DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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

February 12, 2016

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

Re: K151429
Trade/Device Name: Quanta Flash® Jo-1
Quanta Flash® Jo-1 Calibrators
Quanta Flash® Jo-1 Controls
Regulation Number: 21 CFR 866.5100
Regulation Name: Antinuclear Antibodies Immunological Test System
Regulatory Class: II
Product Code: LLL, JIT, JJX
Dated: January 14, 2016
Received: January 15, 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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

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

510(k) Number *(if known)*
K151429

Device Name

QUANTA Flash® Jo-1, QUANTA Flash® Jo-1 Calibrators, QUANTA Flash® Jo-1 Controls

Indications for Use *(Describe)*

QUANTA Flash Jo-1 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Jo-1 antibodies in human serum. The presence of anti-Jo-1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic inflammatory myopathy.

QUANTA Flash Jo-1 Calibrators are intended for use with the QUANTA Flash Jo-1 Reagents for the determination of IgG anti-Jo-1 antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash Jo-1 Controls are intended for use with the QUANTA Flash Jo-1 Reagents for quality control in the determination of IgG anti-Jo-1 antibodies in human serum.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Table of Contents

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

Administrative data

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

Purpose of submission: New device(s)

Devices in the submission:
QUANTA Flash® Jo-1
QUANTA Flash® Jo-1 Calibrators
QUANTA Flash® Jo-1 Controls

Scientific contact:
Gabriella Lakos, Director of Research, Rheumatology
Inova Diagnostics, Inc.
9900 Old Grove Road, San Diego, CA, 92131
Phone: 858-586-9900/1393
Fax: 858-863-0025
email: glakos@inovadx.com

Quality Systems contact:
Ronda Elliott, VP, Quality Systems and RA
Inova Diagnostics, Inc
9900 Old Grove Road, San Diego, CA, 92131
Phone: 858-586-9900/1381
Fax: 858-863-0025
email: relliot@inovadx.com

Device name (assay kit):

Proprietary name:	QUANTA Flash® Jo-1
Common name:	Anti-Jo-1 Chemiluminescent Immunoassay
Classification name:	anti- Jo-1 antibodies, antigen and control

Regulation Description	Extractable Antinuclear Antibody, Antigen and Control
Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	LLL

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

Predicate device

FIDIS Connective 10, 510(k) number: K053653

Device description

The QUANTA Flash Jo-1 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 Jo-1 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

Recombinant Jo-1 antigen is coated onto paramagnetic beads, which is stored in the reagent cartridge

as a suspension. When the cartridge is ready to be used for the first time, the entire cartridge is inverted several times to thoroughly mix the reagents. The reagent cartridge is then loaded onto the BIO-FLASH instrument. Samples are also loaded onto the instrument in sample racks. Serum samples are diluted by the BIO-FLASH with system rinse in a small disposable plastic cuvette. Small amounts of the diluted patient serum, the beads, and assay buffer are 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-Jo-1 antibodies bound to the corresponding beads.

For quantitation, the QUANTA Flash Jo-1 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 Jo-1 Calibrators. Based on the results obtained with the two Calibrators included in the Calibrator Set (sold separately), an instrument specific Working Curve is created, which is used to calculate chemiluminescent units (CU) from the instrument signal (RLU) obtained for each sample.

The QUANTA Flash Jo-1 kit contains the following materials:

One (1) QUANTA Flash Jo-1 Reagent Cartridge

The QUANTA Flash Jo-1 reagent cartridge contains the following reagents for 50 determinations:

a. Jo-1 coated paramagnetic beads, in a suspension containing buffer, protein stabilizers and preservative.
b. Assay buffer – colored pink, containing buffer, Tween 20, protein stabilizers and preservatives.
c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.

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

QUANTA Flash Jo-1 Calibrators:

- QUANTA Flash Jo-1 Calibrator 1: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to Jo-1 in buffer, stabilizer and preservative.
- QUANTA Flash Jo-1 Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL

prediluted, ready to use reagent. Calibrators contain human antibodies to Jo-1 in buffer, stabilizer and preservative.

The QUANTA Flash Jo-1 Controls kit contains two vials of Negative Control and two vials of Positive Control:

QUANTA Flash Jo-1 Controls:

- QUANTA Flash Jo-1 Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to Jo-1 in buffer, stabilizer and preservative.
- QUANTA Flash Jo-1 Positive Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to Jo-1 in buffer, stabilizer and preservative.

