DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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FEB 18 2015

INOVA DIAGNOSTICS, INC.
C/O ROGER ALBESA
SUPERVISOR, RESEARCH AND DEVELOPMENT
9900 OLD GROVE ROAD
SAN DIEGO CA 92131-1638

Re: k141274
Trade/Device Name: QUANTA Flash® β2GP1-Domain1
QUANTA Flash® ß2GP1-Domain1 Controls
HemosIL® Acustar Anti-β2GPI-Domain 1
HemosIL® Acustar Anti-ß2GPI-Domain 1 Controls
Regulation Number: 21 CFR 866.5660
Regulation Name: Multiple autoantibodies immunological test system
Regulatory Class: Class II
Product Code: MSV, JJX
Dated: January 12, 2015
Received: January 14, 2015

Dear Mr. Albesa,

This letter corrects our substantially equivalent letter of February 13, 2015.

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Leonthena R. Carrington -A

Leonthena Carrington, MS, MBA, MT(ASCP)
Director (Acting)
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostics and
Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

# Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
*See PRA Statement below.*

510(k) Number *(if known)*
K141274

Device Name
QUANTA Flash® ß2GP1-Domain1, QUANTA Flash® ß2GP1-Domain1 Controls, HemosIL® AcuStar Anti-ß2GPI Domain 1 and HemosIL® AcuStar Anti-ß2GPI Domain 1 Controls.

Indications for Use *(Describe)*
The QUANTA Flash® ß2GP1-Domain1 is an in vitro chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to ß2GP1-Domain1 in human serum. The presence of anti-ß2GP1- Domain1 autoantibodies is used in conjunction with clinical and other laboratory findings as an aid in the diagnosis of antiphospholipid syndrome. The QUANTA Flash® ß2GP1-Domain1 is not intended to replace assays for antibodies against the whole ß2GP1 molecule. Testing for antibodies to the whole ß2GP1 molecule is required according to the classification criteria for antiphospholipid syndrome.

The QUANTA Flash ß2GP1-Domain1 Controls are intended for quality control purposes of the QUANTA Flash ß2GP1-Domain1 chemiluminescent immunoassay (CIA) kit.

The HemosIL® AcuStar Anti-ß2GPI Domain 1 is an in vitro chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to ß2GPI Domain 1 in human serum. The presence of anti-ß2GPI 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-ß2GPI Domain 1 is not intended to replace assays for antibodies against the whole ß2GPI molecule. Testing for antibodies to the whole ß2GPI molecule is required according to the classification criteria for antiphospholipid syndrome.

The HemosIL AcuStar Anti-ß2GPI Domain 1 Controls are intended for quality control purposes of the HemosIL AcuStar Anti-ß2GPI Domain 1 chemiluminescent immunoassay (CIA) kit.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

**CONTINUE ON A SEPARATE PAGE IF NEEDED.**

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## 510(k) Summary

This summary of the 510(k) (ref. K141274) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

| | |
|---|---|
| **Submitter:** | Inova Diagnostics, Inc.<br>9900 Old Grove Road,<br>San Diego, CA, 92131 |
| **Purpose of submission:** | New device(s) |
| **Devices in the submission:** | QUANTA Flash® ß2GP1-Domain1<br>QUANTA Flash® ß2GP1-Domain1 Controls<br>HemosIL® AcuStar Anti-$ß_2$GPI Domain 1<br>HemosIL® AcuStar Anti-$ß_2$GPI Domain 1 Controls |
| **Scientific contact:** | Roger Albesa, M.S.<br>Supervisor of Research and Development<br>Inova Diagnostics, Inc.<br>9900 Old Grove Road San Diego, CA 92131<br>e-mail: ralbesa@inovadx.com<br>Tel. (858) 586-9900/1394 |
| **Quality Systems contact:** | Ronda Elliott, Vice President, Quality and Regulatory<br>Inova Diagnostics, Inc.<br>9900 Old Grove Road, San Diego, CA, 92131<br>e-mail: relliott@inovadx.com<br>Tel. (858) 586-9900<br>Fax: 858-863-0025/1381 |
| **Preparation date:** | 01/9/2015 |
| **Device name (assay kit):** | Proprietary names: QUANTA Flash® ß2GP1-Domain1, HemosIL® AcuStar Anti-$ß_2$GPI Domain 1<br><br>Common name: Anti-β2GPI Domain 1 Chemiluminescent immunoassay<br>Classification name: Anti-β2GPI Domain 1 antibody, antigen and control |
| **Regulation Description** | Multiple autoantibodies immunological test system |
| **Regulation Medical Specialty** | Immunology |
| **Review Panel** | Immunology |
| **Product Code** | System, Test, Antibodies,B2-Glycoprotein I (B2-GPI) (MSV) |
| **Regulation Number** | 866.5660 (Reagent Kit) |
| **Regulation Number** | 862.1660 (Controls) |
| **Device Class** | 2 |
| **Predicate device:** | QUANTA Lite® β2GPI IgG ELISA, 510(k) number: K970551 |

