**DEPARTMENT OF HEALTH & HUMAN SERVICES** Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

January 29, 2015

INOVA DIAGNOSTICS, INC.
C/O GABRIELLA LAKOS, MD, PhD
DIRECTOR, RHEUMATOLOGY RESEARCH
9900 OLD GROVE ROAD
SAN DIEGO, CA 92131

Re: k141210
Trade/Device Name: QUANTA Flash® SS-B
QUANTA Flash® SS-B Calibrators
QUANTA Flash® SS-B Controls
Regulation Number: 21 CFR § 866.5100
Regulation Name: Antinuclear antibody immunological test system
Regulatory Class: Class II
Product Codes: LLL, JIT, JJX
Dated: December 29, 2014
Received: December 30, 2014

Dear Dr. Lakos:

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)*
k141210

Device Name
QUANTA Flash SS-B, QUANTA Flash SS-B Calibrators, QUANTA Flash SS-B Controls

Indications for Use *(Describe)*

QUANTA Flash SS-B is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-SS-B autoantibodies in human serum. The presence of anti-SS-B autoantibodies, in conjunction with clinical findings and other laboratory tests is an aid in the diagnosis of Sjögren's Syndrome and Systemic Lupus Erythematosus.

QUANTA Flash SS-B Calibrators are intended for use with the QUANTA Flash SS-B Reagents for the determination of IgG anti-SS-B autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash SS-B Controls are intended for use with the QUANTA Flash SS-B reagents for quality control in the determination of IgG anti-SS-B autoantibodies in human serum.

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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**Table of Contents**

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

**Administrative data**

**Submitter:** INOVA Diagnostics, Inc
9900 Old Grove Road,
San Diego, CA, 92131

**Purpose of submission:** New device(s)

**Devices in the submission:** QUANTA Flash® SS-B
QUANTA Flash® SS-B Calibrators
QUANTA Flash® SS-B Controls

**Scientific contact:** Gabriella Lakos, Director of Research, Rheumatology
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Fax: 858-863-0025
email: relliot@inovadx.com

**Preparation date:** 05/05/2014

**Device name (assay kit):**

| | |
|---|---|
| Proprietary name: | QUANTA Flash® SS-B |
| Common name: | Anti-SS-B Chemiluminescent Immunoassay |
| Classification name: | anti-SS-B antibody, antigen and control |

| | |
|---|---|
| **Regulation Description** | Antinuclear antibody immunological test system |
| **Regulation Medical Specialty** | Immunology |
| **Review Panel** | Immunology |
| **Product Code** | LLL, Extractable antinuclear antibody, antigen and control |

| | | |
|---|---|---|
| **Regulation Number** | 866.5100 | |
| **Device Class** | 2 | |
| **Device name (Calibrators):** | Proprietary name: | QUANTA Flash® SS-B Calibrators |
| | Common name: | SS-B Calibrators |
| | Classification name: | Calibrator, secondary |
| **Regulation Description** | Calibrator | |
| **Regulation Medical Specialty** | Clinical Chemistry | |
| **Product Code** | JIT | |
| **Regulation Number** | 862.1150 | |
| **Device Class** | 2 | |
| **Device name (Controls):** | Proprietary name: | QUANTA Flash® SS-B Controls |
| | Common name: | SS-B Controls |
| | Classification name: | single (specified) analyte controls (assayed and unassayed) |
| **Regulation Description** | Quality control material (assayed and unassayed) | |
| **Regulation Medical Specialty** | Clinical Chemistry | |
| **Product Code** | JJX | |
| **Regulation Number** | 862.1660 | |
| **Device Class** | 1 (reserved) | |

**Predicate device:** QUANTA Lite® SS-B ELISA, 510(k) number: K922832

**Device description**

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

Purified recombinant SS-B antigen is coated onto paramagnetic beads. The bead suspension is lyophilized and stored in the bead tube. Prior to use in the BIO-FLASH system, the sealed reagent tubes are pierced with the reagent cartridge lid and the beads are rehydrated and resuspended using

resuspension buffer by pipetting up and down with a transfer pipette. The reagent cartridge is then loaded onto the BIO-FLASH instrument. Samples are also loaded onto the instrument in sample racks. Serum samples are prediluted 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-SS-B antibodies bound to the corresponding beads.

For quantitation, the QUANTA Flash SS-B 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 SS-B Calibrators. Based on the results obtained with the two Calibrators included in the Calibrator Set (sold separately), an instrument specific Working Curve is created, which is used to calculate chemiluminescent units (CU) from the instrument signal (RLU) obtained for each sample.

