

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

**A. 510(k) Number:**

k141274

**B. Purpose for Submission:**

New device

**C. Measurand:**

Anti- $\beta$ 2GP1-Domain 1 autoantibodies

**D. Type of Test:**

Semi-quantitative chemiluminescent immunoassay

**E. Applicant:**

INOVA Diagnostics, Inc.

**F. Proprietary and Established Names:**

QUANTA Flash®  $\beta$ 2GP1-Domain1  
QUANTA Flash®  $\beta$ 2GP1-Domain1 Controls  
HemosIL® Acustar Anti- $\beta$ 2GPI-Domain 1  
HemosIL® Acustar Anti- $\beta$ 2GPI-Domain 1 Controls

**G. Regulatory Information:**

1. Regulation section:  
21 CFR §866.5660, Multiple autoantibodies immunological test system  
21 CFR § 862.1660, Quality control material (assayed and unassayed)
2. Classification:  
Class II (assay)  
Class I (controls)
3. Product code:  
MSV, System, Test, Antibodies, B2-Glycoprotein I (B2-GPI)  
JJX, Analyte Controls (Assayed and Unassayed)

4. Panel:  
Immunology (82)  
Clinical Chemistry (75)

## **H. Intended Use:**

1. Intended use(s):

### QUANTA Flash® $\beta$ 2GP1-Domain1

The QUANTA Flash®  $\beta$ 2GP1-Domain1 is an *in vitro* chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to  $\beta$ 2GP1-Domain1 in human serum. The presence of anti- $\beta$ 2GP1-Domain1 autoantibodies is used in conjunction with clinical and other laboratory findings as an aid in the diagnosis of antiphospholipid syndrome. The QUANTA Flash®  $\beta$ 2GP1-Domain1 is not intended to replace assays for antibodies against the whole  $\beta$ 2GP1 molecule. Testing for antibodies to the whole  $\beta$ 2GP1 molecule is required according to the classification criteria for antiphospholipid syndrome.

### QUANTA Flash® $\beta$ 2GP1-Domain1 Controls

The QUANTA Flash®  $\beta$ 2GP1-Domain1 Controls are intended for quality control purposes of the QUANTA Flash®  $\beta$ 2GP1-Domain1 chemiluminescent immunoassay (CIA) kit.

### HemosIL® AcuStar Anti- $\beta$ 2GPI Domain 1

The HemosIL® Acustar Anti- $\beta$ 2GPI Domain 1 is an *in vitro* chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to  $\beta$ 2GP1-Domain 1 in human serum. The presence of anti- $\beta$ 2GP1-Domain 1 autoantibodies is used in conjunction with clinical and other laboratory findings as an aid in the diagnosis of antiphospholipid syndrome. The HemosIL® Acustar Anti- $\beta$ 2GPI Domain 1 is not intended to replace assays for antibodies against the whole  $\beta$ 2GP1 molecule. Testing for antibodies to the whole  $\beta$ 2GP1 molecule is required according to the classification criteria for antiphospholipid syndrome.

### HemosIL® AcuStar Anti- $\beta$ 2GPI Domain 1 Controls

The HemosIL® AcuStar Anti- $\beta$ 2GPI Domain 1 Controls are intended for quality control purposes of the HemosIL® Anti- $\beta$ 2GPI Domain 1 chemiluminescent immunoassay (CIA) kit.

2. Indication(s) for use:

Same as Intended use

3. Special conditions for use statement(s):

For Prescription use only

4. Special instrument requirements:

BIO-FLASH® or ACL AcuStar instrument (k083518)

**I. Device Description:**

Since the QUANTA Flash®  $\beta$ 2GP1-Domain1 assay and the HemosIL® AcuStar  $\beta$ 2GP1-Domain 1 assay are identical, device description for the QUANTA Flash®  $\beta$ 2GP1-Domain1 assay only is shown below.

The QUANTA Flash®  $\beta$ 2GP1-Domain1 kit contains the following materials:

1. QUANTA Flash  $\beta$ 2GP1-Domain1 Reagent Cartridge, contains the following reagents for 50 determinations:
  - a.  $\beta$ 2GP1-Domain1 coated paramagnetic beads, preserved prior to first time use.
  - b. Assay Buffer – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers, and preservatives.
  - c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies, containing buffer, protein stabilizers and preservative.
  - d. Sample diluent –containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.
2. Resuspension buffer: 1 vial - colorless, containing buffer, protein stabilizers and preservatives.
3. QUANTA Flash  $\beta$ 2GP1-Domain1 Calibrator 1, containing: 1 x 1 mL barcoded tube of a solution with human antibodies to  $\beta$ 2GP1-Domain1 in buffer, stabilizers, and preservatives.
4. QUANTA Flash  $\beta$ 2GP1-Domain1 Calibrator 2, containing: 1 x 1 mL barcoded tube of a solution with human antibodies to  $\beta$ 2GP1-Domain1 in buffer, stabilizers, and preservatives.