Intended use(s)

QUANTA Flash Jo-1 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Jo-1 antibodies in human serum. The presence of anti-Jo-1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic inflammatory myopathies.

QUANTA Flash Jo-1 Calibrators are intended for use with the QUANTA Flash Jo-1 Reagents for the determination of IgG anti-Jo-1 antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash Jo-1 Controls are intended for use with the QUANTA Flash Jo-1 Reagents for quality control in the determination of IgG anti-Jo-1 antibodies in human serum.

Indications for use

Same as Intended use.

Substantial equivalence

The QUANTA Flash Jo-1 Reagent, the QUANTA Flash Jo-1 Calibrators and the QUANTA Flash Jo-1 Controls have the same intended use and assay principle as the predicate device, FIDIS Connective 10.

Comparison to predicate device

QUANTA Flash Jo-1 reagent kit

Similarities

Item	**QUANTA Flash Jo-1**	**Predicate Device**
Intended use	Semi-quantitative determination of anti-Jo-1 antibodies in human serum	Semi-quantitative detection of anti-Jo-1 antibodies in human serum
Assay methodology	Solid phase immunoassay	Solid phase immunoassay
Antigen	Recombinant antigen	Recombinant antigen
Shelf life	One year	One year
Sample Type	Serum	Serum

Differences		
Item	**QUANTA Flash Jo-1**	**Predicate Device**
Detection/ Operating principle	Chemiluminescent immunoassay	Multiplex bead-based flow cytometric fluorescent immunoassay
Solid phase	Paramagnetic microparticles (beads)	Color-coded microspheres
Conjugate	Isoluminol conjugated anti-human IgG	Phycoerythrin conjugated anti-human IgG
Calibration	Lot specific Master Curve + two calibrators (sold separately)	Calibration system interpolates fluorescent intensity (Included in the kit)

QUANTA Flash Jo-1 Calibrators

Item	**QUANTA Flash Jo-1 Calibrators**	**Predicate Device**
Intended use	For use with the QUANTA Flash Jo-1 Reagents 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-Jo-1 antibodies	Anti-Jo-1 antibodies
Method	QUANTA Flash Jo-1 chemiluminescent immunoassay	Included in FIDIS Connective 10
Matrix	Human serum, stabilizer, and preservative	Diluted human serum
Unit	CU (Chemiluminescent units) (arbitrary)	AU/mL (arbitrary)
Physico-chemical characteristics	Liquid, prediluted, ready to use	Liquid, prediluted, ready to use
Storage	2-8 ^{o}C	2-8 ^{o}C
Shelf life	One year	One year

QUANTA Flash Jo-1 Controls

Item	QUANTA Flash Jo-1 Controls	Predicate Device
Intended use	For use with the QUANTA Flash Jo-1 reagents for quality control in the determination of IgG anti-Jo-1 autoantibodies in human serum.	No separate intended use; controls are part of the kit.
Analyte	Anti-Jo-1 antibodies	Anti-Jo-1 antibodies
Method	QUANTA Flash Jo-1 chemiluminescent immunoassay	Included in FIDIS Connective 10
Matrix	Human serum, stabilizers, and preservative	Diluted human serum
Unit	CU (Chemiluminescent units) (arbitrary)	AU/mL (arbitrary)
Physico-chemical characteristics	Liquid, ready to use	Liquid, to be diluted
Levels	2 (negative and positive)	2 (negative and positive)
Storage	2-8 ^{o}C	2-8 ^{o}C
Shelf life	One year	One year

Analytical performance characteristics

Quantitation and units of measure

For quantitation, the QUANTA Flash Jo-1 assay utilizes a lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. The Master Curve for QUANTA Flash Jo-1 consists of 6 Standards. These Master Curve Standards are used to create the lot specific Master Curve during the manufacturing procedure.

List of Jo-1 Standards:

Material	Assigned Value
Jo-1 Master Curve Standard 1	2.2 CU
Jo-1 Master Curve Standard 2	19.0 CU
Jo-1 Master Curve Standard 3	38.9 CU
Jo-1 Master Curve Standard 4	71.1 CU
Jo-1 Master Curve Standard 5	320.2 CU
Jo-1 Master Curve Standard 6	1147.2 CU

Value assignment and traceability of Calibrators and Controls

The QUANTA Flash Jo-1 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-Jo-1 antibodies with stabilizer and 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 Jo-1 assay.