The QUANTA Flash SS-B kit contains the following materials:

One (1) QUANTA Flash SS-B Reagent Cartridge
One (1) vial of Resuspension buffer
One (1) Transfer pipette

The QUANTA Flash SS-B reagent cartridge contains the following reagents for 50 determinations:

a. SS-B antigen coated paramagnetic beads, lyophilized.
b. Assay buffer – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.
c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.

The QUANTA Flash SS-B Calibrators kit contains two vials of Calibrator 1 and two vials of Calibrator 2:

QUANTA Flash SS-B Calibrators:

- QUANTA Flash SS-B Calibrator 1: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to SS-B in stabilizers and preservatives.
- QUANTA Flash SS-B Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL

prediluted, ready to use reagent. Calibrators contain human antibodies to SS-B in stabilizers and preservatives.

The QUANTA Flash SS-B Controls kit contains two vials of Negative Control and two vials of Positive Control:

QUANTA Flash SS-B Controls:

- QUANTA Flash SS-B Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to SS-B in stabilizers and preservatives.
- QUANTA Flash SS-B Positive Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to SS-B in stabilizers and preservatives.

**Intended use(s)**

QUANTA Flash SS-B is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-SS-B autoantibodies in human serum. The presence of anti-SS-B autoantibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Sjögren's Syndrome and Systemic Lupus Erythematosus.

QUANTA Flash SS-B Calibrators are intended for use with the QUANTA Flash SS-B Reagents for the determination of IgG anti-SS-B autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash SS-B Controls are intended for use with the QUANTA Flash SS-B reagents for quality control in the determination of IgG anti-SS-B autoantibodies in human serum.

**Substantial equivalence**

The QUANTA Flash SS-B, the QUANTA Flash SS-B Calibrators and the QUANTA Flash SS-B Controls have the same intended use and assay principle as the predicate device.

**Comparison to predicate device**

*QUANTA Flash SS-B reagent kit*

| ***Similarities*** | | |
|---|---|---|
| **Item** | **QUANTA Flash SS-B** | **Predicate Device** |
| Intended use | Semi-quantitative determination of anti-SS-B antibodies in human serum | Semi-quantitative detection of anti-SS-B antibodies in human serum |
| Assay methodology | Solid phase (heterogenous) | Solid phase (heterogenous) |

| | | |
|---|---|---|
| | immunoassay | immunoassay |
| Traceability | International Reference Preparation is not available<br>Results are traceable to in-house Standards | International Reference Preparation is not available |
| Sample type | Serum | Serum |
| Shelf life | One year | One year |

| ***Differences*** | | |
|---|---|---|
| **Item** | **QUANTA Flash SS-B** | **Predicate Device** |
| Detection/ Operating principle | Chemiluminescent immunoassay | Enzyme-linked immunosorbent assay |
| Solid phase | Paramagnetic microparticles (beads) | 96-well plate |
| Antigen | Purified recombinant SS-B antigen | Native SS-B antigen, purified from bovine thymus |
| Conjugate | Isoluminol conjugated anti-human IgG | HRP conjugated anti-human IgG |
| Calibration | Lot specific Master Curve + two calibrators (Sold separately) | SS-B ELISA Low Positive (Included in the kit) |

*QUANTA Flash SS-B Calibrators*

| **Item** | **QUANTA Flash SS-B Calibrators** | **Predicate Device** |
|---|---|---|
| Intended use | QUANTA Flash SS-B Calibrators are intended for use with the QUANTA Flash SS-B Reagents for the determination of IgG anti-SS-B autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values. | No separate intended use; calibrator is part of the kit. |
| Analyte | Anti-SS-B antibodies | Anti-SS-B antibodies |
| Method | QUANTA Flash SS-B chemiluminescent immunoassay | QUANTA Lite SS-B ELISA |
| Matrix | Human serum, stabilizers, and preservative | Human serum, buffer, stabilizers, preservative |
| Unit | CU (Chemiluminescent units) (arbitrary) | Units (arbitrary) |
| Physico-chemical characteristics | Liquid, prediluted, ready to use | Liquid, prediluted, ready to use |

| | | |
|---|---|---|
| Storage | 2-8 °C | 2-8 °C |
| Shelf life | One year | One year |

