QUANTA Flash® SS-B Reagents

For *In Vitro* Diagnostic Use. CLIA Complexity: Moderate

REF 701153 Rx Only

Intended Use

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.

Summary and Explanation of the Test

Antinuclear antibodies (ANA) are found in a wide variety of connective tissue diseases and as such serve as a sensitive screening assay.[1]

ANA positive sera react with several different nuclear antigens, including small nuclear ribonucleoproteins, such as SS-B, also referred to as lupus antigen La.[2] Small nuclear ribonucleoproteins are RNA-protein complexes that combine with unmodified pre-mRNA and various other proteins to form a spliceosome, a large RNA-protein molecular complex, upon which splicing of pre-mRNA occurs. Anti-SS-B antibodies react with a 47 kDa protein associated with the hY-RNA. Anti-SS-B antibodies are rarely detected without anti-SS-A/Ro60, as both SS-A and SS-B proteins associate with the same type of RNA. Autoantibodies to SS-B antigen are found in 5-15% of Systemic Lupus Erythmatosus (SLE) patients and 30-50% of Sjögren's Syndrome (SS) patients.[3-7]

Positive serum anti-SS-B/La result is one of the three criteria as specified in the American College of Rheumatology Classification Criteria for Sjögren's Syndrome.[8]

SLE patients producing both SS-B and SS-A as opposed to SS-A alone generally have a milder disease with lower incidence of nephritis and antibodies to dsDNA.[9] Anti-SS-B IgG together with anti-Ro60 and Ro52 is transferred across the placenta in the last trimester, and can lead to pathology in the child: neonatal lupus or congenital heart block.[10-13]

A variety of methods including Ouchterlony double diffusion and passive agglutination have been used to detect antibodies to SS-B. Clinically useful ELISA assays for detecting anti-SS-B antibodies have also been developed. The QUANTA Flash SS-B is a highly sensitive chemiluminescent immunoassay for the detection and measurement of anti-SS-B antibodies that provides semi-quantitative results over a wide analytical measuring range with the convenience of random access, continuous sample loading and short assay time.

Principles of the Procedure

Recombinant SS-B protein is covalently coupled to paramagnetic beads, which are stored lyophilized in the reagent cartridge. When the assay cartridge is ready to be used for the first time, a buffer solution is added to the tube containing the preserved beads, and the beads are resuspended with the buffer. The reagent cartridge is then loaded onto the BIO-FLASH instrument.

A patient serum sample is diluted by the instrument 1:23 in a disposable plastic cuvette. Small amounts of the diluted patient serum, the SS-B beads, and the assay buffer are all combined into a second cuvette, and mixed. This cuvette is incubated at 37°C. The beads are then magnetized and washed several times. Isoluminol conjugated anti-human IgG antibody is then added to the cuvette, and incubated at 37°C. Again, the beads are magnetized and washed repeatedly. The isoluminol conjugate produces a luminescent reaction when "Trigger" reagents are added to the cuvette. The

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 bound isoluminol conjugate, which in turn is proportional to the amount of anti-SS-B antibodies bound to the SS-B on the beads.

The QUANTA Flash SS-B assay utilizes a predefined lot specific Master Curve that is uploaded into the instrument through the reagent cartridge barcode. Based on the results obtained by running two Calibrators, an instrument specific Working Curve is created, which is used by the software to calculate chemiluminescent units (CU) from the RLU value obtained for each sample.

Reagents

1. QUANTA Flash SS-B reagent cartridge contains the following reagents for 50 determinations:
    a. SS-B 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 antibody, containing buffer, protein stabilizers and preservative.
2. Resuspension buffer, 1 vial - colored pink, containing buffer, protein stabilizers and preservatives.

Warnings

1. The assay buffer contains a chemical (0.02% chloramphenicol) known to the State of California to cause cancer.
2. Sodium azide is used as a preservative. Sodium azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
3. Use appropriate personal protective equipment while working with the reagents provided.
4. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. This assay is only for use in the BIO-FLASH instrument.
3. Strict adherence to the resuspension protocol is recommended.
4. Once opened, this reagent cartridge must be stored in the instrument's reagent carousel. Care should be taken to avoid spilling the reagents when the reagent cartridge is first placed into the instrument.
5. Chemical contamination of the reagents can result from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BIO-FLASH operator's manual.

