



Food and Drug Administration  
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June 1, 2016

INOVA Diagnostics, Inc.  
Dr. Gabriella Lakos  
Director, Assay Development  
9900 Old Grove Road  
San Diego, CA 92131

Re: K152635

Trade/Device Name: QUANTA Flash® Scl-70  
QUANTA Flash® Scl-70 Calibrators  
QUANTA Flash® Scl-70 Controls

Regulation Number: 21 CFR 866.5100

Regulation Name: Antinuclear Antibodies Immunological Test System

Regulatory Class: II

Product Code: LLL, JIT, JJX

Dated: April 27, 2016

Received: April 29, 2016

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 Parts 801 and 809); 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 regulations (21 CFR Parts 801 and 809), 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” (21 CFR 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,

**Kelly Oliner -S**

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

Enclosure

## Indications for Use

510(k) Number (if known)

Device Name

QUANTA Flash® Scl-70, QUANTA Flash® Scl-70 Calibrators, QUANTA Flash® Scl-70 Controls

Indications for Use (Describe)

QUANTA Flash Scl-70 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Scl-70 autoantibodies in human serum. The presence of anti-Scl-70 autoantibodies, in conjunction with clinical findings and other laboratory tests, aids in the diagnosis of systemic sclerosis.

QUANTA Flash Scl-70 Calibrators are intended for use with the QUANTA Flash Scl-70 chemiluminescent immunoassay for the determination of IgG anti-Scl-70 autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash Scl-70 Controls are intended for use with the QUANTA Flash Scl-70 chemiluminescent immunoassay for quality control in the determination of IgG anti-Scl-70 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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**QUANTA Flash® Scl-70**

**QUANTA Flash® Scl-70 Calibrators**

**QUANTA Flash® Scl-70 Controls**

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**510(k) Summary**

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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® Scl-70  
QUANTA Flash® Scl-70 Calibrators  
QUANTA Flash® Scl-70 Controls

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**Preparation date:** 08/28/2015

**Device name (assay kit):** Proprietary name: QUANTA Flash® Scl-70

	Common name:	Anti-Scl-70 Chemiluminescent Immunoassay
	Classification name:	anti-Scl-70 antibody, antigen and control
<b>Regulation Description</b>		Antinuclear antibody immunological test system
<b>Regulation Medical Specialty</b>		Immunology
<b>Review Panel</b>		Immunology
<b>Product Code</b>		LLL
<b>Regulation Number</b>		866.5100
<b>Device Class</b>		2
<b>Device name (Calibrators):</b>	Proprietary name:	QUANTA Flash® Scl-70 Calibrators
	Common name:	Scl-70 Calibrators
	Classification name:	Calibrator, secondary
<b>Regulation Description</b>		Calibrator
<b>Regulation Medical Specialty</b>		Clinical Chemistry
<b>Product Code</b>		JIT
<b>Regulation Number</b>		862.1150
<b>Device Class</b>		2
<b>Device name (Controls):</b>	Proprietary name:	QUANTA Flash® Scl-70 Controls
	Common name:	Scl-70 Controls
	Classification name:	Single (specified) analyte controls (assayed and unassayed)
<b>Regulation Description</b>		Quality control material (assayed and unassayed)
<b>Regulation Medical Specialty</b>		Clinical Chemistry
<b>Product Code</b>		JJX
<b>Regulation Number</b>		862.1660
<b>Device Class</b>		1 (reserved)

**Predicate device:** QUANTA Lite® Scl-70 ELISA, 510(k) number: K924898

### **Device description**

The QUANTA Flash Scl-70 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 Scl-70 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

Recombinant Scl-70 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 1:23.5 by the BIO-FLASH with system rinse in a 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-Scl-70 antibodies bound to the corresponding Scl-70 on the beads.

For quantitation, the QUANTA Flash Scl-70 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 Scl-70 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)mL from the instrument signal (RLU) obtained for each sample.

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The QUANTA Flash Scl-70 kit contains the following materials:

One (1) QUANTA Flash Scl-70 Reagent Cartridge

One (1) vial of Resuspension buffer

One (1) Transfer pipette

The QUANTA Flash Scl-70 Reagent Cartridge, containing the following reagents for 50 determinations:

- a. Scl-70 antigen coated paramagnetic beads, lyophilized.