*QUANTA Flash SS-B Controls*

| Item | QUANTA Flash SS-B Controls | Predicate Device |
|---|---|---|
| Intended use | QUANTA Flash SS-B Controls are intended for use with the QUANTA Flash SS-B reagents for quality control in the determination of IgG anti-SS-B autoantibodies in human serum. | No separate intended use; controls are part of the kit. |
| Analyte | Anti-SS-B antibodies | Anti-SS-B antibodies |
| Method | QUANTA Flash SS-B chemiluminescent immunoassay | QUANTA Lite SS-B ELISA |
| Matrix | Human serum, stabilizers, and preservative | Human serum, buffer, stabilizers, preservative |
| Unit | CU (Chemiluminescent units) (arbitrary) | Units (arbitrary) |
| Physico-chemical characteristics | Liquid, ready to use | Liquid, prediluted, ready to use |
| Levels | 2 (negative and positive) | 2 (ELISA negative, high positive) |
| Storage | 2-8 °C | 2-8 °C |
| Shelf life | One year | One year |

**Analytical performance characteristics**

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

There is currently no recognized international standard for the measurement of SS-B antibodies.
The CDC ANA reference sera #2 (REFERENCE SERUM FOR HUMAN ANTIBODIES TO SS-B/La) and #3 (REFERENCE SERUM, FLUORESCENCE ANTINUCLEAR ANTIBODY, SPECKLED PATTERN) were tested for SS-B and produced the following results:
CDC ANA #2: >1550.0 CU
CDC ANA #3: 284.1 CU

The QUANTA Flash SS-B Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-SS-B antibodies with commercial antibody stabilizer, 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.

Calibrator and Control values are directly traceable to in-house Standards that are used to create the Master Curves for the QUANTA Flash SS-B assay.

List of SS-B Standards, Calibrators and Controls:

| Material | Assigned Value |
|---|---|
| SS-B Master Curve Standard 1 | 3.3 CU |
| SS-B Master Curve Standard 2 | 15.8 CU |
| SS-B Master Curve Standard 3 | 98.8 CU |
| SS-B Master Curve Standard 4 | 682.1 CU |
| SS-B Master Curve Standard 5 | 1241.6 CU |
| SS-B Master Curve Standard 6 | 1706.8 CU |

| Material | Manufacturing Target Value | Manufacturing Target Range |
|---|---|---|
| SS-B Calibrator 1 | 16 CU | 14 – 18 CU |
| SS-B Calibrator 2 | 600 CU | 540 – 660 CU |
| SS-B Negative Control | 10 CU | 8 – 12 CU |
| SS-B Positive Control | 50 CU | 40 – 60 CU |

***Precision***

The precision of the QUANTA Flash SS-B assay was evaluated first on 10 samples containing various concentrations of SS-B antibodies in accordance with CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Procedures - Approved Guideline: samples were run in duplicates, twice a day, for 21 days. Production reagent lot 131009 was used for the studies.
Data were analyzed with the Analyse-it for Excel method evaluation software, and within run, between run, between day and total precision were calculated.
Acceptance criteria: Total %CV: < 10%
Results are summarized in the Table below.

| QUANTA Flash SS-B | | | Within Run | | Between Runs | | Between Days | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample ID | Mean (CU) | Number of replicates | SD (CU) | CV (%) | SD (CU) | CV (%) | SD (CU) | CV (%) | SD (CU) | CV (%) |
| 120814-70 | 12.4 | 84 | 0.4 | 3.5 | 0.0 | 0.0 | 0.6 | 4.6 | 0.7 | 5.8 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 110689-25 | 24.3 | 84 | 1.4 | 5.8 | 0.0 | 0.0 | 1.6 | 6.7 | 2.2 | 8.9 |
| 110687-120 | 25.0 | 84 | 1.2 | 4.7 | 0.3 | 1.1 | 0.8 | 3.2 | 1.5 | 5.8 |
| 110686-40 | 27.9 | 84 | 1.0 | 3.6 | 0.2 | 0.7 | 1.0 | 3.6 | 1.4 | 5.1 |
| 110684-20 | 132.7 | 84 | 7.6 | 5.8 | 0.0 | 0.0 | 6.1 | 4.6 | 9.8 | 7.4 |
| 120814-02 | 383.5 | 84 | 14.2 | 3.7 | 7.9 | 2.1 | 16.5 | 4.3 | 23.2 | 6.0 |
| 110684-04 | 552.3 | 84 | 15.7 | 2.8 | 18.3 | 3.3 | 14.7 | 2.7 | 28.2 | 5.1 |
| 110687-02 | 883.3 | 84 | 40.3 | 4.6 | 30.2 | 3.4 | 24.3 | 2.7 | 55.9 | 6.3 |
| 000674-12 | 1356.8 | 84 | 92.4 | 6.8 | 0.0 | 0.0 | 60.6 | 4.5 | 110.5 | 8.1 |
| 000674-10 | 1539.6 | 84 | 99.2 | 6.4 | 36.9 | 2.4 | 34.5 | 2.2 | 111.3 | 7.2 |