**Device description:**

The QUANTA Flash® ß2GP1-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® ß2GP1-Domain1 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

The assays included in this submission, the QUANTA Flash® ß2GP1-Domain1 marketed by Inova Diagnostics Inc. (9900 Old Grove Road, San Diego, CA 92131) and HemosIL® AcuStar Anti-ß$_2$GPI Domain 1 marketed by Instrumentation Laboratory (180 Hartwell Road Bedford, MA 01730), are equivalent assays. Therefore all data stated hereafter and referred to as: QUANTA Flash® ß2GP1-Domain1 data is equivalently also valid for HemosIL® AcuStar Anti-ß$_2$GPI Domain 1.

Recombinant ß2GP1-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 prediluted 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 (Fe(III) coproporphyrin in sodium hydroxide solution) and Trigger 2 (urea-hydrogen peroxide in sodium chloride solution) 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- ß2GP1-Domain1 antibodies bound to the corresponding ß2GP1-Domain1 on the beads.

The QUANTA Flash® ß2GP1-Domain1 assay utilizes a predefined 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® ß2GP1-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.

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The QUANTA Flash® ß2GP1-Domain1 kit contains the following materials:

One (1) QUANTA Flash ß2GP1-Domain1 Reagent Cartridge, containing the following reagents for 50 determinations:

a. ß2GP1-Domain1 antigen coated paramagnetic beads in a suspension.
b. Assay Buffer – buffer containing protein stabilizers and preservatives.
c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.
d. Resuspension Buffer 3 – buffer containing protein stabilizers and preservatives.
e. QUANTA Flash ß2GP1-Domain1 Calibrator 1: One (1) barcode labeled tube containing 1.0 mL prediluted, ready to use reagent. Calibrators contain human antibodies to ß2GP1-Domain1 in stabilizers and preservatives.
f. QUANTA Flash ß2GP1-Domain1 Calibrator 2: One (1) barcode labeled tube containing 1.0 mL prediluted, ready to use reagent. Calibrators contain human antibodies to ß2GP1-Domain1 in stabilizers and preservatives

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The QUANTA Flash® ß2GP1-Domain1 Controls kit contains three vials of Low Control and three vials of High Control.

- QUANTA Flash ß2GP1-Domain1 Low Control: Three (3) barcode labeled tubes containing 1.0 mL, ready to use reagent. Controls contain human antibodies to ß2GP1-Domain1 in stabilizers and preservatives.
- QUANTA Flash ß2GP1-Domain1 High Control: Three (3) barcode labeled tubes containing 1.0 mL, ready to use reagent. Controls contain human antibodies to ß2GP1-Domain1 in stabilizers and preservatives.

**Intended use(s):**

**QUANTA Flash® ß2GP1-Domain1**

The QUANTA Flash® ß2GP1-Domain1 is an *in vitro* chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to ß2GP1-Domain1 in human serum. The presence of anti-ß2GP1-Domain1 autoantibodies is used in conjunction with clinical and other laboratory findings as an aid in the diagnosis of antiphospholipid syndrome. The QUANTA Flash® ß2GP1-Domain1 is not intended to replace assays for antibodies against the whole ß2GP1 molecule. Testing for antibodies to the whole ß2GP1 molecule is required according to the classification criteria for antiphospholipid syndrome.

**QUANTA Flash® ß2GP1-Domain1 Controls**

The QUANTA Flash ß2GP1-Domain1 Controls are intended for quality control purposes of the QUANTA Flash ß2GP1-Domain1 chemiluminescent immunoassay (CIA) kit.