- b. Assay Buffer 3 – buffer containing protein stabilizers and preservatives.
- c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.

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The QUANTA Flash Scl-70 Calibrators kit contains two vials of Calibrator 1 and two vials of Calibrator 2.

- QUANTA Flash Scl-70 Calibrator 1: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to Scl-70 in stabilizers and preservatives.
- QUANTA Flash Scl-70 Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to Scl-70 in stabilizers and preservatives.

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The QUANTA Flash Scl-70 Controls kit contains two vials of Negative Control and two vials of Positive Control.

- QUANTA Flash Scl-70 Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to Scl-70 in stabilizers and preservatives.
- QUANTA Flash Scl-70 Positive Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to Scl-70 in stabilizers and preservatives.

#### **Intended use(s)**

QUANTA Flash Scl-70 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Scl-70 autoantibodies in human serum. The presence of anti-Scl-70 autoantibodies, in conjunction with clinical findings and other laboratory tests, aids in the diagnosis of systemic sclerosis.

QUANTA Flash Scl-70 Calibrators are intended for use with the QUANTA Flash Scl-70 chemiluminescent immunoassay for the determination of IgG anti-Scl-70 autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash Scl-70 Controls are intended for use with the QUANTA Flash Scl-70 chemiluminescent immunoassay for quality control in the determination of IgG anti-Scl-70 autoantibodies in human serum.

**Substantial equivalence**

The QUANTA Flash Scl-70, the QUANTA Flash Scl-70 Calibrators and the QUANTA Flash Scl-70 Controls have the same intended use and assay principle as the predicate device.

**Comparison to predicate device**

*QUANTA Flash Scl-70 reagent kit*

<b><i>Similarities</i></b>		
<b>Item</b>	<b>QUANTA Flash Scl-70</b>	<b>Predicate Device</b>
Intended use	QUANTA Flash Scl-70 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Scl-70 autoantibodies in human serum. The presence of anti-Scl-70 autoantibodies, in conjunction with clinical findings and other laboratory tests, aids in the diagnosis of systemic sclerosis.	QUANTA Lite Scl-70 is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of Scl-70 antibodies in human serum. The presence of Scl-70 antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of scleroderma.
Assay methodology	Solid phase (heterogenous) immunoassay	Solid phase (heterogenous) immunoassay
Sample type	Serum	Serum
Shelf life	One year	One year

<b><i>Differences</i></b>		
<b>Item</b>	<b>QUANTA Flash Scl-70</b>	<b>Predicate Device</b>
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well plate
Antigen	Recombinant	Native
Conjugate	Isoluminol conjugated anti-human IgG	HRP conjugated anti-human IgG
Calibration	Lot specific Master Curve + two Calibrators (Sold separately)	Single standard (Included in the kit)

*QUANTA Flash Scl-70 Calibrators*

Item	QUANTA Flash Scl-70 Calibrators	Predicate Device
Intended use	QUANTA Flash Scl-70 Calibrators are intended for use with the QUANTA Flash Scl-70 chemiluminescent immunoassay for the determination of IgG anti-Scl-70 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; calibrators are part of the kit.
Analyte	Anti-Scl-70 antibodies	Anti-Scl-70 antibodies
Method	QUANTA Flash Scl-70 chemiluminescent immunoassay	QUANTA Lite Scl-70 ELISA
Unit	CU (Chemiluminescent units) (arbitrary)	units (arbitrary)
Matrix	Human serum, stabilizers, and preservative	Human serum, stabilizers, and preservative
Physico-chemical characteristics	Liquid, prediluted, ready to use	Liquid, prediluted, ready to use
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

*QUANTA Flash Scl-70 Controls*

Item	QUANTA Flash Scl-70 Controls	Predicate Device
Intended use	QUANTA Flash Scl-70 Controls are intended for use with the QUANTA Flash Scl-70 chemiluminescent immunoassay for quality control in the determination of IgG anti-Scl-70 autoantibodies in human serum.	No separate intended use; controls are part of the kit.
Analyte	Anti-Scl-70 antibodies	Anti-Scl-70 antibodies
Method	QUANTA Flash Scl-70 chemiluminescent immunoassay	QUANTA Lite Scl-70 ELISA
Unit	CU (Chemiluminescent units) (arbitrary)	units (arbitrary)
Matrix	Human serum, stabilizers, and	Human serum, stabilizers, and