***Reproducibility***

Seven samples containing various concentrations of SS-B antibodies were tested on two different reagent lots, using two different lots of Calibrators, by two operators. Samples were run in quadruplicates, two times a day, for 10 days, to generate 80 data points.
Production reagent lots 131009 and 141010, and Calibrator and Control lots 131006 and 131007 were used for the studies.
Data were analyzed with the Analyse-it for Excel method evaluation software, and within run, between reagent lots, between calibrator lots, between operators and total precision were calculated.
Acceptance criteria: Total %CV: < 10%
Results are summarized in the Table below.

| QUANTA Flash SS-B | | | Within Run | | Between Reagent Lots | | Between Calibrator Lots | | Between Operators | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample ID | Mean (CU) | Number of replicates | SD (CU) | CV (%) | SD (CU) | CV (%) | SD (CU) | CV (%) | SD (CU) | CV (%) | SD (CU) | CV (%) |
| 1 | 11.7 | 80 | 0.3 | 2.8 | 0.5 | 4.6 | 0.3 | 2.8 | 0.4 | 3.0 | 0.5 | 3.9% |
| 2 | 26.1 | 80 | 0.7 | 2.6 | 0.6 | 2.1 | 1.0 | 3.6 | 0.9 | 3.3 | 0.9 | 3.4% |
| 3 | 124.2 | 80 | 2.8 | 2.3 | 2.8 | 2.3 | 7.1 | 5.7 | 4.3 | 3.5 | 5.3 | 4.3% |
| B | 252.7 | 80 | 5.1 | 2.0 | 15.9 | 6.3 | 13.1 | 5.2 | 9.6 | 3.8 | 13.4 | 5.3 |
| A | 640.8 | 80 | 23.9 | 3.7 | 50.4 | 7.9 | 50.9 | 7.9 | 43.8 | 6.8 | 50.4 | 7.9 |
| D | 981.9 | 80 | 35.3 | 3.6 | 56.4 | 5.7 | 78.4 | 8.0 | 57.2 | 5.8 | 67.9 | 6.9 |
| C | 1388.7 | 80 | 54.6 | 3.9 | 89.8 | 6.5 | 111.7 | 8.0 | 103.6 | 7.5 | 106.9 | 7.7 |

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

The LoD of the QUANTA Flash SS-B assay is 398 RLU, which is below the analytical measuring range of the assay. It was determined consistent with CLSI EP17-A2 guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 120 determinations, with 60 measurements on blank samples and 60 measurements of low level samples.
For determining the LoB, 4 blank samples (System Rinse) from two different lots were run in replicates of five on two reagent lots, once per day, for 3 days. Production reagent lots 131009 and 141010 were used for the studies. 60 data points were generated on each lot.
The LoB was determined on each lot separately with the *Analyse-it for Excel* software's Reference Interval function, at the 95$^{th}$ percentile, using the parametric method, as the dataset showed normal distribution. The LoB for lot 131009 was determined as 269 RLU, and for lot 141010 as 294 RLU. The final LoB value is 294 RLU.
For determining the LoD, 4 low level samples (prepared by diluting anti-SS-B positive samples with System Rinse) were run in replicates of five on two reagent lots, once per day, for 3 days. Production reagent lots 131009 and 141010 were used for the studies. 60 data points were generated on each lot.
The LoD was determined separately on each lot according to CLSI EP17-A2 guideline. The limit of detection for lot 131009 was determined as 360 RLU, and for lot 141010 as 398 RLU. The final LoD value is 398 RLU.
These values are below the value of the lowest QUANTA Flash SSB Master Curve standard, i.e. below the Analytical Measuring Range.

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

QUANTA Flash SS-B: 3. 3 CU - 1550.0 CU

***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 >1550.0 by further diluting it by 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 1550.0, the highest value that can be reported is 15,500.

***High concentration hook effect***

To assess hook effect, measurement signal (relative light units, RLU) was examined for three high positive samples, with results above the AMR, 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 (that were within the AMR), thereby confirming that high positive specimens above the analytical measuring range do not show hook effect up to 5,004 CU in the SS-B assay (the highest concentration that was tested).

***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 SS-B antibody concentrations were diluted in 10% increments (from 0% to 90% negative base matrix) to obtain values that cover the AMR. The dilutions were assayed in duplicates. Percent recovery of obtained mean results was calculated compared to the expected mean results (based on the dilution factor). Moreover, obtained values were plotted against expected values, and linear regression analysis was performed.
Acceptance criteria:
- Recovery is between 80-120%, or ± 4 CU, whichever is greater.
- For linear regression analysis, slope is between 0.9-1.1, and $R^2$ is ≥ 0.95.

