510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K152875

B. Purpose for Submission:

New device

C. Measurand:

Anti-β2GP1-Domain 1 autoantibodies

D. Type of Test:

Semi-quantitative chemiluminescent immunoassay

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Flash® β2GP1-Domain1
QUANTA Flash® β2GP1-Domain1 Controls
HemosIL® Acustar Anti-β2GPI-Domain 1
HemosIL® Acustar Anti-β2GPI-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® β2GP1-Domain1

   The QUANTA Flash® β2GP1-Domain1 is an *in vitro* chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to β2GP1-Domain1 in human serum or citrated plasma. The presence of anti-β2GP1-Domain1 autoantibodies is used in conjunction with clinical and other laboratory findings as an aid in the diagnosis of antiphospholipid syndrome. The QUANTA Flash® β2GP1-Domain1 is not intended to replace assays for antibodies against the whole β2GP1 molecule. Testing for antibodies to the whole β2GP1 molecule is required according to the classification criteria for antiphospholipid syndrome.

   QUANTA Flash® β2GP1-Domain1 Controls

   The QUANTA Flash ®β2GP1-Domain1 Controls are intended for quality control purposes of the QUANTA Flash ®β2GP1-Domain1 chemiluminescent immunoassay (CIA) kit.

   HemosIL® AcuStar Anti-β2GPI Domain 1

   The HemosIL® Acustar Anti-β2GPI Domain 1 is an *in vitro* chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to β2GP1-Domain 1 in human serum or citrated plasma. The presence of anti-β2GP1-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-β2GPI Domain 1 is not intended to replace assays for antibodies against the whole β2GP1 molecule. Testing for antibodies to the whole β2GP1 molecule is required according to the classification criteria for antiphospholipid syndrome.

   HemosIL® AcuStar Anti-β2GPI Domain 1 Controls

   The HemosIL® AcuStar Anti-β2GPI Domain 1 Controls are intended for quality control purposes of the HemosIL® Anti-β2GPI 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:

Because the QUANTA Flash® β2GP1-Domain1assay and the HemosIL® AcuStar β2GP1-Domain 1 assay are identical, the device description for the QUANTA Flash® β2GP1-Domain1 assay only is shown below.

The QUANTA Flash® β2GP1-Domain1 kit contains the following materials:

1. QUANTA Flash β2GP1-Domain1 Reagent Cartridge, contains the following reagents for 50 determinations:

   a. β2GP1-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 β2GP1-Domain1 Calibrator 1, containing: 1 x 1 mL barcoded tube of a solution with human antibodies to β2GP1-Domain1 in buffer, stabilizers, and preservatives.
4. QUANTA Flash β2GP1-Domain1 Calibrator 2, containing: 1 x 1 mL barcoded tube of a solution with human antibodies to β2GP1-Domain1 in buffer, stabilizers, and preservatives.

The QUANTA Flash® β2GP1-Domain1 Controls kit contains two vials of Negative Control and two vials of Positive Control:

1. QUANTA Flash β2GP1-Domain1 Low Control: Three (3) barcode labeled tubes containing 1.0 mL, ready to use reagent. Controls contain human antibodies to β2GP1-Domain1 in stabilizers and preservatives.
2. QUANTA Flash β2GP1-Domain1 High Control: Three (3) barcode labeled tubes containing 1.0 mL, ready to use reagent. Controls contain human antibodies to β2GP1-Domain1 in stabilizers and preservatives.

The QUANTA Flash® β2GP1-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® β2GP1-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® β2GP1 ELISA

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

   K970551

3. Comparison with predicate:

   QUANTA Flash β2GP1-Domain1 Assay and Calibrators/ HemosIL AcuStar β2GP1-Domain 1 Assay and Calibrators:

Similarities		
Item	New Device: QUANTA Flash® β2GP1-Domain1	Predicate: QUANTA Lite® ß2 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 ^{o}C	2-8 °C

Differences		
Item	New Device: QUANTA Flash® β2GP1-Domain1	Predicate: QUANTA Lite® ß2 GP1 IgG
Intended use	The QUANTA Flash® β2GP1-Domain1 and the HemosIL® Acustar Anti-β2GPI Domain 1 are in vitro chemiluminescent immunoassays (CIA) for the semi-quantitative determination of IgG autoantibodies to β2GP1-Domain1 in human serum or citrated plasma. The presence of anti-β2GP1-Domain1 autoantibodies is used in conjunction with clinical and other laboratory findings as an aid in the diagnosis of	QUANTA Lite® ß2 GP1 IgG is an enzyme linked immunoassay (ELISA) for the semi-quantitative detection of ß2 GP1 IgG antibodies in human serum. The presence of β2GP1 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 those secondary to systemic lupus erythematosus (SLE) or