Item	QUANTA Flash Scl-70 Controls	Predicate Device
	preservative	preservative
Physico-chemical characteristics	Liquid, ready to use	Liquid, prediluted, ready to use
Levels	2 (negative and positive)	2 (negative and positive)
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

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

The QUANTA Flash Scl-70 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-Scl-70 antibodies with antibody stabilizer buffer, containing preservative. The human serum is obtained from commercial sources and it is tested for markers of infectious substances.

The target CU is achieved through trial dilutions on small scale. Once a dilution is selected, the Calibrators and Control are bulked, tested, and adjusted. Upon completion of the manufacturing process, the Calibrators and Controls are tested on at least two instruments, on at least two lots of reagent cartridge, in replicates of 10 to determine final value assignment.

Calibrator and Control values are directly traceable to the in-house Standards that are used to create the Master Curves for the QUANTA Flash Scl-70 assay.

List of Scl-70 Standards, Calibrators and Controls:

Material	Assigned Value (CU)
Scl-70 Master Curve Standard 1	0.0
Scl-70 Master Curve Standard 2	12.6
Scl-70 Master Curve Standard 3	42.4
Scl-70 Master Curve Standard 4	106.4
Scl-70 Master Curve Standard 5	290.8
Scl-70 Master Curve Standard 6	786.3

Material	Manufacturing Target Value	Manufacturing Target Range
Scl-70 Calibrator 1	13 CU	11 – 15 CU
Scl-70 Calibrator 2	290 CU	261 – 319 CU
Scl-70 Negative Control	10 CU	8 – 12 CU
Scl-70 Positive Control	50 CU	40 – 60 CU

### **Analytical performance characteristics**

#### ***Precision***

The precision of the QUANTA Flash Scl-70 assay was evaluated on 13 samples containing various concentrations of Scl-70 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 20 days. Data were analyzed with the *Analyse-it for Excel* method evaluation software, and within run, between run, between day and total imprecisions are summarized in the Table below. Total %CV values were within the acceptance limit, 10%.

Sample	N	Mean	Within Run		Between-Run		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Precision 1	80	22.9	0.5	2.1%	0.5	2.0%	0.5	2.3%	0.9	3.7%
Precision 2	80	89.4	1.4	1.6%	1.9	2.2%	3.3	3.7%	4.1	4.6%
Precision 3	80	22.7	0.5	2.3%	0.5	2.1%	0.6	2.5%	0.9	3.9%
Precision 4	80	28.3	0.6	2.0%	0.7	2.5%	0.3	1.1%	1.0	3.4%
Precision 5	80	700.3	13.9	2.0%	11.9	1.7%	30.4	4.3%	35.5	5.1%
Precision 6	80	10.7	0.3	2.7%	0.4	4.1%	0.0	0.0%	0.5	4.9%
Precision 7	80	10.8	0.6	5.3%	0.2	1.9%	0.2	1.6%	0.6	5.9%
Precision 8	80	58.5	1.0	1.8%	1.4	2.3%	1.6	2.6%	2.3	4.0%
Precision 9	80	23.62	0.4	1.6%	0.5	2.3%	0.9	3.7%	1.1	4.6%
Precision 10	80	21.17	0.5	2.1%	0.6	2.6%	0.7	3.1%	1.0	4.6%
Precision 11	80	23.91	0.4	1.8%	0.5	2.1%	1.0	4.1%	1.2	5.0%
Precision 12	80	368.54	8.0	2.2%	5.2	1.4%	13.2	3.6%	16.3	4.4%
Precision 13	80	534.33	10.2	1.9%	12.0	2.2%	19.1	3.6%	24.7	4.6%

**Reproducibility***Reproducibility between sites (instruments)*

Eight samples were tested on three different instruments at three different sites. Samples were run in replicates of five, once a day for 5 days, to generate 25 data points per sample, per site.

Data were analyzed with the Analyse-it for Excel method evaluation software, between sites imprecision was calculated, and the results are summarized in the Table below. All %CV values were within the acceptance limit, 15%.

