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

A. 510(k) Number:

k141210

B. Purpose for Submission:

New device

C. Measurand:

anti-SS-B IgG antibodies

D. Type of Test:

Semi-quantitative immunoassay

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Flash® SS-B

QUANTA Flash® SS-B Calibrators

QUANTA Flash® SS-B Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR§866.5100, Antinuclear antibody immunological test system

   21 CFR §862.1150, Calibrator

   21 CFR §862.1660, Quality control material (assayed and unassayed)

2. Classification:

   Class II (assay and calibrators)

   Class I (controls)

3. Product code:

   LLL, Extractable antinuclear antibody, antigen and control

   JIT, Calibrator, secondary

JJX, Single (specified) analyte controls (assayed and unassayed)

4. Panel:

   Immunology (82)

   Clinical chemistry (75)

H. Intended Use:

1. 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.

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 Instrument System (k083518)

I. Device Description:

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

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

Each cartridge has a barcode that contains the assay name, the assay ID number, the lot number and expiration date, the four parameters of the lot specific master curve, and the reagent cartridge specific serial number. Also included is a vial of resuspension buffer

containing protein stabilizers and preservative.

The QUANTA Flash® SS-B Calibrator set includes two calibrators (Calibrator 1 and Calibrator 2). These are barcoded tubes containing 0.3 mL pre-diluted, ready-to-use reagent. Calibrators contain human antibodies to SS-B in buffer containing stabilizers and preservatives. They are sold separately.

The QUANTA Flash® SS-B Controls contain two vials (a Negative and a Positive) containing human antibodies to SS-B in buffer containing protein stabilizers and preservatives. They are sold separately.

The following additional consumable items are required for the test and supplied separately: BIO-FLASH system rinse, BIO-FLASH Triggers, and BIO-FLASH Cuvettes.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   QUANTA Lite® SS-B ELISA

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

   k922832

3. Comparison with predicate:

   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	Same
Assay Methodology	Solid phase (heterogeneous) immunoassay	Same
Traceability	International Reference preparation is not available. Results are traceable to in-house standards	Same
Sample Type	Serum	Same
Cut-off	20 CU	Same
Shelf Life	One year	Same

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

Similarities		
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	Same
Matrix	Human serum, buffers, stabilizers, and preservative	Same
Physico-chemical characteristics	Liquid, prediluted, ready to use	Same
Storage	2-8^{0}C	Same
Shelf life	One year	Same

Differences		
Item	**QUANTA Flash SS-B Calibrators**	**Predicate Device**
Method	QUANTA Flash SS-B chemiluminescent immunoassay	QUANTA Lite SS-B ELISA
Unit	CU (chemiluminescent units, arbitrary)	Units (arbitrary)

QUANTA Flash® SS-B Controls

Similarities		
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	Same
Matrix	Human serum, buffer, stabilizers, and preservative	Same
Physico-chemical characteristics	Liquid, ready to use	Same
Levels	2 (negative and positive)	Same
Storage	2-8^{0}C	Same
Shelf life	One year	Same

Differences		
Item	**QUANTA Flash SS-B Controls**	**Predicate Device**
Method	QUANTA Flash SS-B chemiluminescent immunoassay	QUANTA Lite SS-B ELISA
Unit	CU (Chemiluminescent units) (arbitrary)	Units (arbitrary)

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

1. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory (C28-A3)
2. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (EP05-A2)
3. Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition (EP07-A2)
4. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (Interim Revision) (EP09-A2-IR)
5. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (EP6-A)
6. Guidance for Industry - Abbreviated 510(k) Submissions for In Vitro Diagnostic Calibrators; Final

L. Test Principle:

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. A patient serum sample is prediluted by the BIO-FLASH with system rinse buffer in a disposable plastic cuvette. A small amount 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 37^0C. The beads are magnetized and washed several times. Isoluminol conjugated anti-human IgG antibodies are then added to the cuvette, and again incubated at 37^0C. The beads are magnetically bound to the cuvette wall and washed repeatedly. The isoluminol conjugate is oxidized when the "Triggers" 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 SS-B on the beads.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Precision/Reproducibility:

The precision of the QUANTA Flash SS-B assay was evaluated on 10 samples containing various concentrations of SS-B antibodies in accordance with CLSI EP5-A2. Samples were run in duplicate, twice a day, for 21 days (total 84 replicates). Data were analyzed with the Analyse-it for Excel method evaluation software, and within run, between run, between day and total precision were calculated for each sample and are summarized in the Table below. All %CV values were within the manufacturer's pre-determined acceptance limit of <10%.

QUANTA Flash SS-B		**Within Run Precision**		**Between Run Precision**		**Between Day Precision**		**Total Precision**	
Sample ID	**Mean (CU)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
120814-70	12.4	0.4	3.5	0.0	0.0	0.6	4.6	0.7	5.8
110689-25	24.3	1.4	5.8	0.0	0.0	1.6	6.7	2.2	8.9
110687-120	25.0	1.2	4.7	0.3	1.1	0.8	3.2	1.5	5.8
110686-40	27.9	1.0	3.6	0.2	0.7	1.0	3.6	1.4	5.1
110684-20	132.7	7.6	5.8	0.0	0.0	6.1	4.6	9.8	7.4
120814-02	383.5	14.2	3.7	7.9	2.1	16.5	4.3	23.2	6.0
110684-04	552.3	15.7	2.8	18.3	3.3	14.7	2.7	28.2	5.1
110687-02	883.3	40.3	4.6	30.2	3.4	24.3	2.7	55.9	6.3
000674-12	1356.8	92.4	6.8	0.0	0.0	60.6	4.5	110.5	8.1
000674-10	1539.6	99.2	6.4	36.9	2.4	34.5	2.2	111.3	7.2

Reproducibility:

Seven samples were tested on two different reagent lots, using two different lots of Calibrators, by two operators. Samples were run in quadruplicate, twice a day, for 10 days, to generate 80 data points. 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 and the results are summarized in the Tables below. All %CV values were within the manufacturer's pre-determined acceptance limit of < 10%.

Quanta FLASH SS-B		**Within Run**		**Between Reagent Lots**		**Between Calibration**		**Between Operators**		**Total**	
Sample	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
1	11.7	0.3	2.8	0.5	4.6	0.3	2.8	0.4	3.0	0.5	3.9
2	26.1	0.7	2.6	0.6	2.1	1.0	3.6	0.9	3.3	0.9	3.4
3	124.2	2.8	2.3	2.8	2.3	7.1	5.7	4.3	3.5	5.3	4.3
4	640.8	23.9	3.7	50.4	7.9	50.9	7.9	43.8	6.8	50.4	7.9
5	252.7	5.1	2.0	15.9	6.3	13.1	5.2	9.6	3.8	13.4	5.3
6	1388.7	54.6	3.9	89.8	6.5	111.7	8.0	103.6	7.5	106.9	7.7
7	981.9	35.3	3.6	56.4	5.7	78.4	8.0	57.2	5.8	67.9	6.9

b. Linearity/assay reportable range:

The linearity of the AMR was evaluated by a study according to CLSI EP6-A. Five serum samples with various SS-B antibody concentrations were diluted in 10% increments (from 0% to 90% diluent) with System Rinse to obtain values that cover the AMR. The resulting dilutions were assayed in duplicates, and obtained values were plotted against the expected values, calculated based on the dilution factor. Linear regression analysis was performed on each dataset with the Analyse-it for Excel method evaluation software, and the slope and intercept of the regression line, as well as the R2 values were calculated.