**HemosIL AcuStar Anti-ß$_2$GPI Domain 1**

The HemosIL® AcuStar Anti-ß$_2$GPI Domain 1 is an *in vitro* chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to ß$_2$GPI Domain 1 in human serum. The presence of anti-ß$_2$GPI 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-ß$_2$GPI Domain 1 is not intended to replace assays for antibodies against the whole ß$_2$GPI molecule. Testing for

antibodies to the whole ß$_2$GPI molecule is required according to the classification criteria for antiphospholipid syndrome.

**HemosIL AcuStar Anti-ß$_2$GPI Domain 1 Controls**

The HemosIL AcuStar Anti-ß2GPI Domain 1 Controls are intended for quality control purposes of the HemosIL Anti-ß2GPI Domain 1 chemiluminescent immunoassay (CIA) kit.

**Substantial equivalence:** The QUANTA Flash ß2GP1-Domain1 and the QUANTA Flash ß2GP1-Domain1 Controls have a similar intended use and assay principle as the predicate device.

**Comparison to predicate device:**

QUANTA Flash® ß2GP1-Domain1 reagent kit

| ***Similarities*** | | |
|---|---|---|
| **Item** | **Applicant** | **Predicate Device** |
| | QUANTA Flash® ß2GP1-Domain1 | QUANTA Lite® $ß_2$ GPI IgG ELISA |
| Intended use | The QUANTA Flash® ß2GP1-Domain1 is an *in vitro* chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgG autoantibodies to ß2GP1-Domain1 in human serum. The presence of anti-ß2GP1-Domain1 autoantibodies is used in conjunction with clinical and other laboratory findings as an aid in the diagnosis of antiphospholipid syndrome. The QUANTA Flash® ß2GP1-Domain1 is not intended to replace assays for antibodies against the whole ß2GP1 molecule. Testing for antibodies to the whole ß2GP1 molecule is required according to the classification criteria for antiphospholipid syndrome. | QUANTA Lite® ß2 GPI IgG is an enzyme linked immunoassay (ELISA) for the semi-quantitative detection of $ß_2$ GPI IgG antibodies in human serum. The presence of $ß_2$ GPI 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 other lupus-like thrombotic diseases. |
| Assay methodology | Solid phase (heterogeneous) immunoassay | Solid phase (heterogeneous) immunoassay |
| Shelf life | One year | One year |
| Sample type | Serum | Serum |

| ***Differences*** | | |
|---|---|---|
| **Item** | **QUANTA Flash ß2GP1-Domain1** | **Predicate Device** |
| Detection/ Operating principle | Chemiluminescent immunoassay | Enzyme-linked immunosorbent assay |
| Solid phase | Paramagnetic microparticles (beads) | 96-well plate |
| Antigen | Recombinant domain1 of ß2-Glycoprotein1 | purified ß2-Glycoprotein1 |
| Conjugate | Isoluminol conjugated anti-human IgG | HRP conjugated anti-human IgG |
| Calibration | Lot specific Master Curve + two Calibrators (included in kit) | Five lot specific calibrators (Included in the kit) |
| Units | < 20 CU Negative result<br>≥ 20 CU Positive result | < 20 SGU Negative result<br>≥ 20 SGU Positive result |
| Analytical Measuring Range | 3.6 – 1380.4 CU<br>Reportable range = up to 13804.0 CU | 9.4 – 150.0 SGU |

QUANTA Flash ß2GP1-Domain1 Calibrators

| **Item** | **QUANTA Flash ß2GP1-Domain1 Calibrators** | **Predicate Device** |
|---|---|---|
| Intended use | No separate intended use; calibrators are part of the kit. | No separate intended use; calibrators are part of the kit. |
| Analyte | Anti-ß2GP1-Domain 1 antibodies | Anti-ß2GP1 antibodies |
| Method | QUANTA Flash® ß2GP1-Domain1 | QUANTA Lite® $ß_2$ GPI IgG ELISA |
| Unit | Chemiluminescent Units (CU)<br>QUANTA Flash<br>Arbitrary Units (U/ml)<br>HemosIL AcuStar | SGU |
| Matrix | Human serum, stabilizers, and preservative | Human serum, stabilizers, and preservative |
| Physico-chemical characteristics | Liquid, prediluted, ready to use | Liquid, prediluted, ready to use |
| Storage | 2-8 $^{o}$C | 2-8 $^{o}$C |
| Shelf life | One year | One year |