QUANTA Flash Scl-70			Between Site Precision (Reproducibility)	
Sample ID	Number of Replicates	Mean (CU)	SD (CU)	CV (%)
1	75	682.4	11.7	1.7
2	75	404.9	15.6	3.9
3	75	109.6	5.0	4.5
4	75	35.8	0.5	1.3
5	75	10.1	0.5	4.5
6	75	20.0	1.7	8.3
7	75	18.5	0.9	5.0
8	75	20.0	1.2	6.1

*Reproducibility between lots*

Lot to lot reproducibility study was performed according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, by testing eight samples with three different lots of reagents in five replicates for 5 days, to generate 25 data points per lot, 75 data points total.

Data were analyzed with the Analyse-it for Excel method evaluation software, between lots imprecision was calculated, and the results are summarized in the Table below. All %CV values were within the acceptance limit, 10%.

Sample ID	N	Mean (CU)	Between Lot Imprecision	
			SD (CU)	CV
1	75	14.1	1.0	6.8%
2	75	61.1	0.9	1.5%
3	75	202.4	12.9	6.4%
4	75	513.9	39.4	7.7%
5	75	564.2	36.2	6.4%
6	75	20.7	1.4	6.8%
7	75	19.8	0.8	4.2%
8	75	19.6	1.9	9.6%

#### ***Limit of Quantitation (LoQ), Limit of Blank (LoB) and Limit of Detection (LoD)***

The Limit of Quantitation (LoQ) of the QUANTA Flash Scl-70 assay is 1.2 CU, which defines the lower limit of the AMR. The LoQ was determined consistent with CLSI EP17-A2 guideline by calculating the total error (TE) of each sample per reagent lot, using the Westgard model ( $TE = \text{Bias} + 2s$ ) based on 120 measurements of four low level samples. The total error (TE) for the LoQ is < 25% (accuracy goal). The sample with the lowest CU that met the accuracy goal specification is assigned LoQ.

The LoD of the QUANTA Flash Scl-70 assay is 0.2 CU, which is below the analytical measuring range of the assay. It was determined consistent with CLSI EP17-A guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 240 determinations, with 120 measurements on blank samples and 120 measurements of low level samples, per reagent lot. The LoB is 0.1 CU.

For the LoB study, four different aliquots of System Rinse were tested in five replicates over 3 days, on two reagent lots (60 data points per lot). The LoB was calculated separately on the two lots, and the higher value was used as the final LoB.

For the LoD study, four low level samples were tested in five replicates for 3 days on two reagent lots (60 data points per lot). The LoD was calculated separately on the two lots, and the higher value was used as the final LoD.

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

1.2 CU – 786.3 CU

The AMR is defined by the values of the Limit of Quantitation and highest Master Curve Standard.

**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 re-test any sample that has a result of result >786.3 CU by further diluting it by a factor specified in the assay definition file (20 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 786.3 CU, the highest value that can be reported is 15726 CU.

To validate the Auto-rerun function, three high positive specimens with results above the analytical measuring range were selected. The samples were run with the Auto-rerun function enabled on the BIO-FLASH. Then the specimens were manually diluted 20 fold and tested on the BIO-FLASH. The results were within the analytical measuring range after auto-rerun or manual dilution for all specimens. The % recovery values for results obtained with the auto-rerun results compared to the results obtained by manual dilution were 104%, 101% and 91% (average 102%) and are within the  $\pm 20\%$  acceptance limit.

**High concentration hook effect**

N/A

**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 Scl-70 antibody concentrations were diluted with negative serum in 10% increments (from 0% to 90% negative serum) to obtain values that cover the AMR. Diluted samples were assayed in duplicate. Percent recovery was calculated compared to expected results (based on dilution). Percent recovery for all data points ranged from 80.2% to 118.4%. Obtained values were plotted against expected values, and linear regression analysis was performed on each samples, and also on the combined results. Acceptance criteria were 80%-120% recovery, 0.9-1.1 slope and  $\geq 0.95 R^2$ . Linear regression results are shown in the Table below.