Storage Conditions

1. Store unopened reagent cartridges and resuspension buffer at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Opened reagent cartridges should be stored onboard the instrument. The BIO-FLASH software monitors the onboard (in-use) expiration as well as the reagent lot expiration (shelf-life) of the reagent cartridge. The system will not allow use of a cartridge which has expired.

Specimen Collection

This procedure should be performed on a serum specimen. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Samples containing up to 10 mg/dL bilirubin, 200 mg/dL hemoglobin, 1000 mg/dL triglycerides, 224 mg/dL cholesterol, or 500 IU/mL IgM rheumatoid factor did not show interference in the QUANTA Flash SS-B assay.

Following collection, the serum should be separated from the clot. The following storage conditions for samples are recommended:

1. Samples can be stored at room temperature for up to 48 hours.
2. Samples can be stored at 2-8°C for up to 14 days.
3. If the assay will not be completed within 14 days, or for shipment of the sample, freeze at -20°C or lower. Samples may be frozen and thawed up to 3 times. Frozen samples must be mixed well after thawing and prior to testing.

Procedure

Materials Provided

1 QUANTA Flash SS-B Reagent Cartridge

1 Resuspension buffer

1 Transfer pipette

Additional Materials Required But Not Provided

BIO-FLASH instrument with operating computer

BIO-FLASH System Rinse (Part Number: 3000-8205)

BIO-FLASH Triggers (Part Number: 3000-8204)

BIO-FLASH Cuvettes (Part Number: 3000-8206)

QUANTA Flash SS-B Calibrators (Part Number: 701151)

QUANTA Flash SS-B Controls (Part Number: 701152)

Using the BIO-FLASH Chemiluminescent Analyzer

1. Refer to the operator's manual provided with the BIO-FLASH system for detailed operating instructions of the BIO-FLASH chemiluminescent analyzer and the BIO-FLASH software. For additional information and for troubleshooting problems with this assay, contact Inova Diagnostics, Inc. technical service at the address or telephone number found at the end of this Direction Insert.

2. To empty the solid waste container, open the waste drawer. Remove the solid waste container and dispose of the used cuvettes properly. Replace the solid waste container, close the waste drawer, and click **Yes** in the **Empty Waste Drawer** window.

3. To replace the triggers, click the **Bulks Inventory F9** button (upper right).

   a. In the **Inventory – Bulks** screen, click the **Triggers** button on the left. A new window will pop up titled **Add Triggers – Remove old bottles.**

   b. Open and remove the waste drawer on the BIO-FLASH instrument. Dispose of any cuvettes in the dry waste drawer. Click **Yes** on the **Empty Waste Drawer** window. Remove the trigger bottles from their holders and click the **Next** button. Unscrew the old trigger bottles from their caps and replace with new triggers. Be sure to do them one at a time, and match the color-coded caps (white to white and red to red).

   c. Follow the instructions in the new window **Add Triggers – Add Trigger 2 bottle.** Once the barcode has been accepted, place Trigger 2 into the color-coded white holder. Click **Next**.

   d. Follow the instructions in the window **Add Triggers – Add Trigger 1 bottle.** Once the barcode has been accepted, place Trigger 1 into the color-coded red holder. Click **Finish.** Replace and close the waste drawer.

4. To replace the system rinse container, click the **Bulks Inventory F9** button (upper right corner). In the **Inventory – Bulks** screen, click the **Sys. Rinse** button. In the new window **Add System Rinse – Remove bottles**, click **Next.** Follow the instructions in the new window **Add System Rinse – Add bottle.** Once the barcode has been accepted, click **Finish** if necessary.

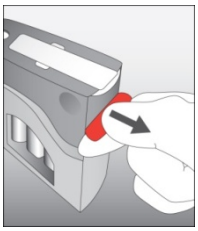
5. To empty the fluid waste container, from the **Inventory – Bulks** screen, click the **Fluid Waste** button. Remove and dispose of the fluid waste. Click **Next**. Once the empty bottle has been replaced, click **Finish**.