The QUANTA Flash®  $\beta$ 2GP1-Domain1 Controls kit contains two vials of Negative Control and two vials of Positive Control:

1. QUANTA Flash  $\beta$ 2GP1-Domain1 Low Control: Three (3) barcode labeled tubes containing 1.0 mL, ready to use reagent. Controls contain human antibodies to  $\beta$ 2GP1-Domain1 in stabilizers and preservatives.
2. QUANTA Flash  $\beta$ 2GP1-Domain1 High Control: Three (3) barcode labeled tubes containing 1.0 mL, ready to use reagent. Controls contain human antibodies to  $\beta$ 2GP1-Domain1 in stabilizers and preservatives.

The QUANTA Flash®  $\beta$ 2GP1-Domain1 assay is designed to run on the BIO-FLASH®

instrument. This platform is a fully automated closed system with continuous load and random access capabilities that automatically processes the samples, runs the assay and reports the results. It includes liquid handling hardware, luminometer and computer with software-user interface. The QUANTA Flash®  $\beta$ 2GP1-Domain1 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

QUANTA Lite®  $\beta$ 2GP1 ELISA

2. Predicate 510(k) number(s):

k970551

3. Comparison with predicate:

QUANTA Flash  $\beta$ 2GP1-Domain1 Assay and Calibrators/ HemosIL AcuStar  $\beta$ 2GPI-Domain 1 Assay and Calibrators:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	QUANTA Flash® $\beta$ 2GP1-Domain1	QUANTA Lite® $\beta$ 2 GP1 IgG
Assay methodology	Solid phase (heterogeneous) immunoassay	Solid phase (heterogeneous) immunoassay
Assay and Calibrator Shelf life	One year	One year
Assay and Calibrator Storage	2-8 °C	2-8 °C
Sample type	Serum	Serum

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	QUANTA Flash® $\beta$ 2GP1-Domain1	QUANTA Lite® $\beta$ 2 GP1 IgG
Intended use	The QUANTA Flash® $\beta$ 2GP1-Domain1 and the HemosIL® Acustar Anti- $\beta$ 2GPI Domain 1 are in vitro chemiluminescent immunoassays (CIA) for the semi-quantitative determination of IgG autoantibodies to $\beta$ 2GP1-Domain1 in human serum or citrated plasma. The presence of anti- $\beta$ 2GP1-Domain1 autoantibodies is used in conjunction with clinical and	QUANTA Lite® $\beta$ 2 GP1 IgG is an enzyme linked immunoassay (ELISA) for the semi-quantitative detection of $\beta$ 2 GP1 IgG antibodies in human serum. The presence of $\beta$ 2Gp1 IgG antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of certain autoimmune disease thrombotic disorders such as

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	other laboratory findings as an aid in the diagnosis of antiphospholipid syndrome. The QUANTA Flash® $\beta$ 2GPI-Domain1 and the HemosIL® Acustar Anti- $\beta$ 2GPI Domain 1 are not intended to replace assays for antibodies against the whole $\beta$ 2GPI molecule. Testing for antibodies to the whole $\beta$ 2GPI molecule is required according to the classification criteria for antiphospholipid syndrome.	those secondary to systemic lupus erythematosus (SLE) or other lupus-like thrombotic diseases.
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well plate
Instrumentation	Automated on BIO-FLASH® instrument	Manual
Antigen	Recombinant domain1 of $\beta$ 2-Glycoprotein1	Purified $\beta$ 2-Glycoprotein1
Conjugate	Isoluminol conjugated anti-human IgG	HRP conjugated anti-human IgG
Signal Detected	Luminescence (visible light)	Absorbance at 450 nm
Calibration	Lot specific Master Curve + two calibrators (included in kit)	Five lot specific calibrators (included in kit)
Units	< 20 CU: Negative result  $\geq$ 20 CU: Positive result	< 20 SGU: Negative result  $\geq$ 20 SGU: Positive result
Analytical Measuring Range	3.6 – 1380.4 CU	9.4 – 150.0 SGU