QUANTA Flash ß2GP1-Domain1 Controls

| **Item** | **QUANTA Flash ß2GP1-Domain1 Controls** | **Predicate Device** |
|---|---|---|
| Intended use | The QUANTA Flash ß2GP1-Domain1 Controls are intended for quality control purposes of the QUANTA Flash ß2GP1-Domain1 chemiluminescent immunoassay (CIA) kit. | No separate intended use; controls are part of the kit. |
| Analyte | Anti-ß2GP1-Domain1 antibodies | Anti- ß2GP1- antibodies |
| Method | QUANTA Flash ß2GP1-Domain1 chemiluminescent immunoassay | QUANTA Lite® $ß_2$ GPI IgG ELISA |
| Unit | QUANTA Flash (CU)<br>HemosIL AcuStar (U/ml) | SGU |
| Matrix | Human serum, stabilizers, and preservative | Human serum, stabilizers, and preservative |
| Physico-chemical characteristics | Liquid, ready to use | Liquid, prediluted, ready to use |
| Levels | 2 (low and high) | 2 (negative and positive) |
| Storage | 2-8$^{o}$C | 2-8$^{o}$C |
| Shelf life | One year | One year |

***Value assignment and traceability of Calibrators and Controls***

The QUANTA Flash ß2GP1-Domain1 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-ß2GP1-Domain1 antibodies with antibody stabilizer buffer, containing preservative. The human serum is obtained from commercial sources and it is tested for markers of infectious substances.

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. Upon completion of the manufacturing process, the 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.

There are no international reference standards for anti- ß2GP1 IgG. Calibrators and controls are assigned values based on a 20 CU cut-off between positive (high) and negative (low) during assay development. Calibrators are specified in the labeling and supplied with the assay. Control materials are sold separately. The table below summarizes the control and calibrators target values.

List of ß2GP1-Domain1 Standards, Calibrators and Controls:

| **Points on master curve** | Assigned Value (CU) |
|---|---|
| Point 1 | 3.6 |
| Point 2 | 18.5 |
| Point 3 | 43.7 |
| Point 4 | 90.0 |
| Point 5 | 316.0 |
| Point 6 | 1380.4 |

| Material | Manufacturing Target Value (CU) |
|---|---|
| Calibrator 1 | 18.5 |
| Calibrator 2 | 316.0 |
| Control Low | 10.0 |
| Control High | 50.0 |

## ***Analytical performance characteristics***

***Precision***

Testing for precision was performed in accordance with CLSI EP5-A2, and analyzed using Analyze-it which is based on CLSI EP5 1 & 2. 8 Samples 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. 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 acceptance limit, 15%.

Precision study QUANTA Flash® ß2GP1-Domain1 according to the CLSI EP5-A2 guideline

| QUANTA Flash ß2GP1-Domain1 Precision Study Decision Summary | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Within Run | | Between-Run | | Between-Day | | Total | |
| **Sample** | **N** | **Mean (CU)** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| Precision 1 | 80 | 10.4 | 0.9 | 8.3% | 0.0 | 0.0% | 0.5 | 4.3% | 1.0 | 9.4% |
| Precision 2 | 80 | 18.4 | 0.9 | 4.6% | 0.5 | 2.9% | 0.5 | 2.6% | 1.1 | 6.0% |
| Precision 3 | 80 | 22.8 | 1.4 | 6.1% | 0.9 | 3.8% | 0.0 | 0.0% | 1.6 | 7.2% |
| Precision 4 | 80 | 54.3 | 2.5 | 4.6% | 1.4 | 2.5% | 1.2 | 2.2% | 3.1 | 5.6% |
| Precision 5 | 80 | 99.6 | 6.4 | 6.5% | 0.0 | 0.0% | 0.0 | 0.0% | 6.4 | 6.5% |
| Precision 6 | 80 | 318.3 | 18.6 | 5.9% | 0.0 | 0.0% | 4.9 | 1.6% | 19.3 | 6.1% |
| Precision 7 | 80 | 538.1 | 30.3 | 5.6% | 7.9 | 1.5% | 28.7 | 5.3% | 42.5 | 7.9% |
| Precision 8 | 80 | 947.8 | 82.8 | 8.7% | 57.3 | 6.0% | 0.0 | 0.0% | 100.7 | 10.6% |