Method

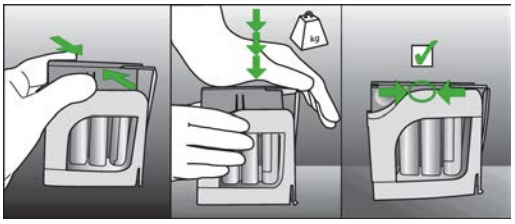
Reagent Cartridge Preparation

The first time the reagent cartridge is to be used, the following steps must be followed to accurately install the cartridge onto the BIO-FLASH instrument. Note: Do not use the reagent cartridge if any signs of damage are observed.

1. Place the reagent cartridge on a solid surface. Hold the reagent cartridge in place with one hand. With your other hand, firmly grasp the red pull-tab on the back of the reagent cartridge and pull it out completely.



2. Press the two tabs on the sides of the piercing cap (grey part) and apply pressure to the top portion of the reagent cartridge until it snaps down into a locked position. The tabs should no longer be visible. DO NOT INVERT THE OPEN CARTRIDGE.



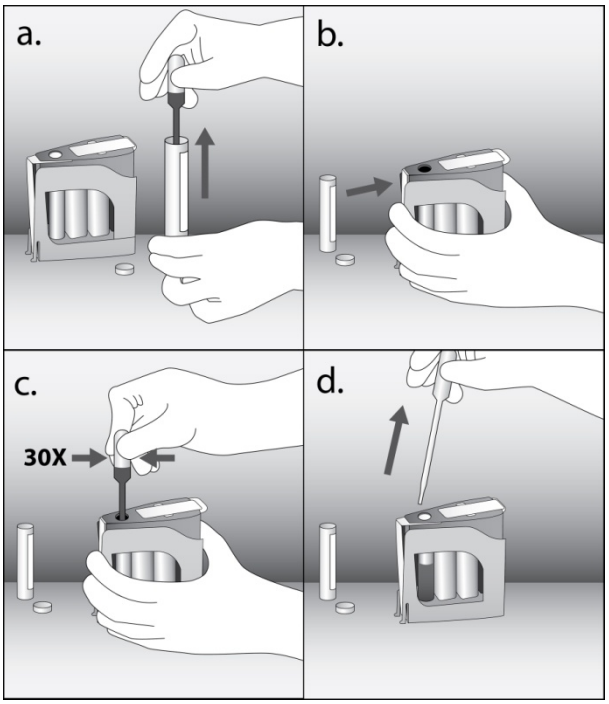
3. Resuspend the SS-B microparticles:

   a. Uncap the resuspension buffer vial and collect fluid into the transfer pipette provided. The entire contents of the vial will be used.

   b. Slide the door in the reagent cartridge lid to the open position by gently pressing the narrow side on the reagent cartridge, and hold it in this position. Carefully transfer the entire contents of the vial into the microparticle reagent tube through the one single hole on the top of the reagent cartridge.

   c. Mix the contents of the microparticle reagent tube by aspirating and dispensing the liquid at least 30 times. If visible clumps of beads are observed, continue to mix the solution for another 30 times. If the microparticles do not resuspend, DO NOT USE THE CARTRIDGE.

   d. Be sure to dispense all the liquid before removing the pipette from the tube and discarding it.



4. Peel the sticker off the top of the reagent cartridge to reveal the other three holes.

5. Place the reagent cartridge into any open slot on the reagent carousel of the BIO-FLASH instrument.

Assay Calibration

1. Each new lot of reagent cartridge must be calibrated prior to first time use. The software will not allow a new lot to be used until it is calibrated.
2. Refer to the section titled **QUANTA Flash® SS-B Calibrators 701151** of this Direction Insert for detailed instructions of how to calibrate the reagent cartridge.
3. Once the calibration is validated, the reagent cartridge lot on which the calibration was performed is ready for use.