QUANTA Flash  $\beta$ 2GPI-Domain1 Controls/ HemosIL AcuStar  $\beta$ 2GPI-Domain 1 Controls:

<b>Similarities</b>		
<b>Item</b>	<b>QUANTA Flash <math>\beta</math>2GPI-Domain1 Controls</b>	<b>Predicate Device</b>
Matrix	Human serum, stabilizers, and preservative	Human serum, stabilizers, and preservative
Physico-chemical characteristics	Liquid, ready to use	Liquid, pre-diluted, ready to use
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

<b>Differences</b>		
<b>Item</b>	<b>QUANTA Flash <math>\beta</math>2GP1-Domain1 Controls</b>	<b>Predicate Device</b>
Intended use	The QUANTA Flash $\beta$ 2GP1-Domain1 Controls are intended for quality control purposes of the QUANTA Flash $\beta$ 2GP1-Domain1 chemiluminescent immunoassay (CIA) kit.	No separate intended use; controls are part of the QUANTA Lite® $\beta$ 2GP1 kit.
Analyte	Anti- $\beta$ 2GP1-Domain 1 antibodies	Anti- $\beta$ 2GP1 antibodies
Method	QUANTA Flash® $\beta$ 2GP1-Domain1 Chemiluminescent immunoassay	QUANTA Lite® $\beta$ 2GP1 ELISA
Unit	QUANTA Flash (CU) Arbitrary Units (U/mL)	SGU
Levels	2 (low and high)	2 (negative and positive)

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

CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition

CLSI EP17-A, Protocols for determination of limits of detection and limits of quantitation

CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

CLSI EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (Interim Revision)

CLSI EP07-A, Interference Testing in Clinical Chemistry; Approved Guideline

**L. Test Principle:**

Recombinant  $\beta$ 2GP1-Domain1 protein is coated onto paramagnetic beads, which are stored lyophilized in the reagent cartridge. The reagent pack is prepared for use in the BIO-FLASH® system by pressing down on the grey lid of the reagent pack to pierce the induction seals on the reagent tubes. Once the seals are broken, the beads are rehydrated by adding the entire contents of the vial of resuspension buffer to the bead reagent tube using the transfer pipette supplied with the kit. Only the hole above the bead reagent tube is accessible at this point. The beads are then mixed with the resuspension buffer by pipetting up and down 30 times. This amount of mixing ensures complete resuspension of the beads. The label covering the remaining three reagent holes is now removed, and the reagent cartridge is then loaded onto the BIO-FLASH instrument. Samples are also loaded onto the instrument in sample racks. A patient serum sample is pre-diluted 1:10 by the BIO-FLASH with system rinse in a small disposable plastic cuvette. Small amounts of the diluted patient serum, the

beads, and assay buffer are all combined into a second cuvette, and mixed. This cuvette is then incubated at 37°C. The beads are magnetized and washed several times. Isoluminol conjugated anti-human IgG antibodies are then added to the cuvette, and again incubated at 37°C. The beads are magnetized and washed repeatedly. The isoluminol conjugate is oxidized when Trigger 1 and Trigger 2 are added to the cuvette, and the flash of light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH optical system. The RLU are proportional to the amount of isoluminol conjugate that is bound to the human IgG, which is in turn proportional to the amount of anti-  $\beta$ 2GP1-Domain1 antibodies bound to the corresponding  $\beta$ 2GP1-Domain1 on the beads.

The QUANTA Flash®  $\beta$ 2GP1-Domain1 assay utilizes a pre-defined lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. Every new lot number of reagent cartridge must be calibrated before first use, with the QUANTA Flash®  $\beta$ 2GP1-Domain1 Calibrators. Based on the results obtained with the two Calibrators included in the reagent kit, an instrument specific Working Curve is created, which is used to calculate chemiluminescent units (CU) from the instrument signal (RLU) obtained for each sample.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

Since the QUANTA Flash  $\beta$ 2GP1-Domain1 assay and the HemosIL AcuStar  $\beta$ 2GPI-Domain 1 assay are identical, performance data for only the QUANTA Flash  $\beta$ 2GP1-Domain1 assay is shown below.

*a. Precision/Reproducibility:*

Precision of the QUANTA Flash®  $\beta$ 2GP1-Domain1 assay was assessed in accordance with CLSI EP5-A2, by running 8 serum samples that were selected to cover the analytical measuring range of the assay, including samples around the medical decision point. Patients were run using duplicate aliquots, twice a day, for 20 days for a total of 80 replicates. Controls were run as quality control during each run. A working curve was generated using calibrators prior to run 1 on day 1. Total %CV values were within the manufacturer’s pre-determined acceptance limit of  $\leq 10\%$  - 15%. The results were analyzed by Analyse-It.