Differences		
Item	New Device: QUANTA Flash® β2GP1-Domain1	Predicate: QUANTA Lite® ß2 GP1 IgG
	antiphospholipid syndrome. The QUANTA Flash® β2GP1-Domain1 and the HemosIL® Acustar Anti-β2GPI Domain 1 are not intended to replace assays for antibodies against the whole β2GP1 molecule. Testing for antibodies to the whole β2GP1 molecule is required according to the classification criteria for antiphospholipid syndrome.	other lupus-like thrombotic diseases.
Sample Type	Serum and citrated plasma	Serum only
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 ß2-Glycoprotein1	Purified ß2-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 ≥ 20 CU: Positive result	< 20 SGU: Negative result ≥ 20 SGU: Positive result
Analytical Measuring Range	3.6 – 1380.4 CU	9.4 – 150.0 SGU

QUANTA Flash β2GP1-Domain1 Controls/ HemosIL AcuStar β2GP1-Domain 1 Controls:

Similarities		
Item	QUANTA Flash β2GP1-Domain1 Controls	Predicate Device
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

Differences		
Item	QUANTA Flash β2GP1-Domain1 Controls	Predicate Device
Intended use	The QUANTA Flash β2GP1-Domain1 Controls are intended for quality control purposes of the QUANTA Flash β2GP1-Domain1 chemiluminescent immunoassay (CIA) kit.	No separate intended use; controls are part of the QUANTA Lite® β2GP1 kit.
Analyte	Anti-β2GP1-Domain 1 antibodies	Anti-β2GP1 antibodies
Method	QUANTA Flash® β2GP1-Domain1 Chemiluminescent immunoassay	QUANTA Lite® β2GP1 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 EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

CLSI EP09-A3, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Third Edition

CLSI EP15-A3, User Verification of Precision and Estimation of Bias; Approved Guideline—Third Edition

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

CLSI C28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

L. Test Principle:

Recombinant β2GP1-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 sample is pre-diluted by the BIO-FLASH with system rinse in a small disposable plastic cuvette. Small amounts of the diluted patient sample, 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- β2GP1-Domain1 antibodies bound to the corresponding β2GP1-Domain1 on the beads.

The QUANTA Flash® β2GP1-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® β2GP1-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:

   Because the QUANTA Flash β2GP1-Domain1 assay and the HemosIL AcuStar β2GP1-Domain 1 assay are identical, there is only one set of performance data and it is shown below (labeled QUANTA Flash β2GP1-Domain1 assay).

   The analytical performance of the QUANTA Flash β2GP1-Domain1 assay for use with human serum samples was demonstrated in K141274, as indicated in the sections below. This submission tested the performance of the QUANTA Flash β2GP1-Domain1 assay in human citrated plasma, and included a matrix comparison study and testing of precision and linearity in citrated plasma.

   a. Precision/Reproducibility:

   The manufacturer completed precision studies using human serum samples in submission K141274. These studies measured repeatability, within-laboratory, and lot-to-lot precision and all data met the manufacturer's predetermined acceptance criteria.

Precision of the QUANTA Flash® β2GP1-Domain1 assay in citrated plasma was assessed in accordance with CLSI EP15-A3, *User Verification of Precision and Estimation of Bias; Approved Guideline—Third Edition*. Three plasma samples with concentrations of measurand near the medical decision point, and at low and high positive values were tested with five replicates in a single run for five days (n = 25). Controls were run as quality control during each run. Total %CV values were within the manufacturer's pre-determined acceptance criteria. The acceptance criteria for the plasma precision studies were the same as for the serum precision studies performed in K141274.

		Within Run		Between-Day		Total	
Sample	Mean (CU)	SD	%CV	SD	%CV	SD	%CV
1	18.70	0.87	4.6%	1.16	6.2%	1.45	7.8%
2	91.98	2.32	2.5%	0.27	0.3%	2.34	2.5%
3	444.62	14.97	3.4%	7.93	1.8%	16.94	3.8%

b. Linearity/assay reportable range:

Linearity: The manufacturer completed linearity studies using human serum samples in submission K141274. These studies included five serially diluted serum samples that covered the analytical measuring range and all data met the manufacturer's predetermined acceptance criteria.