Programming and Running Samples

1. Press the **Worklist** button at the top of the screen and select the **Racks** tab at the bottom.
2. Select the sample rack to be used by highlighting the rack on the screen or by scanning its barcode with the handheld barcode reader. Scan or type in the sample name, select the sample type, container type (tube/cup) and select SS-B from the assay panel. Repeat these steps for all samples.
3. Load the samples into the selected positions in the sample rack, and load the rack into the sample carousel of the instrument.
4. If all required materials are onboard the instrument, the start icon will be available, in green, at the top of the screen. Press the **Start F4** icon to begin the run.

Quality Control

The QUANTA Flash SS-B Controls (sold separately - Inova Item Number 701152) contains both SS-B Positive and Negative Controls. Refer to the section titled **QUANTA Flash® SS-B Controls 701152** of the Direction Insert for detailed instructions on how to input all required information of each control into the software, as well as how to run the controls. Controls are recommended to be run once every day that the assay is used; however, users should also consider national/local regulatory requirements and recommendations.

Calculation of Results

A six point Master Curve is created at Inova for each new lot of QUANTA Flash SS-B. The parameters of the curve are encoded in the barcode of each reagent cartridge. During calibration, an instrument specific Working Curve is created based on the Master Curve, and is used to convert the RLU values to CU. The antibody reactivity to SS-B can then be classified according to the table below.

Reactivity	**CU**
Negative	<20
Positive	≥20

Reactivity in CU is directly related to the titer of the autoantibody in the patient sample. Increases and decreases in patient autoantibody concentrations will be reflected in a corresponding rise or fall in CU, which is proportional to the amount of antibody.

The analytical measuring range (AMR) of the assay is 3.3 CU to 1550.0 CU, which corresponds to the linear range of the assay. If a patient result is less than 3.3 CU, the BIO-FLASH system will report it as “<3.3 CU”. Since this is less than 20 CU, it is considered a negative result. If a patient result is greater than 1550.0 CU, the BIO-FLASH system will report it as “>1550.0 CU”. This is considered a positive result. 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 >1550.0 CU after additional 10 fold dilution, 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 CU, the highest value that can be reported is 15500 CU.

Interpretation of Results

Each laboratory is advised to verify the manufacturer provided reference range, and may establish its own normal range based upon its own controls and patient population according to their own established procedures.

It is suggested that the results reported by the laboratory should include the statement: “The following results were obtained with the Inova QUANTA Flash SS-B chemiluminescent immunoassay. Values obtained with different manufacturers’ assay methods must not be used interchangeably.”

Limitations of the Procedure

1. Not all SLE and Sjögren’s syndrome patients are positive for SS-B. In our validation studies, 13% of SLE and 35% of Sjögren’s syndrome patients were positive for SS-B antibodies.
2. Results of this assay should be used in conjunction with clinical findings and other serological tests.
3. Failure to adequately resuspend the SS-B coated beads may yield lower values than if the beads are properly resuspended.
4. The performance characteristics of this assay have not been established for matrices other than serum.

Cut-off (Reference Range)

The assay cut-off was determined by testing samples form a reference population of 187 subjects consisting of 162 apparently healthy blood donors, 10 viral hepatitis positive samples, 5 syphilis serology positive samples, 5 HIV serology positive samples, and 5 rheumatoid arthritis samples. The cut-off was established based on the 99th percentile of the results obtained on the reference subjects and was assigned a value of 20 CU.

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). 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.

Traceability

No international standard serum for anti-SS-B antibodies is available that allows for the standardization of anti-SS-B antibody assays.

The Reference sera IS2073 ANA #2 and IS2074 ANA #3 from the Center of Disease Control and Prevention have been tested and showed a concentration of 1634.9 CU and 284.1 CU, respectively.

Clinical Sensitivity and Specificity

A total of 761 characterized samples were included in the clinical validation study, including 290 samples from SLE patients and 40 from Sjögren’s syndrome (SS) patients. A total of 431 samples from patients with other autoimmune and rheumatic diseases were included as controls. See Table 4 below for a detailed distribution of the disease control samples. Samples from patients with secondary antiphospholipid syndrome (APS) were excluded from the sensitivity and specificity calculations as primary diagnosis is not known.

