mid-range of the assays (about 75 units) were serially diluted and run on each of the PS/PT IgG and PS/PT IgM assays. Observed vs. expected values were plotted. R^2 values for the curves generated ranged from 0.932 to 0.992 for PS/PT IgG and 0.967 to 0.986 for PS/PT IgM. The linear range is 5.1-150 units for IgG and 3.6-150 units for IgM.

PS/PT IgG

Dilution (neg. serum + sample)	**Sample H1 (Units)**		**Sample H2 (Units)**		**Sample H4 (Units)**		**Sample H5 (Units)**	
	Obs	**Exp**	**Obs**	**Exp**	**Obs**	**Exp**	**Obs**	**Exp**
0.875+0.125	38.6	22.9	22.0	22.3	9.4	12.8	8.8	9.2
0.750+0.250	58.8	45.9	32.4	44.7	21.8	25.6	17.0	18.5
0.625+0.375	79.7	69.1	59.4	67.3	34.9	38.6	24.8	27.8
0.500+0.500	107.5	91.9	76.1	89.5	45.1	51.4	29.7	36.9
0.375+0.625	115.7	114.9	88.7	111.8	56.3	64.3	40.9	46.2
0.250+0.750	151.1	138.3	111.9	134.5	71.0	77.2	51.9	55.6
0.125+0.875	139.2	161.3	112.6	157	81.1	90.1	54.6	64.8
H1	183.9	183.9	179	179	102.7	102.7	73.9	73.9
Regression eq.	y=1.13x– 20.34		y=1.05x+ 11.19		y=1.00x + 4.92		y=1.03x + 2.72	
R^2	0.962		0.932		0.992		0.976	

PS/PT IgM

Dilution (neg. serum + sample)	Sample H1 (Units)		Sample H2 (Units)		Sample H3 (Units)		Sample H5 (Units)	
	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
0.875+0.125	31.7	19.5	21.1	19.3	28.1	19.9	8.8	11.1
0.750+0.250	46.7	38.9	38.7	38.6	51.9	39.9	21.7	22.2
0.625+0.375	78.2	58.6	65.7	57.9	74.4	60.1	34.9	33.3
0.500+0.500	85.1	77.9	75.6	77.1	88.1	79.9	40.9	44.3
0.375+0.625	115.4	97.4	90.6	96.4	104.9	99.9	49.0	55.4
0.250+0.750	108	117.2	103.8	115.9	122.4	120.2	57.1	66.6
0.125+0.875	145.1	136.8	126.5	135.2	124.5	140.3	64.6	77.7
H1	155.9	155.9	154.2	154.2	159.9	159.9	88.6	88.6
Regression eq.	y=1.07x– 14.62		y=1.065x-3.23		y=1.14x-17.2		y=1.06x 1.27	
R^2	0.966		0.986		0.978		0.968	

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no internationally recognized standard for PS/PT. Calibrators and controls are prepared in-house. The results are reported in arbitrary units.

Stability:

Microwell plates:

Three separate lots of coated plates were placed in 37°C incubators and then tested at 7, 14, 21, and 28 days with 5 standards for varying reactivity and a positive control. Optical densities at each time point were compared back to a plate stored at 4°C. For all test points, opened, but resealable foil mylar pouches were used. An average remaining activity of over 85% was maintained through the 28 day period.

Standard and controls:

Two different lots of standards and controls were tested at 7, 14, 21, and 28 days at 37°C and compared to material kept at 4°C. Over 90% activity was maintained at 21 days.

d. *Detection limit:*
The limit of blank was determined by running three different lots of Reagent blank (sample diluent) five times each, calculating the average and the standard deviation. The results are as follows:

PS/PT IgG		PS/PT IgM	
	UNITS		UNITS
AVG	2.5	AVG	0.70
STDEV	0.23368	STDEV	0.04522

The limit of detection was assessed by serially diluting 2 high positive PS/PT IgG and 2 high positive PS/PT IgM sera (1:100 to 1:3,276,800), and the diluted

samples were run on the PS/PT IgG and PS/PT IgM tests. The dilution below which the OD plateaus out was noted. For both the IgG and IgM tests, the dilution of 1:25,600 was empirically determined to yield the lowest limit of detection. All four sera were run 16 times at this dilution. The results are below:

PS/PT IgG		**PS/PT IgM**	
Sample #1978	**UNITS**	**Sample #62**	**UNITS**
AVG	7.2	AVG	3.2
STDEV	1.172	STDEV	0.516
Sample #343	**UNITS**	**Sample #676**	**UNITS**
AVG	3.172	AVG	4.5
STDEV	0.419	STDEV	0.361

The limit of detection for the PS/PT IgG assay was calculated to be 5.1 (0.795 SD) and the limit of detection for the PS/PT IgM assay is 3.8 (0.438 SD).

e. Analytical specificity:

1. Interference:
   Three serum samples were selected for each PS/PT assay. One sample was negative, one near the clinical decision point and one a high positive after spiking with pooled NHS (normal human serum) @ 1:1 dilution. Interferents tested were bilirubin; triglyceride; RF IgM positive serum (final concentration- 77 RLU and hemoglobin (final concentration – 200 mg/dL).

Interferent	PS/PT IgG Units	PS/PT IgM Units
RF IgM Pos (77 RLU)	5.7	4.1
Triglyceride (508 mg/dL)	4.8	10.5
Bilirubin (15 mg/dL)	6.8	4.3
NHS (pooled normal human serum for diluting test samples)	2.7	4.8
Hemoglobin		

PS/PT IgG

Sample	Control (Units)	Sample + Triglyceride (Units)	% Change	Sample + Bilirubin (Units)	% Change	Sample + Hemoglobin (Units)	% Change	Sample + RF IgM (Units)	% Change
1	4.1	5.3	+29.2%	5.6	+36.5%	4	-2.4%	4.9	+19.5%
2	32.4	37.2	+14.8%	37.2	+14.8%	34.5	+6.5%	33	+1.8%
3	151	140.9	-6.7%	171.8	+13.7%	173.5	+14.9%	165.8	+9.8%

PS/PT IgM

Sample	Control (Units)	Sample + Triglyceride (Units)	% Change	Sample + Bilirubin (Units)	% Change	Sample + Hemoglobin (Units)	% Change	Sample + RF IgM (Units)	% Change
1	4.9	7.9	+61%	5.5	+12.2%	4.1	+16.0%	4.5	-8.1%
2	28.4	31	+9%	28.9	+1.8%	25.9	-8.8%	28.6	+0.7%
3	131.1	105.8	-19.8%	136.5	+4.1%	125.7	-4.1%	116.5	-10.9%

f. Assay cut-off:

A total of 382 clinically defined samples; including 247 normals, 24 known Lupus Anticoagulant (LA) positive, 71 anti-phospholipid syndrome (APS) positive and 40 non-APS disease controls; were tested with the PS/PT IgG and IgM kits. A non-parametric rank order analysis of the data was performed and a cut-off of 30 units was chosen.

2. Comparison studies:

a. Method comparison with predicate device:

Two method comparison studies were performed at two different sites, one internal and one external. In both studies, the PS/PT IgG and IgM assays were compared with the predicate assay.

A total of 62 samples were used for the IgG kit comparison and 63 samples were used for the IgM kit comparison. All samples had values within the claimed linear range of the assays. The results are below:

IgG PS/PT ELISA				
		Positive	Negative	Total
IgG ß2 GPI ELISA	Positive	13	7	20
	Negative	6	36	42
	Total	19	43	62
Positive Percent Agreement		65% (95% CI: 44.1-85.9)		
Negative Percent Agreement		83.7% (95% CI: 75.1-96.3)		
Relative Agreement		77.8% (95% CI: 70.5-87.5)		

IgM PS/PT ELISA				
		Positive	Negative	Total
IgG ß2 GPI ELISA	Positive	13	3	16
	Negative	11	36	47
	Total	24	39	63
Positive Percent Agreement		81.3% (95% CI: 62.1-100)		
Negative Percent Agreement		76.6% (95% CI: 64.5-88.7)		
Relative Agreement		77.8% (95%CI: 68.2-86.4)		

The low percent agreements between the predicate and the new devices are due to the fact that different analytes are being measured.

b. Matrix comparison:

Twelve samples submitted for LAC testing at an outside laboratory (6 positive and 6 negative) were tested. Half of each sample was converted to serum by addition of calcium, followed by freeze-thawing and then centrifugation. The 24 paired citrated plasma/serum samples were then run on both the IgG and IgM PS/PT kits. The results are shown below. The values between serum and plasma samples had coefficients of correlation of over 0.99% with slopes ranging from 0.92-1.04.