**Summary of Precision study QUANTA Flash® ß2GP1-Domain1, according to the CLSI EP5-A2 guideline**

| | No. of samples | Within Run | Between-Day | Between-Run | Total |
|---|---|---|---|---|---|
| ß2GP1-Domain1 | 8 | 4.6-8.7% | 0.0-5.3% | 0.0-6.0% | 5.6-10.6% |

***Limit of Blank (LoB) and Limit of Detection (LoD)***

The LoD of the QUANTA Flash® ß2GP1-Domain1 assay is 1296 RLU, which is below the analytical measuring range of the assay. It was determined consistent with 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 LoB is 425.7 RLU.

***Analytical Measuring Range (AMR)***

3.6 CU – 1380.4 CU

The AMR is defined by the values of the lowest and highest Master Curve Standards.

***Auto-rerun function and reportable results***

The BIO-FLASH software has an Auto-rerun option available. If this option is selected, the instrument will automatically rerun any sample that has a result of result >1380.4 CU by further diluting it by a factor specified in the assay definition file (10 fold), thereby bringing the measured value within the AMR. The final result will be calculated by the software by taking into account the additional dilution factor. As the highest value that can be measured is 1380.4 CU, the highest value that can be reported is 13804 CU.

To validate the Auto-rerun function, three high positive specimens with results above the analytical measuring range were selected. The samples were run with the Auto-rerun function enabled on the BIO-FLASH. Then the specimens were manually diluted the same way as it happens in the Auto-rerun function (10 fold), and tested on the BIO-FLASH. The results were within the analytical measuring range after auto-rerun or manual dilution for all specimens. The % recovery values for results obtained with the auto-rerun results compared to the results obtained by manual dilution were between 91.9% and 99% (average 94%) and are within the ± 20% acceptance limit.

***High concentration hook effect***

To assess hook effect, the measurement signal (relative light units, RLU) was examined for the above mentioned 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 ones, thereby confirming that high positive specimens above the analytical measuring range do not show hook effect up to 435,000 RLU.

***Linearity***

The linearity of the AMR was evaluated by a study according to CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Five serum samples with various ß2GP1-Domain1 antibody concentrations were diluted with assay buffer to obtain values that cover the AMR. Percent recovery for all 100 data points ranged from 83.9% to 113.9%, or less than 4 CU. All specimens showed dilution linearity individually, and the combined data yielded the following results with linear regression:

| Assay | Test Range (CU) | Slope (95% CI) | Y-intercept (95% CI) | $R^2$ | Percent Recovery |
|---|---|---|---|---|---|
| ß2GP1-Domain1 | 1.0 to 1640.7 | 1.01 (0.99 to 1.02) | -3.18 (-9.20 to 2.83) | 1.00 | 83.9-113.9% |

***Interference***

Interfering substances were spiked into every specimen at three different concentrations in 10% of total specimen volume, and the resulting samples were assessed in triplicates with the ß2GP1-Domain1 assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluents (10% of total). Acceptance criteria for the interference studies were 85%-115% recovery, or ±4 CU difference, whichever is greater.

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%).

***Cross-reactivity***

A potential cross-reactivity of the QUANTA Flash® ß2GP1 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 tested for various autoantibodies using other QUANTA Flash® immunoassays: Sm, RNP, SS-A/Ro60, Ro52, SS-B, Jo-1, Scl-70, CENP, Ribosomal P, DFS70 and the QUANTA Flash® ß2GP1-Domain1 CIA. These are autoantibodies found in individuals with autoimmune diseases such as SLE, Sjögren's Syndrome, scleroderma, and polymyositis patients. All except for two samples were negative for QUANTA Flash® ß2GP1 Domain1 CIA.
Fisher test revealed no significant association between the other positive reactivities and Domain1. This verified that there is no significant cross-reactivity on the QUANTA Flash® ß2GP1 Domain1 CIA.