Clinical sensitivity and specificity for SS (n=40) and SLE (n=290) separately and combined were calculated and shown in the three tables below:

Table 1 - Clinical sensitivity and specificity of the QUANTA Flash SS-B in SS:

Clinical Analysis 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	

Table 2 - Clinical sensitivity and specificity of the QUANTA Flash SS-B in SLE:

Clinical Analysis n=706		Diagnosis			Analysis (95% confidence)
		SLE	Controls (excluding SS)	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	

Table 3 - Clinical sensitivity and specificity of the QUANTA Flash SS-B in SS and SLE:

Clinical Analysis n=746		Diagnosis			Analysis (95% confidence)
		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	

Table 4 - Distribution of the disease control population used in the validation study:

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%
Hepatitis C infection	10	0	0.0%
Hepatitis B infection	10	0	0.0%
HIV infection	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%

Patient group	N	Number positive	% positive
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 with autoimmune liver disease specific antibodies (SLA, F-actin, M2)

Method Comparison with Predicate Device

Samples for method comparison analysis included 639 clinically characterized specimens. The cohort consisted of Sjögren's syndrome (n=40) and SLE patients (n=240) and relevant disease controls (359) with autoimmune or rheumatic diseases. No healthy controls were included in this cohort. These samples were tested on both the QUANTA Flash SS-B and on the predicate ELISA. Results of 142 samples out of the 639 samples fell within the analytical measuring range of the QUANTA Flash SS-B.

Table 5 - Method comparison, all samples:

Method Comparison (N=639)		SS-B ELISA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Flash® SS-B CIA	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%)

Table 6 - Method comparison, samples within the analytical measuring range:

Method Comparison (N=142)		SS-B ELISA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Flash® SS-B CIA	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%)

Precision and Reproducibility

Precision performance of the QUANTA Flash SS-B assay was evaluated by testing 10 serum samples 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. Within run, between run, between day and total precision were calculated and are summarized in the table below.

Table 7 – Precision

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 (%)
Sample #1	12.4	84	0.4	3.5	0.0	0.0	0.6	4.6	0.7	5.8
Sample #2	24.3	84	1.4	5.8	0.0	0.0	1.6	6.7	2.2	8.9

QUANTA Flash SS-B			Within Run		Between Runs		Between Days		Total	
Sample #3	25.0	84	1.2	4.7	0.3	1.1	0.8	3.2	1.5	5.8
Sample #4	27.9	84	1.0	3.6	0.2	0.7	1.0	3.6	1.4	5.1
Sample #5	132.7	84	7.6	5.8	0.0	0.0	6.1	4.6	9.8	7.4
Sample #6	383.5	84	14.2	3.7	7.9	2.1	16.5	4.3	23.2	6.0
Sample #7	552.3	84	15.7	2.8	18.3	3.3	14.7	2.7	28.2	5.1
Sample #8	883.3	84	40.3	4.6	30.2	3.4	24.3	2.7	55.9	6.3
Sample #9	1356.8	84	92.4	6.8	0.0	0.0	60.6	4.5	110.5	8.1
Sample #10	1539.6	84	99.2	6.4	36.9	2.4	34.5	2.2	111.3	7.2

Analytical Measuring Range

The lower limit of detection of this assay is 398 RLU, which is below the bottom of the AMR (3.3 CU). 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, performed on two lots of reagents. The LoB is 294 RLU.

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 serially diluted to obtain 10 dilutions of each sample, and dilutions were tested with QUANTA Flash SS-B. Dilutions covered the AMR of the assay. Obtained antibody levels were plotted against expected antibody levels. All five specimens showed dilution linearity individually, and yielded the following results with linear regression:

Table 8

AMR (CU)	Slope (95% CI)	R^2
3.3 – 1550.0	0.98 (0.96 to 1.00)	0.99

QUANTA Flash® SS-B Calibrators

For *In Vitro* Diagnostic Use

REF **701151** Rx Only

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.