Sample ID	Test Range (CU)	Slope (95% CI)	R <sup>2</sup>
Sample 2	72.0 - 727.5	1.00 (0.96 to 1.04)	0.99
Sample 3	7.9 - 101.3	1.03 (1.00 to 1.05)	1.00
Sample 4	1.8 - 20.2	1.01 (0.98 to 1.04)	1.00
Sample 5	1.7 - 9.7	1.01 (0.98 to 1.04)	1.00
Sample 6	81.2 - 739.6	0.96 (0.92 to 1.00)	1.00
All	1.7 - 739.6	1.01(0.99 to 1.02)	0.99

**Interference**

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Three specimens were tested (negative: 10.7 CU; low: 21.5 CU; positive: 49.4 CU). 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 Scl-70 assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluent (10% of total). Acceptance criteria for the interference studies were 85% - 115% recovery. The following interfering substances were tested:

Interfering substance	concentration #1 tested	concentration #2 tested	concentration #3 tested
Bilirubin, conjugated	10 mg/dL	5 mg/dL	2.5 mg/dL
Hemoglobin	200 mg/dL	100 mg/dL	50 mg/dL
Triglycerides	1000 mg/dL	500 mg/dL	250 mg/dL
Cholesterol	332.5 mg/dL	166.3 mg/mL	83.1 mg/mL
Human IgG	70 mg/mL	35 mg/mL	17.5 mg/mL
RF IgM	500 IU/mL	300 IU/mL	100 IU/mL
Prednisone	0.3 mg/mL	0.15 mg/mL	0.08 mg/mL
Naproxen	25.6 mg/mL	12.8 mg/mL	6.4 mg/mL

No interference was detected with bilirubin up to 10 mg/dL (recovery: 88% to 101%), hemoglobin up to 200 mg/dL (recovery: 93% to 105%), triglycerides up to 1000 mg/dL (recovery: 89% to 97%), cholesterol up to 224.3 mg/dL (recovery: 93% to 106%), human IgG up to 70 mg/mL (recovery 90-109% or < 4 CU), RF IgM up to 500 IU/mL (recovery: 97% to 111%), prednisone up to 0.3 mg/mL (recovery: 100.0 to 109.7) and naproxen up to 25.6 mg/mL (recovery: 97.7 to 109.7%).

### ***Cross-reactivity***

To test potential cross-reactivity with autoantibodies and infection-induced antibodies, results obtained on 375 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 infection. The composition of the cohort and the anti-Scl-70 positivity rate is shown in the Table below:

Patient group	Number of samples	# Positive	% Positive
Systemic Lupus Erythematosus	32	0	0.0%
Rheumatoid Arthritis	31	0	0.0%
Idiopathic Inflammatory Myopathy	25	0	0.0%
Mixed Connective Tissue Disease	25	0	0.0%
Celiac disease	25	0	0.0%
Autoimmune thyroiditis	25	0	0.0%
Sjögren's syndrome	20	0	0.0%
Infectious disease*	30	1	3.3%

<b>Crohn's disease</b>	<b>54</b>	<b>2</b>	<b>3.7%</b>
<b>Osteoarthritis</b>	<b>28</b>	<b>1</b>	<b>3.6%</b>
<b>COPD</b>	<b>15</b>	<b>0</b>	<b>0.0%</b>
<b>Chronic Kidney Disease</b>	<b>10</b>	<b>0</b>	<b>0.0%</b>
<b>Vasculitis</b>	<b>15</b>	<b>0</b>	<b>0.0%</b>
<b>Raynaud's</b>	<b>10</b>	<b>0</b>	<b>0.0%</b>
<b>Diabetes</b>	<b>5</b>	<b>0</b>	<b>0.0%</b>
<b>Asthma</b>	<b>15</b>	<b>0</b>	<b>0.0%</b>
<b>Skin Disease</b>	<b>10</b>	<b>1</b>	<b>10.0%</b>
<b>Total controls</b>	<b>375</b>	<b>4</b>	<b>1.4%</b>

### ***Sample stability***

Six samples, encompassing negative, around the cutoff and low to high positive samples were tested in duplicates for up to 21 days at 2-8°C, up to 48 hours at room temperature, moreover, after repeated freeze/thaw cycles up to 3 cycles. Results were compared to those obtained on control samples (day zero, at 2-8°C)

Acceptance criteria: 90-110% average recovery.