Sample	Test Range (CU)	Slope (95% CI)	R^2	% Recovery
1	204.4 - 1507.0	1.03 (0.98 to 1.08)	0.99	81.4-100.0
2	155.3 - 1546.8	1.04 (0.99 to 1.09)	0.98	80.3-105.0
3	99.0 - 1048.6	0.96 (0.92 to 1.00)	0.99	92.6-100.0
4	17.4 - 163.8	1.01 (0.97 to 1.05)	0.99	100.0-111.7
5	3.6 - 29.4	0.95 (0.91 to 0.99)	0.99	95.7-110.5
All	3.6 - 1546.8	0.98 (0.96 to 1.00)	0.99	80.3-111.7

The data indicate that for the claimed analytical measuring range of 3.3 – 1550 CU, all specimens met the manufacturer's pre-determined linearity and dilutional recovery acceptance criteria.

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

Traceability:

There are currently no recognized international standards for the measurement of anti-SS-B antibodies.

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.

Value Assignment:

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.

Stability:

Shelf life stability:

Real time stability testing performed at 3, 6, 9 and 12 months on Calibrators, Controls and reagent cartridge supports a one year expiration claim.

On-board stability:

Calibrators:

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. The manufacturer tested 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. Each Calibrator is measured in triplicate during calibration. The acceptance criteria were met, and the data support the claim that calibrators can be used for up to 4 calibrations over an 8 hour period.

Controls:

During assessment of on-board stability, two 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 an 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 by the manufacturer when all replicates are within their stated range, and the linear regression line obtained by plotting percent 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 and 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, when stored at the manufacturer's recommended temperature.

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 six serum specimens along with the Negative and Positive Controls. The specimens were tested periodically up to 69 days.

Using the above criteria, each of the three lots met the manufacturer's pre-determined acceptance criteria for stability at for 57, 60, and 68 days. Based on this, 57 days stability is claimed for the reagent cartridge.

d. Detection limit:

Limit of Blank:

Four blank samples (SS-B: system rinse) from two different lots were run in replicates of five on two reagent lots, once per day, for 3 days. Sixty (60) data points per lot were generated. As the results followed a normal distribution, the LoB for each lot was calculated separately with the Analyse-it for Excel software's Reference Interval function, at the 95th percentile, using the parametric method. The LoB for the two lots was determined to be 269 RLU and 294 RLU. The claimed LoB value is 294 RLU.

Limit of Detection:

Four 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. Sixty (60) data points per lot were generated for both the LoB and LoD determination on each lot.

LoD was calculated based on the formula published in the CLSI Guideline EP17-A:

$LoD = LoB + (c_p * SD_L)$, where:

$c_p = 1.645/(1-1/(4*f))$

f = (# of total data points) – (# of samples used)

SD_L = Average of the SD's for all samples

The LoD of the QUANTA Flash SS-B assay for the two lots were determined to be 360 and 398 RLU, which are below the value of the lowest QUANTA Flash SSB Master Curve standard, and therefore below the Analytical Measuring Range of the assay, e.g. < 3.3 CU. The claimed LoD is 398 RLU.

e. Analytical specificity:

Interference:

The studies were performed according to CLSI EP07-A2, *Interference Testing in Clinical Chemistry, Approved Guideline- Second Edition*. Three specimens, a high positive (200.3 CU), a low positive (25.5 CU) and a negative (15.4 CU) were tested.

Interfering substances were spiked into every specimen at three different concentrations (final min and max concentrations as follows: hemoglobin 50-200 mg/dL; bilirubin 2.5-10 mg/dL; triglycerides 250-1000 mg/dL; rheumatoid factor 100-500 IU/mL) in 10% of total specimen volume, and the resulting samples were assessed in triplicate 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).

No interference ($\leq$ 10%) was detected with bilirubin up to 10 mg/dL; hemoglobin up to 200 mg/dL, triglycerides up to 1,000 mg/dL. cholesterol up to 224.3 mg/dL. The three specimens spiked with Rheumatoid Factor 1gM up to 500 IU/mL showed recovery from 91% to 112%.