Summary and Principles of the Procedure

The QUANTA Flash SS-B CIA utilizes a predefined lot specific Master Curve that is stored in the reagent cartridge barcode. The QUANTA Flash SS-B Calibrators are designed to produce an instrument specific Working Curve from the parameters of the Master Curve, with a decision point based on the performance characteristics and clinical evaluation of the QUANTA Flash SS-B CIA. Calibrators are tested on multiple instruments with multiple lots of reagents prior to value assignment.

Reagents

1. 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.
2. 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.

Warnings

1. All human source material used in the preparation of calibrators for this product has been tested and found negative with FDA cleared methods for antibody to HIV and HCV, and for HBsAg No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the QUANTA Flash SS-B Calibrators should be handled in the same manner as potentially infectious material.[14]
2. Use appropriate personal protective equipment while working with the reagents provided.
3. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. The QUANTA Flash SS-B Calibrators are for use with the QUANTA Flash SS-B assay.
3. Do not transfer the calibrator reagents to secondary tubes. The barcodes on the tubes are used by the instrument to match the calibrators to the proper assay type.
4. Once a calibrator tube is opened, it is good for up to 8 hours kept uncapped, onboard the instrument, after which the reagent must be discarded.
5. Chemical contamination of the reagents can result from improper cleaning or rinsing of the

instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BIO-FLASH operator's manual.

Storage Conditions

1 Store unopened calibrators at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.

2. Opened calibrators must be discarded after 8 hours kept uncapped, onboard the instrument.

Procedure

1. Each new lot of reagent cartridge must be calibrated prior to first time use. The software will not allow a new lot to be used until it is calibrated.

2. Each calibrator must be gently mixed before use to insure homogeneity. Avoid foam formation, as bubbles may interfere with the instruments liquid level detection. Uncap each calibrator tube and place both into a sample rack, with the barcodes facing forward through the gaps in the rack. Place the sample rack into the sample carousel of the BIO-FLASH instrument, and close the door. The instrument will read the barcodes on the calibrator tubes, and identify the required reagent cartridge. Refer to the operator's manual provided with the BIO-FLASH system for detailed operating instructions of the BIO-FLASH chemiluminescent analyzer and the BIO-FLASH software.

3. The instrument will run each calibrator in triplicate. After the Calibrators have been run, the software will require the calibration to be validated. From the **Instrument Summary** screen, click the **Choose more options – Ctrl-M (▼)** arrow button. Select **Calibration Ctrl-F3**. In the **Calibration** window, highlight the desired assay, and click **Details**.

4. In the new **Calibration Details** window, select the calibration that was just performed. The Master Curve appears as a dashed line, while the new Working Curve appears as a solid line. If the calibration results are valid, a validation button will appear in the lower left of the screen. Click the **Validate Calibration** button.

5. Once the calibration is validated, the reagent cartridge lot on which the calibration was performed is ready for use. It is recommended that the QUANTA SS-B Controls (sold separately – part number 701152) be run after a reagent cartridge lot is calibrated.

Traceability

No international standard serum for anti-SS-B antibodies is available that allows for the standardization of anti-SS-B antibody assays.

The Reference sera IS2073 ANA #2 and IS2074 ANA #3 from the Center of Disease Control and Prevention have been tested and showed a concentration of 1634.9 CU and 284.1 CU, respectively.

Limitations

These calibrators are designed for 4 calibrations. The total time the calibrator tubes can be uncapped onboard the instrument is 8 hours. If the calibrators are left uncapped, onboard, for any longer period of time, they should be discarded. Using the same calibrator tubes for more than 8 hours can result in improper calibration of the assay, which in turn could give erroneous results.

QUANTA Flash® SS-B Controls

For *In Vitro* Diagnostic Use

REF 701152 Rx Only

Intended Use

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

Summary and Principles of the Procedure

The QUANTA Flash SS-B Controls are made up of a Negative Control and a Positive Control. Each contains a different amount of anti-SS-B antibodies. The Negative Control and Positive Control are used to monitor the analytical performance of the QUANTA Flash SS-B chemiluminescent immunoassay.

Reagents

1. 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.
2. 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.