All four specimens showed dilution linearity individually.

| **Sample** | **Test Range (CU)** | **Slope (95% CI)** | **$R^2$** |
|---|---|---|---|
| 1 | 204.4 - 1507.0 | 1.03 (0.98 to 1.08) | 0.99 |
| 2 | 155.3 - 1546.8 | 1.04 (0.99 to 1.09) | 0.98 |
| 3 | 99.0 - 1048.6 | 0.96 (0.92 to 1.00) | 0.99 |
| 4 | 17.4 - 163.8 | 1.01 (0.97 to 1.05) | 0.99 |
| 5 | 3.6 - 29.4 | 0.95 (0.91 to 0.99) | 0.99 |

The combined data yielded the following results with linear regression:

| **Sample** | Test Range (CU) | Slope (95% CI) | $R^2$ |
|---|---|---|---|
| All | 3.6 - 1546.8 | 0.98 (0.96 to 1.00) | 0.99 |

The upper limit of the analytical measuring range was limited to 1550 CU.

***Interference***

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Three specimens were tested (near-the–cutoff negative: 15.4 CU; weak positive: 25.5 CU; high positive: 200.3). 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 SS-B assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluent (10% of total).

Acceptance criteria: 85% - 115% recovery, or ± 4 CU difference, whichever is greater.

No interference was detected with bilirubin up to 10 mg/dL (recovery: 95.0% to 108.4%), hemoglobin up to 200 mg/dL (recovery: 96.0% to 105.8%), triglycerides up to 1000 mg/dL (recovery: 96.0% to 104.8%), cholesterol up to 224.3 mg/dL (recovery: 96.0% to 104.8%), and RF IgM up to 500 IU/mL (recovery: 90.7% to 111.5%).

***Cross-reactivity***

To test potential cross-reactivity with autoantibodies and infection-induced antibodies, results obtained on altogether 273 of the total 431 control samples that were included in the clinical validation study were assessed. These samples were from patients with autoimmune diseases that are characterized with disease specific autoantibodies, or from patients with positive infectious disease serology. The composition of the cohort and the anti-SS-B positivity rate is shown in the Table below:

| Diagnosis | Number of samples | # pos | % pos |
|---|---|---|---|
| Graves' Disease | 19 | 0 | 0.0% |
| Hashimoto Thyroiditis | 21 | 0 | 0.0% |
| HCV | 10 | 0 | 0.0% |
| HBV | 10 | 0 | 0.0% |
| HIV | 5 | 0 | 0.0% |
| Syphilis | 5 | 0 | 0.0% |
| Primary Antiphospholipid Syndrome | 15 | 0 | 0.0% |
| Vasculitis | 1 | 0 | 0.0% |
| Systemic sclerosis | 89 | 1 | 1.1% |
| Autoimmune myositis | 4 | 0 | 0.0% |
| Rheumatoid arthritis | 70 | 4 | 5.7% |
| Autoimmune liver disease group#1 | 2 | 1 | 50.0% |
| Autoimmune liver disease group#2** | 22 | 1 | 4.5% |
| | | | |
| Total controls | 273 | 7 | 2.6% |

** Samples contain autoimmune liver disease specific antibodies (SLA, F-actin, M2)

Based on the results, the QUANTA Flash SS-B assay does not show cross-reactivity with autoantibodies that are present in various autoimmune diseases, or antibodies against infectious agents.

***Lot to lot comparison***

Twenty-two unique samples and the Positive and Negative Controls (altogether 24 specimens) with various reactivity levels were tested in triplicates with three different reagent lots: 131009, 14010 and

14011. The samples covered the total analytical measuring range of the assay. Results were processed by linear regression analysis and bias calculation according to CLSI EP09-A2, Method Comparison and Bias Calculation Using Patient Samples; Approved Guideline - Second Edition.
Pair-wise comparisons were performed between lot 131009 vs 14010, lot 131009 vs 14011 and lot 141010 vs 141011, considering individual replicates instead of the mean of replicates.
Acceptance criteria and results are in the Table below. All results were within the acceptance limits.