| # of Samples tested = 111 | # of Positives (%) | # of Domain1 Positives (%) |
|---|---|---|
| Sm | 6 (5.4%) | 0 (0.0%) |
| RNP | 10 (9.0%) | 0 (0.0%) |
| Ro60 | 11 (9.9%) | 0 (0.0%) |
| Ro52 | 20 (18.0%) | 0 (0.0%) |
| SSB | 8 (7.2%) | 0 (0.0%) |
| Jo-1 | 1 (0.9%) | 0 (0.0%) |
| Scl-70 | 25 (22.5%) | 2 (1.8%) |
| CENP | 0 (0.0%) | 0 (0.0%) |
| Ribosomal P | 5 (4.5%) | 0 (0.0%) |
| DFS70 | 5 (4.5%) | 0 (0.0%) |

***Stability***

*Shelf life*

To establish the initial claim for shelf life, accelerated stability studies were performed for 4 weeks at 37 °C.

Accelerated stability testing was performed on each of the following sealed components of the QUANTA Flash® ß2GP1-Domain1 to establish initial stability claim:

- QUANTA Flash® ß2GP1-Domain1 Reagent Kit (1 Lot)
- ß2GP1-Domain1 beads (3 Lots)
- Resuspension Buffer (3 Lots)
- Calibrators 1 and 2 (3 Lots)
- Low and High controls (3 Lots)

Each week a new sealed component was placed in the incubator, and all components were tested at the end of the experiment together with the one that was stored at 5 ± 3°C. The recovery of the measured values was calculated for each time point (compared to those obtained with 5 ± 3°C stored reagent). All calculations were performed by comparing results of sealed components stored at 5 ± 3°C (control) to those stored at 37 ± 3°C (test) for 1, 2, 3, and 4 weeks, where one week is equal to six months at 5 ± 3°C. Linear regression analysis was performed between recovery values and the number of days.

Acceptance criteria for one year preliminary expiration dating were:

-Microparticles (beads), Resuspension Buffer, and Reagent Kit:
With regression analysis, the lower 95% CI interval of the regression line is ≥ 85% at 2 weeks, and no individual data point has ≤ 75% recovery at 2 weeks.

- Controls and Calibrators:
With regression analysis, the lower 95% CI interval of the regression line is ≥ 90% at 2 weeks, and no individual data point has ≤ 80% recovery at 2 weeks.

All components tested fulfilled the acceptance criteria above, so one year expiration dating was assigned to each component

*In-use (onboard) stability*

*Calibrators*

During assessing on-board stability, Calibrators were placed, uncapped, onboard the instrument, and calibration was performed altogether five times, then a panel of characterized patient specimens were run on each calibration curve.

Acceptance criteria were: Calibrators are considered stable if all five calibrations performed in the 8.5 hour period are successful, and Calibrator average RLU recovery values are between 90% and 110% compared to the first use.

A total of 5 successful calibrations were performed over an 8.5 hour period. Calibrator RLU values remained within the 90-110% range. Moreover, all characterized patient samples ran within their expected range. This supports the claim that calibrators can be used for up to 4 calibrations over an 8 hour period.

*Controls*

During assessing on-board stability, Low and high Controls were assayed for a total of 20 runs, over 9 days. The controls were left uncapped, onboard the instrument for 15 minutes per run. When not in use, the controls were capped, and stored at 5 ± 3°C for at least 2 hours between runs.

Acceptance criteria: Controls are considered stable when all replicates run within their established range, moreover, the linear regression line obtained by plotting %recovery values against the number of runs stays between 85% and 115% at run 15.

Low and high controls ran within their respective acceptable range for all 20 runs. The linear regression line obtained by plotting %recovery values against the number of runs was within 85% and 115% at run 15 for both Controls. The controls were given a maximum of 15 uses with a maximum of 10 minutes onboard per use.

*Reagent Cartridge*

To determine the in-use stability of the QUANTA Flash® ß2GP1-Domain1 reagent cartridge, four serum specimens (with different reactivity levels) along with the Low and high Control were tested. The specimens were tested periodically for 64 days. Recoveries were calculated compared to the day zero average values, and linear regression analysis was performed. The claim was established using the following criteria (using the one that is fulfilled first):

a) The stability claim is established at the day where the 95% confidence interval of the regression line reaches 85% or 115% recovery, or
b) When 2 data points or ≥2% of the recovery data (whichever is greater) is ≤ 75% or ≥ 125%.