Sample	Mean (CU)	Within Run		Between-Run		Between-Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	10.4	0.9	8.3%	0.0	0.0%	0.5	4.3%	1.0	9.4%
2	18.4	0.9	4.6%	0.5	2.9%	0.5	2.6%	1.1	6.0%
3	22.8	1.4	6.1%	0.9	3.8%	0.0	0.0%	1.6	7.2%
4	54.3	2.5	4.6%	1.4	2.5%	1.2	2.2%	3.1	5.6%
5	99.6	6.4	6.5%	0.0	0.0%	0.0	0.0%	6.4	6.5%
6	318.3	18.6	5.9%	0.0	0.0%	4.9	1.6%	19.3	6.1%

7	538.1	30.3	5.6%	7.9	1.5%	28.7	5.3%	42.5	7.9%
8	947.8	82.8	8.7%	57.3	6.0%	0.0	0.0%	100.7	10.6%

**Lot-to-Lot:**

A separate lot-to-lot comparison study was performed using 26 samples from INOVA's serum bank covering the analytical measuring range of the assay and positive and negative controls. Samples were tested in duplicate with three different reagent lots. All results were within the manufacturer's pre-defined acceptance.

*b. Linearity/assay reportable range:*

The linearity of the analytical measuring range (AMR, 3.6 – 1380.4 CU) was evaluated by a study according to CLSI EP6-A. Five serum samples with various  $\beta$ 2GP1-Domain1 antibody concentrations were serially diluted to obtain 10 dilutions of each sample. Percent recovery of obtained results was calculated compared to the expected results. Obtained values were plotted against expected values, and linear regression analysis was performed using Analyse-It software. The slope and intercept of the regression line were calculated with 95% CI as well as the R<sup>2</sup> values. All samples met the manufacturer's pre-defined acceptance criteria. Results of the study are summarized below:

Sample	Test Range (CU)	Slope (95% CI)	y-intercept (95% CI)	R <sup>2</sup>	Recovery Range
1	164.1 – 1640.7	1.04 (0.98 – 1.09)	-36.8 (-93.8 to -0.2)	0.99	95.1 – 106.2%
2	45.5 – 454.7	0.99 (0.96 – 1.03)	0.2 (-10.3 to 10.6)	0.99	95.5 – 104.1%
3	12.5 – 124.9	1.03 (1.00 – 1.05)	-1.9 (-3.9 to 0.1)	1.00	94.3 – 103.3%
4	8.1 – 81.1	1.01 (0.95 – 1.08)	-2.1 (-5.4 to 1.3)	0.98	83.9 – 106.7 %
5	1.0 – 10.3	0.96 (0.91 – 1.01)	0.2 (-0.1 to 0.5)	0.99	90.9 – 113.9 %

The AMR is defined by the values of the lowest and highest Master Curve Standards. The QUANTA Flash®  $\beta$ 2GP1-Domain1 AMR is 3.6 CU to 1380.4 CU.

High dose hook effect:

To assess hook effect, the measurement signal (relative light units, RLU) was examined for four high positive specimens, before and after automatic or manual dilution. All sera produced significantly higher RLU values (above the AMR) when used "as is" compared to the manually or automatically diluted values, thereby confirming that high positive specimens above the analytical measuring range do not show hook effect up to 435,000 RLU.

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

Traceability:

There are currently no international reference standards for anti-β2GP1-Domain1 antibodies. The assay is calibrated in relative arbitrary units (CU or U/mL). The QUANTA Flash® β2GP1-Domain1 Calibrators and Controls are manufactured by diluting human serum containing high titers of anti- β2GP1-Domain1 antibodies with antibody stabilizer buffer. The human serum is obtained from commercial sources and is tested for markers of infectious substances.

Value Assignment:

The QUANTA Flash® β2GP1-Domain1 assay utilizes a pre-defined lot specific Master Curve that is stored in the reagent pack barcode. The target CU is achieved through trial dilutions on small scale. Once a dilution is selected, the Calibrators and Control are bulked, tested, and adjusted. Calibrators and Controls are tested on at least two instruments, on at least two lots of reagent cartridge, in replicates of 10 to determine final value assignment.