Warnings

1. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the QUANTA Flash SS-B Controls should be handled in the same manner as potentially infectious material.[14]
2. Use appropriate personal protective equipment while working with the reagents provided.
3. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. The QUANTA Flash SS-B Controls are for use with the QUANTA Flash SS-B assay.
3. Do not transfer the control reagents to secondary tubes. The barcodes on the tubes are used by the instrument to identify the control.
4. Once opened, each control tube is good for up to 15 uses with an average time of 10 minutes onboard the instrument per use, for a total of 2 ½ hours.
5. Chemical contamination of the reagents can result from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BIO-FLASH operator's manual.

Storage Conditions

1. Store unopened controls at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Opened controls can be used for up to 15 times, with an average time of 10 minutes onboard the instrument per use. The total time the control tubes can be uncapped, onboard the instrument is 2 ½ hours. If the controls are left uncapped, onboard, for a total time greater than 2 ½ hours, they should be discarded.
3. For optimal stability, remove controls from the system immediately after sampling and store them at 2-8°C capped in the original vial.

Procedure

To Create New QC Materials for the SS-B Assay:

1. Prior to using QUANTA Flash SS-B Controls for the first time on the instrument, enter the name, lot, expiration, value (or dose), and target standard deviation (SD) information into the software.
2. From the **Instrument Summary** screen, click the **Choose more options – Ctrl-M (▼)** arrow button. Select **QC Ctrl-F2**. Click the **New QC Material** button.
3. A lot specific data sheet is included with each Control kit. First enter the name, lot number, expiration from this data sheet into the software. Next, click the **Add Assay** button. In the new window, make sure the **Show All Assays** box is checked. Select the SS-B assay from the list and click **Add**. Finally, enter in the target dose and target SD. Click **Save**. Perform this process for both controls.

To Create a New Lot for Existing QC Materials:

1. Prior to using a new lot of QUANTA Flash SS-B Controls for the first time, enter the lot, expiration, value (or dose), and target SD information into the software.
2. From the **Instrument Summary** screen, click the **Choose more options – Ctrl-M (▼)** arrow button. Select **QC Ctrl-F2**. Highlight the SS-B assay in the column on the left. Then highlight the appropriate control material on the right (either “SSBN” for the Negative Control or “SSBP” for the Positive Control). Click the **New QC Lot** button.
3. A lot specific data sheet is included with each Control kit. Enter the information from this data sheet into the software. This should include the lot number, expiration, target dose, and target SD. If necessary, click the **Add Assay** button. In the new window, make sure the **Show All Assays** box is checked. Select the SS-B assay from the list and click **Add**. Click **Save**. Perform this process for both controls.

It is recommended that the QUANTA Flash SS-B Controls be run once every day that the assay is used; however, users should also consider national/local regulatory requirements.

Each control must be gently mixed before use to insure homogeneity. Avoid foam formation, as bubbles may interfere with the instruments liquid level detection. Uncap each control tube and place both into a sample rack, with the barcodes facing forward through the gaps in the rack. Place the sample rack into the sample carousel of the BIO-FLASH instrument, and close the door. The instrument will read the barcodes on the control tubes, and identify the required reagent cartridge. Refer to the operator’s manual provided with the BIO-FLASH system for detailed operating instructions of the BIO-FLASH chemiluminescent analyzer and the BIO-FLASH software.

Traceability

No international standard serum for anti-SS-B antibodies is available that allows for the standardization of anti-SS-B antibody assays.

The Reference sera IS2073 ANA #2 and IS2074 ANA #3 from the Center of Disease Control and Prevention have been tested and showed a concentration of 1634.9 CU and 284.1 CU, respectively.

Limitations

These controls are designed for 15 uses. The label of each control tube has a row of 15 boxes that may be checked off so as to track the number of uses. The total time the control tubes can be uncapped onboard the instrument is 2 ½ hours. If the controls are left uncapped, onboard, for any longer period of time, they should be discarded.

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Symbols Used

In Vitro diagnostic medical device

For prescription use only

Consult instructions for use

Temperature limitation

Do not reuse

Biological risks

Batch code

Catalog number

Use by

Manufacturer

Authorized representative

Contains sufficient for < n > tests

Positive Control

Negative Control

Calibrator 1

Calibrator 2

Recycle paper box

This end up

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