Jo-1 Calibrators and Controls with target manufacturing values:

Material	Manufacturing Target Value	Manufacturing Target Range
Jo-1 Calibrator 1	19 CU	17 – 21 CU
Jo-1 Calibrator 2	320 CU	280 – 360 CU
Jo-1 Negative Control	10 CU	8 – 12 CU
Jo-1 Positive Control	50 CU	40 – 60 CU

Precision

The precision of the QUANTA Flash Jo-1 assay was evaluated on 9 samples containing various concentrations of Jo-1 antibodies in accordance with CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; 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 precision were calculated.

Acceptance criteria: Total %CV: < 10%

Results are summarized in the Table below.

QUANTA Flash® Jo-1			Within Run		Between-Run		Between-Day		Total	
Sample ID	N	Mean (CU)	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Pt A	80	5.5	0.2	3.4%	0.0	0.7%	0.4	6.8%	0.4	7.6%
Pt B	80	19.5	0.6	3.1%	0.5	2.6%	0.9	4.7%	1.2	6.2%
Pt I	80	20.7	0.8	3.7%	0.3	1.4%	1.2	5.9%	1.5	7.1%
Pt C	80	37.1	1.6	4.4%	0.0	0.0%	2.3	6.1%	2.8	7.6%
Pt D	80	42.0	1.2	2.9%	1.0	2.3%	2.4	5.6%	2.8	6.7%
Pt E	80	97.5	3.1	3.2%	2.4	2.4%	6.0	6.1%	7.1	7.3%
Pt F	80	288.2	10.0	3.5%	8.5	3.0%	19.1	6.6%	23.2	8.0%
Pt G	80	445.6	18.7	4.2%	0.0	0.0%	29.4	6.6%	34.8	7.8%
Pt H	80	879.3	43.6	5.0%	13.2	1.5%	43.8	5.0%	63.2	7.2%

Reproducibility

Five 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, and within days, between days, between site, and total precision were calculated.
Acceptance criteria: Total %CV: < 15%
Results are summarized in the Table below.

QUANTA Flash® Jo-1			Within Days		Between-Days		Between-Sites		Total	
Sample ID	Number of Replicates	Mean (CU)	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Sample 1	75	854.6	34.4	4.0%	29.7	4.0%	0.4	6.8%	100.0	11.7%
Sample 2	75	32.4	1.0	3.1%	1.9	5.9%	2.3	7.0%	3.1	9.6%
Sample 3	75	168.3	6.6	3.9%	8.1	4.8%	14.7	8.8%	18.0	10.7%
Sample 4	75	77.3	3.1	4.0%	3.2	4.2%	6.4	8.3%	7.8	10.1%
Sample 5	75	9.8	0.5	4.7%	0.5	4.6%	0.8	8.0%	1.0	10.4%

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

The LoD of the QUANTA Flash Jo-1 assay is 409 RLU, which is below the analytical measuring range of the assay. It was determined consistent with CLSI EP17-A2 guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 240 determinations, with 60 measurements on blank samples and 60 measurements of low level samples, per reagent lot. The LoB is 337 RLU.

These values are below the value of the lowest QUANTA Flash Jo-1 Master Curve standard, i.e. below the Analytical Measuring Range.

Analytical Measuring Range (AMR)

QUANTA Flash Jo-1: 2.2 CU – 1147.2 CU
The AMR is defined by the values of the lowest and highest Master Curve Standards.

Auto-rerun function and reportable results

The BIO-FLASH software has an Auto-rerun option available. If this option is selected, the instrument will automatically rerun any sample that has a result of >1147.2 CU after further diluting it by 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 directly measured is 1147.2 CU, the highest value that can be reported is 22944 CU.

High concentration hook effect

To assess hook effect, measurement signal (relative light units, RLU) was examined by performing serial dilutions of two high positive samples (with results above the AMR when tested as neat samples). RLU values showed increase with increasing antibody concentrations above the AMR, thereby confirming that high positive specimens above the analytical measuring range do not show hook effect up to 65625.5 CU in the Jo-1 assay (the highest concentration that was tested).