| **Acceptance criteria** | **131009 vs 14010** | **131009 vs 14011** | **141010 vs 141011** |
|---|---|---|---|
| Weighted r: ≥0.975 for linear regression | 1.00 | 0.99 | 0.99 |
| Intercept of the regression line (constant bias): ± 15% of cut-off (3 CU) | 0.5 | -0.6 | -1.0 |
| Slope of the regression line (proportional bias): 0.9-1.1 | 1.0 | 1.0 | 1.0 |
| Weighted S y/x: ≤ 0.5 | 0.06 | 0.11 | 0.11 |
| Predicted bias (difference) at cut-off: ±15 (3 CU) | 0.4 | -0.6 | -1.0 |

***Stability***

*Shelf life*
To establish the initial claim for shelf life, accelerated stability studies were performed for 4 weeks at 37°C ± 3°C, where one week is equal to six months at 5 ± 3°C.
Accelerated stability testing was performed on each of the following sealed components of the QUANTA Flash SS-B to establish initial stability claim: the beads, the two Calibrators, and the negative and positive Controls. 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:
- Beads:
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.

*Beads*

Testing was performed on three lots of SS-B coupled beads using up to 7 characterized samples with various reactivity levels.

All three lots of beads retained > 85% reactivity (considering the 95% CI) after two weeks at 37 ± 3ºC, and therefore pass the acceptance criteria for one year expiration date.

*Calibrators and Controls*

Testing was performed on three lots of SS-B Calibrators and Controls. All Calibrators and Controls maintained > 90% reactivity (considering the 95% CI) when sored at 37 ± 3ºC for 2 weeks, and therefore pass the acceptance criteria for one year expiration dating.

*In-use (onboard) stability*

*Calibrators*

Onboard stability claim: 4 calibrations, or 8 hours onboard

During assessing on-board stability, Calibrators were placed uncapped, onboard the instrument, and calibration was performed altogether five times over 8.5 hours. Controls and a panel of characterized patient specimens were run on each calibration curve.

Calibrators are considered stable if all five calibrations performed in the 8.5 hour period are successful, and average Calibrator 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 Controls and patient panel 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*

Onboard stability claim: up to 15 uses, at 10 minutes onboard per use

During assessing on-board stability, 2 vials of each Control were assayed twice a day for a total of 21 runs. The first run was used to establish baseline value, and then additional 20 runs were performed. During runs, the Controls were left uncapped, onboard the instrument for 15 minutes per run. When not in use, the controls were capped, and stored at 5º ± 3°.

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

All controls ran within their respective acceptable ranges for all runs. Moreover, the regression line remained between 85% and 115% at run 15 for both Controls. These results support the claim that controls can be used for up to 15 times, at 10 minutes per use.

*Reagent Cartridge*

To establish the in-use stability of the QUANTA Flash SS-B reagent cartridge, three lots of cartridges were tested with up to 6 serum specimens (with different reactivity levels) along with the Negative and

Positive Controls. The specimens were tested periodically up to 69 days. Percent recoveries were calculated compared to the day zero average values, and linear regression analysis was performed by plotting %recovery against the number of days. The claim was established using the following criteria (using the one that is fulfilled first):
- The stability claim is established at the actual measurement day proceeding the day when the 95% confidence interval of the regression line reaches 85% or 115% recovery, or
- At the actual measurement day preceding the day when 2 data points or ≥2% of the recovery data (whichever is greater) is ≤ 75% or ≥ 125% recovery.

The onboard stability results of the three lots are as follows:
RP0008: 68 days
121006: 57 days
131009: 60 days
Using these criteria, the in-use (onboard) stability of SS-B reagent cartridge was set at 57 days.

*Real time stability*

Real time stability testing was performed at 3, 6, 9 and 12 months on Calibrators, Controls and reagent cartridge to support the one year expiration.

For Controls, each control was tested in triplicates at each time point.
- Acceptance criteria: results should fall within their acceptable ranges as it was established at the release of the controls.

Calibrators were tested in triplicates at each time point as it is done during calibration. Averages of the triplicates were compared to the value that was assigned to the Calibrators at release.
- Acceptance criteria: % recovery of the average of the triplicates is between 85 and 115%, and %CV of the triplicates is < 10%

For reagent cartridge, the panel of QC samples was tested at each time point. This QC panel is used by the QC Department for reagent release and QC.
- Acceptance criteria: results should fall within their respective QC ranges.

All results were within the acceptance limits.