All samples fulfilled the acceptance criteria at each time point for each condition.

Based on these result, we recommend that samples are stored up to 48 hours at RT, up to 21 days of at 2-8 °C, and can be subjected to up to 3 freeze/thaw cycles (when samples are stored at or below -20 °C).

### ***Reagent stability***

#### ***Shelf life***

##### ***Accelerated Stability***

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

Accelerated stability testing was performed on three lots of each of the following sealed components of the QUANTA Flash Scl-70 to establish initial stability claim:

- Scl-70 beads
- Calibrators 1 and 2
- Low and High 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  $5 \pm 3^\circ\text{C}$ . The recovery of the measured values was calculated for each time point (compared to those obtained with  $5 \pm 3^\circ\text{C}$  stored reagent). All calculations were performed by comparing results of sealed components stored at  $5 \pm 3^\circ\text{C}$  (control) to those stored at  $37 \pm 3^\circ\text{C}$  (test) for 1, 2, 3, and 4 weeks, where one week is equal to six months at  $5 \pm 3^\circ\text{C}$ . Linear regression analysis was performed between recovery values and the number of days.

Acceptance criteria for one year preliminary expiration dating were:

-Microparticles (beads):

With regression analysis, the 95% CI interval of the regression line is between 85 and 115 % at 2 weeks, and no individual data point is outside the 75-125 %recovery range at 2 weeks.

- Controls and Calibrators:

With regression analysis, the lower 95% CI interval of the regression line is between 90 and 110 % at 2 weeks, and no individual data point is outside the 80-120 %recovery range at 2 weeks.

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

#### *Real time stability*

To confirm the shelf life that was assigned based on accelerated stability studies, real time stability studies have been initiated.

Real time stability testing will be performed on Calibrators, Controls and Reagents at regular time points to support the one year expiration. We are providing real time stability data for Calibrators, Controls, and Reagents up to 6 months

Controls were 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 samples. 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, 3 samples were tested at each time point. At every time point, all samples must be tested in three replicates.

Acceptance criteria for results obtained at consecutive time points:

- %CV of the replicates is < 15% at each time point.

- The percent recovery of each sample is between 80-120%.

All results were within the acceptance limits.

#### *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.

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

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

#### *Controls*

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

During assessing on-board stability, Low and High Controls were assayed for a total of 21 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  $5 \pm 3^{\circ}\text{C}$  for at least 2 hours between runs.

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

Low and High Controls ran within their respective acceptable range for all runs. The linear regression line obtained by plotting %recovery values against the number of runs was within 85 % and 115 % at run 15 for both Controls.

#### *Reagent Cartridge*

To determine the in-use stability of the QUANTA Flash Scl-70 reagent cartridge, two lots of reagent were tested with four to six serum specimens (with different reactivity levels) along with the Negative and Positive Controls were tested. The specimens were tested periodically for up to 60 days. Recoveries were calculated compared to the day zero average values, and linear regression analysis was performed. The claim is established using the following criteria (using the one that is fulfilled first):

- The stability claim is established on the actual measurement day preceding the day when the 95% confidence interval of the regression line reaches 85% or 115% recovery, or
- On the actual measurement day preceding the day when 2 data points or  $\geq 2\%$  of the recovery data (whichever is greater) is  $\leq 75\%$  or  $\geq 125\%$  recovery.

The in-use (onboard) stability of Scl-70 reagent cartridge was set at 60 days.

#### ***Cut-off (reference range) establishment and verification***

The reference population for establishing and verifying the reference interval for the Scl-70 assay consisted of 254 subjects:

Sample Group	N
Rheumatoid Arthritis	49
Systemic Lupus Erythematosus	50
Hashimoto's Thyroiditis	30
Hepatitis B Virus	26
Hepatitis C Virus	10
Inflammatory Bowel Disease	17
Drug Induced Lupus	16
Autoimmune atrophic gastritis	4
Biliary anastomatic stricture	2
Healthy Individuals	50
Total	254

All specimens were the same matrix (serum) as specified in the Intended Use. All specimens were unaltered. The cut-off was established in accordance to CLSI C28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition. Using Analyse-it for Excel, the upper 99th percentile Reference Interval limit was calculated as 7387.3 RLU.