Stability:

Stability studies have been performed and the results support the following claims:

*Sample Stability:*

Samples were shown to be stable (defined by the manufacturer as recovery of  $\leq \pm 15\%$ ) for up to 48 hours at room temperature, 21 days at 2-8°C, and can undergo freeze/thaw up to 3 times.

*Reagent Pack Stability:*

*Shelf Life:* All components tested by accelerated stability testing (4 weeks at 37°C) fulfilled the manufacturer's pre-defined acceptance criteria, therefore one year expiration dating was assigned to each component stored, unopened, at 2-8°C. Real time stability is ongoing and currently supports a claim of 12 months.

*Open/In use (onboard) Stability:*

Opened reagent packs must be stored on-board the instrument. The in-use (on-board) stability of the reagent cartridge was set at 60 days.

*Calibration Stability:*

*Shelf Life:* Calibrators tested by accelerated stability testing (4 weeks at 37°C) fulfilled the manufacturer's pre-defined acceptance criteria, supporting assignment of a one year expiration dating to each component at 2-8°C. Real time stability is ongoing and currently supports a claim of 12 months.

*In use (onboard) Stability:*

Calibrators can be used for up to 4 calibrations over an 8 hour period onboard the instrument.

*Control Stability:*

*Shelf Life:* Controls tested by accelerated stability testing (4 weeks at 37°C) fulfilled the manufacturer's acceptance criteria, so one year expiration dating was assigned to each component at 2-8°C. Real time stability is ongoing and currently supports a claim of 12 months.

*Open/In use (onboard) Stability:* The controls were given a maximum of 15 uses with a maximum of 10 minutes onboard the instrument per use. The total time the control tubes can be used is 2.5 hours or 10 minutes per use.

*d. Detection limit:*

Limit of Blank (LoB):

For the QUANTA Flash  $\beta$ 2GP1-Domain1 assay, the LoB was determined according to CLSI EP17-A guideline by running 58 measurements on blank samples.

For the QUANTA Flash  $\beta$ 2GP1-Domain1 assay the LoB was determined to 425.7 RLU. The LoB value is below the bottom limit of the curve that the instrument uses to calculate chemiluminescent units (CU), and therefore cannot be converted into CU.

Limit of Detection (LoD):

The LoD of the QUANTA Flash®  $\beta$ 2GP1-Domain1 assay is 1296 RLU, which is below the analytical measuring range of the assay. LoD was determined using the CLSI EP17-A guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 264 determinations, with 144 measurements on blank samples and 120 measurements of low level samples. The LoD value is below the bottom limit of the curve that the instrument uses to calculate chemiluminescent units (CU), and therefore cannot be converted into CU. The LoD is below the lower limit of the reportable range, e.g. < 3.6 CU.

*e. Analytical specificity:*

Interference:

The interference study was performed according to CLSI EP07-A2. Three specimens were tested (negative: 10.7 CU; positive: 55.9 CU; high positive: 446.6 CU). Each interfering substance was spiked into each specimen at three different concentrations per CLSI EP07-A2 and the resulting samples were assessed in triplicate. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluents (10% of total).

No interference (defined by the manufacturer as  $\leq \pm 15\%$ , or  $\pm 4$  CU difference, whichever is greater) was detected with bilirubin up to 100 mg/dL (recovery: 92.9% to 97.6%), hemoglobin up to 200 mg/dL (recovery: 95.6% to 97.2%), triglycerides up to 1000 mg/dL (recovery: 92.5% to 100%), cholesterol up to 224.3 mg/dL (recovery: 92.5% to 100%), and RF IgM up to 500 IU/mL (recovery: 86.2% to 98.3%).

A statement indicating that grossly hemolyzed or icteric serum should not be used is included in the direction insert.

Cross-Reactivity:

A potential cross-reactivity of the QUANTA Flash®  $\beta$ 2GP1 Domain1 CIA with other autoantibodies was evaluated with 111 clinical samples with unknown disease states, most having high levels of various other autoantibodies. These samples were known to be positive for various other autoantibodies including: Sm, RNP, SS-A/Ro60, Ro52, SS-B, Jo-1, Scl-70, CENP, Ribosomal P, and DFS70. These are autoantibodies found in individuals with autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Sjögren’s Syndrome, scleroderma, and polymyositis patients. All samples, except for two, scleroderma samples, were negative for QUANTA Flash®  $\beta$ 2GP1 Domain1 CIA.

*f. Assay cut-off:*

The reference population for establishing the reference interval for the  $\beta$ 2GP1-Domain1 assay consisted of 30 subjects with various conditions including infectious diseases and SLE without a history of thrombotic events.