Cross Reactivity:

Cross reactivity was investigated using international reference sera from the Center of Disease Control and Prevention (CDC).The CDC ANA reference sera #2 (reference serum for human antibodies to SS-B/La) and #3 (reference serum, fluorescence antinuclear antibody, speckled pattern) was tested for SS-B and produced the following results: CDC ANA #2: 1634.9 CU; CDC ANA #3: 284.1 CU. The other 10 reference sera in the panel were analyzed and found to be negative for SS-B by this assay.

f. Assay cut-off:

The assay cut-off is 20 CU. Results <20 CU should be reported as negative and results ≥20 CU should be reported as positive.

2. Comparison studies:

a. Method comparison with predicate device:
Samples for method comparison analysis included 639 samples from the clinical validation studies (below) that had predicate ELISA results available. The cohort consisted of Sjögren's syndrome (n = 40) and SLE patients (n = 240) and relevant disease controls (n = 359). No healthy controls were included in this cohort. Of the 639 samples, 142 samples fell within the analytical measuring range of the QUANTA Flash SS-B.

		SS-B ELISA		
		Positive	Negative	Total
QUANTA Flash® SS-B CIA	Positive	48	7	55
	Negative	6	81	87
	Total	54	88	142

Positive Agreement (48/54) = 88.9 % (95% C.I .= 77.4 – 95.8%)
Negative Agreement (81/88) = 92.0% (95% C.I.= 84.3 – 96.7%)
Total Agreement (129/142) = 90.8% (95% C.I. = 84.9 – 95.0%)

b. Matrix comparison:

Not applicable since human serum is the only claimed specimen matrix.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A cohort of characterized samples from serum libraries or on leftover serum specimens 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. Samples from patients with secondary antiphospholipid syndrome (APS) were excluded from the sensitivity and specificity calculations as primary diagnosis is not known.

		Diagnosis - SLE		
		SLE	Controls (excluding SS)	Total
QUANTA Flash SS-B	Positive	38	9	47
	Negative	252	407	659
	Total	290	416	706

Sensitivity = 13.1% (95% C.I .= 9.4 – 17.5%)
Specificity = 98.0% (95% C.I .= 96.0 – 99.1%)

		Diagnosis - SS		
		SS	Controls (excluding SLE)	Total
QUANTA Flash SS-B	Positive	14	9	23
	Negative	26	407	433
	Total	40	416	456

Sensitivity = 35.0% (95% C.I .= 20.6–1.7%)
Specificity = 97.8% (95% C.I .= 96.0–99.1%)

The distribution of the cohort and the SS-B positivity rate is in the Table below:

Cohort	**#**	**# pos**	**% pos**
SLE	290	38	13.1%
SS	40	14	35.0%
Ulcerative colitis	20	0	0.0%
Graves' Disease	19	0	0.0%
Hashimoto's 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 (SSc)	89	1	1.1%
Autoimmune Myositis	2	0	0.0%
Rheumatoid Arthritis	70	4	5.7%
Autoimmune liver disease group #1	2	1	50.0%
Autoimmune liver disease group #2**	24	1	4.2

*Samples from patients with secondary antiphospholipid syndrome (APS) were excluded from the sensitivity and specificity calculations as primary diagnosis is not known. Other Rheumatic Diseases category was comprised of the following entities:

Gout, n=2; Gonarthrosis, Polymyalgia rheumatica, n=1; Poluarteriitis nodosa, n=2; Polymyalgia rheumatica, n=10; Psoriatic arthritis , n=13; Psoriatic arthritis, Synovitis Acne Pustulosis Hyperostosis Osteitis, n=1; Sarcoidosis, n=1; Seronegative spondylarthritis, n=10.

4. Clinical cut-off**:**

   There is no clinically accepted cut-off defined for this analyte.

5. Expected values/Reference range:

   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 that used to establish the cut-off, and was only used to assess expected values. At the cut-off of 20 CU, one (0.7 %) sample was positive. The mean concentration was < 3.3 CU, and the values ranged from <3.3 to 28.7 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.