Additionally, 19 systemic sclerosis samples that were positive on the predicate device were tested to aid in the determination of the cutoff. Taking into account the results of these samples, the cutoff was increased to 15,000 RLU to ensure optimal differentiation between negatives and positives, and a 20 CU value was assigned to this RLU value.

### **Clinical performance characteristics**

#### ***Clinical sensitivity, specificity***

A separate set of samples, none of which were used in establishing the reference range, was used to validate the clinical performance of the QUANTA Flash Scl-70. A total of 498 samples were included in the Validation Set for the QUANTA Flash Scl-70.

Distribution of the cohort used in the QUANTA Flash Scl-70 validation study:

Patient group	N	# Positive	% Positive
Systemic Lupus Erythematosus	32	0	0.0%
Rheumatoid Arthritis	31	0	0.0%
Idiopathic Inflammatory Myopathy	25	0	0.0%
Mixed Connective Tissue Disease	25	0	0.0%
Celiac disease	25	0	0.0%
Autoimmune thyroiditis	25	0	0.0%
Sjögren's syndrome	20	0	0.0%

Infectious disease*	30	1	3.3%
Crohn's disease	54	2	3.7%
Osteoarthritis	28	1	3.6%
COPD	15	0	0.0%
Chronic Kidney Disease	10	0	0.0%
Vasculitis	15	0	0.0%
Raynaud's	10	0	0.0%
Diabetes	5	0	0.0%
Asthma	15	0	0.0%
Skin Disease	10	1	10.0%
Total controls	375	5	1.3%
Systemic Sclerosis (SSc)	123	52	42.3%
Total	498		

\* The specific infectious disease samples tested are outlined in the table below.

Patient group	N	# Positive	% Positive
Hepatitis C virus	10	1	10.0%
Epstein-Barr virus	10	0	0.0%
Toxoplasmosis	4	0	0.0%
Cytomegalovirus	4	0	0.0%
Mycoplasma infection	1	0	0.0%
Borrelia virus	1	0	0.0%

Clinical sensitivity and specificity of the QUANTA Flash Scl-70 in systemic sclerosis

Clinical Study (N=498)		QUANTA Flash® Scl-70			Analysis (95% confidence)
		Positive	Negative	Total	
Diagnosis	SSc	52	71	123	Sensitivity = 42.3% (33.9 – 51.1%)
	Controls	5	370	375	Specificity = 98.7% (96.9 – 99.4%)
	Total	57	441	498	

#### **Expected values**

The expected value in the normal population is “negative”. Anti-Scl-70 antibody levels were analyzed in a cohort of 100 apparently healthy blood donors (42 females and 58 males, ages 21 to 67 years, with an average and median age of 46 years) using the QUANTA Flash Scl-70. This patient population was

different from the one that was used to establish the cutoff. The mean concentration was <1.2 CU, and the values ranged from <1.2 to 2.2 CU.

**Comparison with predicate device**

All samples from the Validation Set study were tested on both the QUANTA Flash Scl-70 and on the predicate ELISA, along with 41 additional samples. These additional samples were contrived by diluting Scl-70 positive serum with negative serum. The comparison is shown in the tables below.

Method comparison on all clinical samples:

All Samples (n=539)		QUANTA Flash® Scl-70			Percent Agreement (95% confidence)
		Negative	Positive	Total	
Scl-70 ELISA	Negative	441	11	452	Negative Agreement = 97.6% (95.78 – 98.6%)
	Positive	2	85	87	Positive Agreement = 97.7 % (92.0 – 99.4%)
	Total	443	96	539	Total Agreement = 97.6% (95.9 – 98.6%)

Out of the 539 samples, results were within the AMR of both the QUANTA Flash assay and of the predicate ELISA for 193 samples. Agreement on samples within the AMR is shown below:

Samples within the AMR (n=193)		QUANTA Flash® Scl-70			Percent Agreement (95% confidence)
		Negative	Positive	Total	
Scl-70 ELISA	Negative	139	10	149	Negative Agreement = 93.3% (88.1 – 96.3%)
	Positive	2	42	44	Positive Agreement = 95.5 % (84.9 – 98.7%)
	Total	141	52	193	Total Agreement = 93.8% (89.4 – 96.4%)