All specimens were the same matrix as specified in the Intended Use. All specimens were unaltered. The cut-off was established Using Analyse-it for Excel, to ensure optimal differentiation between negatives and positives, and found at 7880 RLU. This point was defined as 20 CU. A result below 20 CU is considered negative and greater than 20 CU is considered positive.

2. Comparison studies:

*a. Method comparison with predicate device:*

Samples for method comparison analysis included those samples from the clinical validation study (below) that were within the reportable range of the assay. For the first table for the analyte, only samples within the measuring ranges defined by the lowest and highest calibrators of the QUANTA Flash  $\beta$ 2GP1-Domain1 and predicate assay is included in the method comparison. The 238 samples in this cohort included 183 APS samples and eight contrived samples within  $\pm 25\%$  of the assay cut-off. Twenty-two samples (9.2%) were within  $\pm 25\%$  of the assay cut-off.

		Predicate ELISA		
		Positive	Negative	Total
QUANTA Flash $\beta$ 2GP1- Domain1 CIA	Positive	101	28	129
	Negative	10	99	109
	Total	111	127	238

Positive Percent Agreement (101/111): 91.0 % (95% CI: 84.1-95.6%)

Negative Percent Agreement (99/127): 78.0 % (95% CI: 69.7-84.8%)

Overall Percent Agreement (200/238): 84.0% (95%CI: 78.7-88.4%)

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and Clinical specificity:*

A set of 1090 samples, none of which were used in establishing the reference range, was used to validate the clinical performance of the QUANTA Flash  $\beta$ 2GP1-Domain1 CIA. The validation set consists of a set of clinically characterized sera from Primary APS, Secondary APS, and non-APS diseased controls. The results of the QUANTA Flash  $\beta$ 2GP1-Domain1 CIA device in each disease category are shown below:

APS Sub-Diagnosis	N	QUANTA Flash $\beta$ 2GP1-Domain1 % Positive (N)
Primary APS (pAPS)	180	56.6%(102)
Secondary APS (sAPS)*	90	40.0% (36)
<b>Total APS</b>	<b>270</b>	<b>51.1% (138)</b>

\* All patients with sAPS were diagnosed with SLE

Non-APS Diagnosis	N	QUANTA Flash $\beta$ 2GP1-Domain1 % Positive (N)
Hepatitis B virus (HBV)	21	0.0% (0.0)
Hepatitis C virus (HCV)	10	0.0% (0.0)
Syphilis	40	0.0% (0.0)
Crohn's Disease	104	1.0% (1)
Ulcerative Colitis	94	0.0% (0.0)
Rheumatoid Arthritis	168	0.0% (0.0)
Osteoarthritis	49	0.0% (0.0)
Scleroderma	127	1.6 % (2)
Others	24	0.0% (0.0)
Pre-eclampsia/eclampsia	34	0.0% (0.0)
Fetal loss no APS	45	0.0% (0.0)
SLE no APS	37	0.0% (0.0)
Thrombosis no APS	41	0.0% (0.0)
Atopic Dermatitis	26	0.0% (0.0)
<b>Total</b>	<b>820</b>	<b>0.5% (3)</b>

		Clinical Diagnosis		
		APS	Not APS	Total
QUANTA Flash β2GP1-Domain1	Positive	138	3	141
	Negative	132	817	949
	Total	270	820	1090

Sensitivity = 51.1% (95% CI: 45.0-57.2%)

Specificity = 99.6 % (95% CI: 98.9-99.9%)

While antibodies to Domain 1 of β2GP1 may be important in the pathology of APS, the test should be used in conjunction with whole β2GP1 molecule testing to aid in establishing a diagnosis of APS per the current practice guidelines outlined in the APS Classification Criteria<sup>1</sup> (Sydney 2006).

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

Not Applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The expected value in the normal population is “negative”. Anti- β2GP1-Domain1 antibody levels were analyzed in a cohort of 400 apparently healthy blood donors (191 females, ages 17 to 60 years, average age 32.2 years, and 209 males ages 17 to 60 years, average age 34.7 years) using the QUANTA Flash® β2GP1-Domain1. This patient population was different from the one that was used to establish the cut-off. With a cut-off of 20 CU, one sample (0.2%) was positive (34.2 CU) on the QUANTA Flash β2GP1-Domain1. The mean concentration was 3.8 CU, the median concentration 3.6 CU, and the values ranged from <3.6 to 34.2 CU.

#### **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.

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<sup>1</sup> Miyakis S. et. al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006, 4:295 – 306.