The onboard study was ended at 64 days, where all three lots of Reagent Cartridge tested still fulfill the acceptance criteria. The in-use (onboard) stability of the reagent cartridge was set at 60 days.

***Cut-off, reference range***

The reference population for establishing the reference interval for the ß2GP1-Domain1 assay consisted of 30 subjects:

| | |
|---|---|
| Apparently healthy blood donors | 5 |
| Viral hepatitis | 4 |
| Systemic Lupus erythematosus (without history of thrombotic events) | 10 |
| Syphilis | 10 |
| HIV | 1 |

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 equal or greater than 20 CU is considered positive.

## ***Clinical performance characteristics***

### *Clinical sensitivity, specificity*

A separate set of samples, none of which were used in establishing the reference range, was used to validate the clinical performance of the QUANTA Flash ß2GP1-Domain1 CIA. A total of 1090 samples were included in the Validation Set for the QUANTA Flash ß2GP1-Domain1. This Validation Set includes:

- 270 samples from APS patients
- 71 infectious disease samples (40 syphilis, 10 HCV, 21 HBV)
- 104 samples from Crohn's Disease (CD) patients
- 94 samples from Ulcerative Colitis (UC) patients
- 127 samples from autoimmune scleroderma patients
- 168 samples from patients with rheumatoid arthritis
- 49 samples from patients with osteoarthritis
- 24 samples from patients with other diseases (Polymyalgia Rheumatica, Degenerative Spine Disease)
- 183 samples from different conditions "without APS" (Pre-eclampsia/eclampsia, fetal loss, systemic lupus erythematosus (SLE), thrombosis and atopic dermatitis)

Clinical sensitivity and specificity for APS (n=270) using the control population (n=820) is calculated in the table below.

| **Cohort 1 (n=938)** | | **Diagnosis** | | | **Percent Agreement (95% confidence)** |
|---|---|---|---|---|---|
| | | APS | Not APS | Total | |
| **QUANTA Flash® Domain 1 CIA** | Positive | 138 | 3 | 141 | Sensitivity = 51.1% (45.0-57.2%) |
| | Negative | 132 | 817 | 949 | Specificity = 99.6% (98.9-99.9%) |
| | Total | 270 | 820 | 1090 | |

To assess diagnostic efficiency, ROC analysis was performed on the validation sample pool for APS. The results are below:

| Test | Area | 95% CI | SE | Z | p |
|---|---|---|---|---|---|
| QUANTA Flash B2GP1-Domain1 (CU) | 0.84 | 0.81 to 0.86 | 0.015 | 22.24 | <0.0001 |

***Expected values***

The expected value in the normal population is "negative". Anti- ß2GP1-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® ß2GP1-Domain1. This patient population was different from the one that was used to establish the cutoff, and was only used to validate the cutoff. With a cut-off of 20 CU, one sample (0.2%) was positive (34.2 CU) on the QUANTA Flash ß2GP1-Domain1. The mean concentration was 3.8 CU, and the values ranged from <3.6 to 34.2 CU.

***Comparison with predicate device***

Of the samples tested in the validation set, 238 samples, within the analytical measuring range of the QUANTA Flash® ß2GP1-Domain1 CIA were tested also by the QUANTA Lite® $\beta_2$GPI IgG ELISA. 129 samples were positive by QUANTA Flash ß2GP1-Domain1 CIA, 111 samples were positive by QUANTA Lite® $\beta_2$GPI IgG ELISA. The positive, negative, and total agreements were 91.0%, 78.0% and 84.0% respectively. The seroreactivity of 22 sera in an area ±5CU of the cut off, the calculated positive, negative, and total agreements were 80.0%, 82.4% and 81.8% respectively.

| **All (n=238)** | | **QUANTA Lite® $\beta_2$GPI IgG ELISA** | | | **Percent Agreement (95% confidence)** |
|---|---|---|---|---|---|
| | | Positive | Negative | Total | |
| **QUANTA Flash® ß2GP1-Domain1 CIA** | Positive | 101 | 28 | 129 | Pos. Agree = 91.0% (84.1-95.6%) |
| | Negative | 10 | 99 | 109 | Neg. Agree = 78.0% (69.7-84.8%) |
| | Total | 111 | 127 | 238 | Total Agree = 84.0% (78.7-88.4%) |