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

| | | |
|---|---|---|
| QUANTA Flash SS-SB: | Negative | <20 CU |
| | Positive | ≥20 CU |

The reference population for establishing the reference interval for the SS-B assay consisted of 187 subjects:

| | |
|---|---|
| Apparently healthy blood donors | 162 |
| Viral hepatitis positive samples | 10 |
| HIV positive samples | 5 |
| Syphilis positive samples | 5 |
| Rheumatoid arthritis patients | 5 |

All specimens were the same matrix (serum) as specified in the Intended Use. All specimens were unaltered. The cut off was established in accordance with CLSI C28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition. The Analyse-it for Excel software was used to make the calculations. The distribution of the results was non-normal (Saphiro-Wilk $p<0.0001$), so the non-parametric percentile method was used. One sample from a blood donor was excluded, as it was considered outlier. The RLU value of this sample was 138,062, and it was also positive with the predicate ELISA. The 99th percentile Reference Interval was calculated as 4097 RLU. One reference sample tested positive at this threshold.
Additionally, 32 samples characterized as positive for SS-B antibodies by IIF, FIA, and ELISA were assayed to aid in the determination of the cutoff. Based on the distribution of RLU values in these (known) positive samples (37,743-942,882 RLU), the cutoff was increased to 12,000 RLU to ensure optimal differentiation between negatives and positives, and 20 CU value was assigned to this RLU value.

**Clinical performance characteristics**

***Clinical sensitivity, specificity***

A cohort of characterized samples, none of which were used for establishing the reference range, was used to validate the clinical performance of the QUANTA Flash SS-B. A total of 761 characterized samples were included in the Validation Set for the QUANTA Flash SS-B. All samples were run on the QUANTA Flash SS-B. The distribution of the cohort and the SS-B positivity rate is in the Table below:

| Patient group | N | Number positive | % positive |
|---|---|---|---|
| Ulcerative colitis | 20 | 0 | 0.0% |
| Graves' Disease | 19 | 0 | 0.0% |
| Hashimoto Thyroiditis | 21 | 0 | 0.0% |
| Non-autoimmune thyroid disease | 43 | 0 | 0.0% |
| Crohn's disease | 20 | 0 | 0.0% |
| HCV | 10 | 0 | 0.0% |
| HBV | 10 | 0 | 0.0% |
| HIV | 5 | 0 | 0.0% |
| Syphilis | 5 | 0 | 0.0% |
| Osteoarthritis | 20 | 1 | 5.0% |

| | | | |
|---|---|---|---|
| Primary Antiphospholipid Syndrome | 15 | 0 | 0.0% |
| Secondary Antiphospholipid Syndrome* | 15 | 0 | 0.0% |
| Other rheumatic diseases | 40 | 1 | 2.5% |
| Vasculitis | 1 | 0 | 0.0% |
| Systemic sclerosis | 89 | 1 | 1.1% |
| Autoimmune myositis | 4 | 0 | 0.0% |
| Rheumatoid arthritis | 70 | 4 | 5.7% |
| Autoimmune liver disease group#1 | 2 | 1 | 50.0% |
| Autoimmune liver disease group#2** | 22 | 1 | 4.5% |
| Sjögren's Syndrome | 40 | 14 | 35.0% |
| SLE | 290 | 38 | 13.1% |
| **Total** | **761** | | |
| **Total controls** | **431** | **9** | **2.1%** |

*Patients may have SLE

** Samples contain autoimmune liver disease specific antibodies (SLA, F-actin, M2)

The results were analyzed to calculate sensitivity and specificity for SLE (n=290) and Sjögren's syndrome (SS) (n=40) separately, and SLE and SS combined.

Clinical sensitivity and specificity of the QUANTA Flash SS-B in Sjögren's syndrome

| n=456 | | Diagnosis | | | Analysis (95% confidence) |
|---|---|---|---|---|---|
| | | SS | Controls (excluding SLE) | Total | |
| QUANTA Flash SS-B | Positive | 14 | 9 | 23 | Sensitivity = 35.0% (20.6-51.7%) |
| | Negative | 26 | 407 | 433 | Specificity = 97.8% (95.9-99.0%) |
| | Total | 40 | 416 | 456 | |

Clinical sensitivity and specificity of the QUANTA Flash SS-B in SLE

| n=706 | | Diagnosis | | | Analysis (95% confidence) |
|---|---|---|---|---|---|
| | | SLE | Controls (excluding Sjögren's) | Total | |
| QUANTA Flash SS-B | Positive | 38 | 9 | 47 | Sensitivity = 13.1% (9.4-17.5%) |
| | Negative | 252 | 407 | 659 | Specificity = 98.0% (96.0-99.1%) |
| | Total | 290 | 416 | 706 | |

Clinical sensitivity and specificity of the QUANTA Flash SS-B in SLE+SS

| n=746 | Diagnosis | Analysis |
|---|---|---|

| | | SS or SLE | Controls | Total | |
|---|---|---|---|---|---|
| QUANTA Flash SS-B | Positive | 52 | 9 | 61 | Sensitivity = 15.8% (12.0-20.1%) |
| | Negative | 278 | 407 | 685 | Specificity = 97.8% (95.9-99.0%) |
| | Total | 330 | 416 | 746 | |