The linearity of the analytical measuring range (AMR, 3.6–1380.4 CU) in citrated plasma was evaluated by a study according to CLSI EP6-A, *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. Three citrated plasma samples with various β2GPβ2GP1-Domain1 antibody concentrations were serially diluted to obtain 10 dilutions of each sample. Percent recovery was calculated compared to the expected results. Obtained values were plotted against expected values, and linear regression analysis was used to analyze the results. The slope and intercept of the regression line were calculated with the 95% confidence intervals (95% CI) as well as the correlation coefficient (R^2) values. All samples met the manufacturer's pre-defined acceptance criteria. The acceptance criteria for the plasma linearity studies were the same as for the serum precision studies performed in K141274. Results of the study are summarized below:

Sample	Test Range (CU)	Slope (95% CI)	y-intercept (95% CI)	R^2	Recovery Range
1	135.8–1357.6	1.02 (0.96–1.08)	−58.04 (−108–−8.08)	0.99	86.6%–102.6%
2	18.2–182.2	0.97 (0.93–1.01)	−2.09 (−7.04–2.87)	0.99	90.1%–100.0%
3	3.5–17.7	0.96 (0.93–0.99)	0.49 (0.11–0.86)	1.00	98.2%–111.9%

The AMR is defined by the values of the lowest and highest Master Curve Standards. The QUANTA Flash® β2GP1-Domain1 AMR is 3.6 CU to 1380.4 CU.

High dose hook effect:

The manufacturer completed studies to measure the high dose hook effect in submission K141274.

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

The manufacturer completed studies to determine the traceability, value assignment, stability, and expected values in submission K141274. Stability studies included evaluation of sample stability, and the shelf life and on-board stability of the reagent pack, calibrators, and controls. All data met the manufacturer's predetermined acceptance criteria.

d. Detection limit:

The manufacturer completed studies to determine the limit of blank and limit of detection in submission K141274.

e. Analytical specificity:

The manufacturer completed studies to determine interferences and cross-reactivity with other auto-antibodies in submission K141274. In these studies, no interference 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 package insert.

f. Assay cut-off:

The manufacturer completed studies to determine the assay cut-off in submission K141274. 30 serum samples were tested and the cut-off was set to 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:

The manufacturer completed a method comparison with the predicate device in human serum samples in submission K141274. This included a total of 238 serum samples: 230 native samples and eight contrived samples, with 183 of the samples from patients with primary or secondary antiphospholipid syndrome, and 47 samples from patients with other autoimmune diseases. The percent agreement between the predicate and the QUANTA Flash β2GP1-Domain1 assay are summarized below:

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:

The manufacturer completed a matrix comparison study using 59 matched serum and citrated plasma samples. 29 of these matched samples were native, i.e., they were tested without any modification. 25 matched samples were spiked with anti-Domain1 antibodies. Five matched samples that were originally running above the AMR were tested after diluting with normal serum and plasma to obtain values within the AMR. Finally, 10 matched samples that were also tested as neat samples were diluted with normal serum and plasma to obtain two contrived matched pairs per sample. A total of 79 native, spiked, and diluted matched samples were tested. Analysis using weighted ($1/x^2$) linear regression showed that all the data met the manufacturer's predetermined acceptance criteria. The data are presented in the table below:

Equation of Regression Line	95% Confidence Interval of Slope	95% Confidence Interval of y-intercept
y = 1.009x + 0.11	(0.991–1.027)	(−0.13–0.34)

3. Clinical studies:

a. Clinical Sensitivity and Clinical specificity:

The manufacturer measured the clinical sensitivity and specificity of the QUANTA Flash β2GP1-Domain1 assay in submission K141274. A set of 1090 samples, none of which were used in establishing the reference range, was used to validate the clinical performance. The validation set consisted of a set of clinically characterized sera from Primary APS, Secondary APS, and non-APS diseased controls. The data

are summarized below:

		Predicate ELISA		
		Positive	Negative	Total
QUANTA Flash β2GP1- 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. Other clinical supportive data:

Not Applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

The manufacturer completed studies to determine the expected value and reference range in submission K141274. The expected value in the normal population is "negative". Anti- β2GPβ2GP1-Domain1 antibody levels were analyzed in a cohort of 400 apparently healthy blood donors. With a cut-off of 20 CU, one sample (0.2%) was positive (34.2 CU) using the QUANTA Flash β2GPβ2GP1-Domain1 assay. 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.