Sample	**PS/PT IgG (Units)**		**PS/PT IgM (Units)**	
	Plasma	Serum	Plasma	Serum
Ca	134.1	129.4	52.5	49.3
C2	149.3	148.5	61.1	61.2
C5	135.7	125.7	53.3	53.3
L0	26.2	26	103.1	113
V1	251.9	239.9	9.6	9.7
V2	245.6	236	9.1	8.3
1	2.4	2.5	8.7	8
2	15.7	14.2	22.1	15.5
3	2.8	2.4	7.1	5.4
5	5.5	5.9	4.9	4.4
6	2.4	2.3	7.3	5.8
7	3.2	3.7	6.7	5.4
R value	**0.999**		**0.996**	
Intercept	**0.203**		**2.73**	
Slope	**1.043**		**0.921**	

3. Clinical studies:
   a. Clinical Sensitivity and Specificity:
      Two hundred forty-seven normal samples along with 24 lupus anticoagulant positive, 71 Anti-Phospholipid Syndrome (APS) positive samples, and samples from other similar conditions were tested with the QUANTA Lite™ aPS/PT IgG and IgM kits. Testing was done at one internal and one external site. The results are tabulated in the table below.

      The sensitivity of the IgG Assay is 58% (95% CI: 47%-68%) and the sensitivity of the IgM assay is 56% (95% CI: 45.2%-66%). The combined sensitivity of both is 76%.

      The specificity of the IgG Assay is calculated to be 99% (95% CI: 97.1%-99.8%), and the specificity of the IgM assay is 98.3% (95% CI: 96%-99.5%). The combined specificity is 97.2%.

	Number Positive (percent)			
Patient Group	No. Samples	PS/PT IgG	PS/PT IgM	IgG and/or IgM
Normals	247	3 (1.2)	4 (1.6)	7 (2.8)
Lupus Anticoagulant (LAC) Positive	24	21 (87.5)	19 (79.2)	24 (100)
Anti-Phospholipid Syndrome (APS)	71	33 (46.5)	34 (47.9)	48 (67.6)
Rheumatoid Arthritis	6	0	0	0
Crohn's Disease	2	0	0	0
Ulcerative Colitis	2	0	0	0
Celiac disease	5	0	0	0

	Number Positive (percent)			
Patient Group	No. Samples	PS/PT IgG	PS/PT IgM	IgG and/or IgM
Lupus Anticoagulant (LAC) negative	8	0	1 (12.5)	1 (12.5)
Infectious disease (CMV, Toxo, rubella, HSV, HBV, HCV)	14	0	0	0
Syphilis	12	0	0	0
Actin antibody positive	1	0	0	0
H. Pylori	2	0	0	0

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

Clinical Concordance with ß2GPI IgG and ß2GPI IgM assays - The QUANTA Lite™ aPS/PT IgG and IgM kits were compared with the QUANTA Lite™ ß2GPI IgG and ß2GPI IgM assays on clinical samples. The devices measure different analytes, but they are used as aids in the diagnosis of autoimmune thrombotic diseases in the SLE and lupus-like disease populations. The samples tested included 71 APS patients, 24 Lupus anticoagulant positive patients, and 85 normals. The results are summarized below:

IgG PS/PT ELISA				
		Positive	Negative	Total
IgG ß2 GPI ELISA	Positive	38	10**	48
	Negative	16*	116	132
	Total	54	126	180
Positive Percent Agreement		79.2%		
Negative Percent Agreement		87.9%		
Overall Agreement		85.6%		

*1/16 was LAC positive, 15/16 were APS patients

**All 10 were from the APS group

IgM PS/PT ELISA				
		Positive	Negative	Total
IgM ß2 GPI ELISA	Positive	28	7*	35
	Negative	25**	120	155
	Total	53	127	180
Positive Percent Agreement		80%		
Negative Percent Agreement		77.4%		
Overall Agreement		82.2%		

*1 was normal and 6 were APS patients

**22 were APS patients and 3 were LAC positives

4. Clinical cut-off**:**
   Not applicable
5. Expected values/Reference range:
   Not applicable

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.