***Expected values***

The expected value in the normal population is "negative". Anti-SS-B autoantibody levels were analyzed in a cohort of 138 apparently healthy blood donors (118 females and 20 males, ages 17 to 60 years, with an average age of 32.8 years and median age of 31 years) using the QUANTA Flash SS-B. This patient population was different from the one that was used to establish the cutoff, and was only used to assess expected values. With the cut-off of 20 CU, 1 (0.7 %) of the samples was positive on the QUANTA Flash SS-B. The mean concentration was < 3.3 CU, and the values ranged from <3.3 to 28.7 CU.

***Comparison with predicate device***

Samples for method comparison analysis included 639 samples from the total of 761 samples that were used in the clinical validation studies, and had predicate ELISA results available. The cohort consisted of Sjögren's syndrome (n=40) and SLE patients (n=240) and relevant disease controls (359). No healthy controls were included in this cohort.

Results obtained with the predicate device are shown in the Table below:

| **Patient group** | **N** | **Number positive** | **% positive** |
|---|---|---|---|
| Ulcerative colitis | 20 | 0 | 0% |
| Graves' Disease | 19 | 0 | 0% |
| Hashimoto Thyroiditis | 21 | 0 | 0% |
| Non-autoimmune thyroid disease | 43 | 0 | 0% |
| Crohn's disease | 20 | 0 | 0% |
| HCV | 10 | 0 | 0% |
| HBV | 10 | 0 | 0% |
| HIV | 5 | 0 | 0% |
| Syphilis | 5 | 0 | 0% |
| Osteoarthritis | 20 | 2 | 10% |
| Primary Antiphospholipid Syndrome | 15 | 0 | 0% |
| Secondary Antiphospholipid Syndrome | 15 | 1 | 7% |
| Other rheumatic diseases and arthropathies | 40 | 0 | 0% |
| Vasculitis | 1 | 0 | 0% |

| **Patient group** | **N** | **Number positive** | **% positive** |
|---|---|---|---|
| Systemic sclerosis | 41 | 6 | 15% |
| Autoimmune myositis | 2 | 0 | 0% |
| Rheumatoid arthritis | 70 | 1 | 1% |
| Autoimmune liver disease group 1 | 2 | 1 | 50% |
| Autoimmune liver disease group 2** | 0 | n/a | n/a |
| Sjogren's Syndrome | 40 | 15 | 38% |
| SLE | 240 | 33 | 14% |

** Samples contain autoimmune liver disease specific antibodies (SLA, F-actin, M2). For distribution, please see table in Section #9.

Agreement between the QUANTA Flash SS-B and the predicate ELISA was calculated three ways:

Method comparison, all samples

| Method Comparison (N=639) | | SS-B ELISA | | | Percent Agreement (95% Confidence) |
|---|---|---|---|---|---|
| | | Negative | Positive | Total | |
| QUANTA Flash SS-B | Negative | 573 | 11 | 584 | Pos. Agree = 81.4% (69.1 – 90.3%) |
| | Positive | 7 | 48 | 55 | Neg. Agree = 98.8% (97.5 – 99.5%) |
| | Total | 580 | 59 | 639 | Total Agree = 97.2% (95.6 – 98.3%) |

Method comparison, data within reportable range

| Method Comparison (N=142) | | SS-B ELISA | | | Percent Agreement (95% Confidence) |
|---|---|---|---|---|---|
| | | Negative | Positive | Total | |
| QUANTA Flash SS-B | Negative | 81 | 6 | 87 | Pos. Agree = 88.9% (77.4 – 95.8%) |
| | Positive | 7 | 48 | 55 | Neg. Agree = 92.0% (84.3 – 96.7%) |
| | Total | 88 | 54 | 142 | Total Agree = 90.8% (84.9 – 95.0%) |

Method comparison, data around the cutoff

| Method Comparison (N=10) | | SS-B ELISA | | | Percent Agreement (95% Confidence) |
|---|---|---|---|---|---|
| | | Negative | Positive | Total | |
| QUANTA Flash® SS-B | Negative | 5 | 2 | 7 | Pos. Agree = 33.3% (0.8 – 90.6%) |
| | Positive | 2 | 1 | 3 | Neg. Agree = 71.4% (29.0 – 96.3%) |
| | Total | 7 | 3 | 10 | Total Agree = 60.0% (26.2 – 87.8%) |