Linearity

The linearity of the AMR was evaluated by a study according to CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Fiver serum samples with various Jo-1 antibody concentrations were diluted with negative serum in 10% increments (from 0% to 90% negative serum) to obtain values that cover the AMR. The dilutions were assayed in duplicates. Percent recovery of obtained mean results was calculated compared to the expected mean results (based on the dilution factor). Moreover, obtained values of individual replicates were plotted against expected values, and linear regression analysis was performed.
Acceptance criteria:
- Recovery is between 80-120%, or ± 4 CU, whichever is greater.
- For linear regression analysis, slope is between 0.9-1.1, and R^2 is ≥ 0.95.

All five specimens showed dilution linearity individually.

Sample	Test Range (CU)	Slope (95% CI)	R^2
Sample 1	3.0 - 14.4	0.99 (0.96 to 1.02)	1.00
Sample 2	4.9 - 44.3	0.99 (0.95 to 1.02)	0.99
Sample 3	10.1 - 110.5	0.99 (0.98 to 1.01)	0.99
Sample 4	60.1 - 686.8	1.00 (0.95 to 1.04)	0.99
Sample 5	139.3 - 1126.4	0.92 (0.89 to 0.96)	1.00

The combined data yielded the following results with linear regression:

Sample	Test Range (CU)	Slope (95% CI)	R^2
All	3.0 – 1126.4	0.96 (0.95 to 0.97)	1.00

These data demonstrate the linearity of the analytical measuring range (2.2 CU – 1147.2 CU) of the QUANTA Flash Jo-1 assay.

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: 11.3 CU; around cutoff: 21.1 CU; positive: 117.0 CU). Interfering substances (hemoglobin, bilirubin, and triglycerides/cholesterol) 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 QUANTA Flash Jo-1 assay. Moreover, 3 additional samples (negative: 7.3 CU; low positive: 32.5 CU; high positive: 123 CU) were tested for RF interference by combining them with different proportions of a high positive RF IgM serum sample (1894 IU/mL). Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluents (10% of total sample volume, except for RF). For the RF interference study, recovery values were calculated compared to control samples created by adding negative serum to the test serum in the same proportions as the RF serum was used). Acceptance criteria for the interference studies were 85% - 115% recovery for samples above the cutoff, and ± 4 CU difference for samples below the cutoff.

No interference was detected with bilirubin up to 10 mg/dL (recovery: 89% to 98%), hemoglobin up to 200 mg/dL (recovery: 86% to 101% or < 4 CU), triglycerides up to 1000 mg/dL (recovery: 86% to 99% or < 4 CU), cholesterol up to 224.3 mg/dL (recovery: 86% to 99% or < 4 CU) and RF up to 947 IU/mL (recovery: 100% to 114% or < 4 CU).

Cross-reactivity

To test potential cross-reactivity with autoantibodies and infection-induced antibodies, results obtained on 201 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-Jo-1 positivity rate is shown in the Table below:

Diagnosis	**Number of samples**	**# pos**	**% pos**
Systemic Sclerosis	44	0	0.0%
Rheumatoid Arthritis	59	1	1.7%
Systemic Lupus Erythematosus	41	0	0.0%
Septicaemia	19	0	0.0%
Mixed Connective Tissue Disease	103	1	0.9%
Sjögren's syndrome	15	0	0.0%
Total controls	**281**	**2**	**0.7%**

Based on the results, the QUANTA Flash Jo-1 assay does not show cross-reactivity with autoantibodies that are present in various autoimmune diseases or with antibodies against infectious agents.

Lot to lot comparison

Five unique samples with various reactivity levels were tested with three different reagent lots: RP0007, 141011, 151012. The samples covered the analytical measuring range of the assay. Samples were tested in replicates of 5, once per day, for 5 days according to CLSI EP05-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline.
Data were analyzed with the Analyse-it for Excel method evaluation software to calculate between lot precision.
Acceptance criteria: Between lot %CV: < 10%
Results are summarized in the Table below.

QUANTA Flash Jo-1			Between Lot Precision	
Sample ID	Mean (CU)	N	SD (CU)	CV (%)
1	18.6	75	1.4	7.7%
2	23.3	75	1.3	5.5%
3	73.3	75	3.7	5.0%
4	382.5	75	11.0	2.9%
5	911.9	75	67.5	7.4%

Sample Stability

Seven samples, encompassing negative, around the cutoff and moderate to high positive samples were tested in duplicates after storing for up to 21 days at 2-8°C, up to 48 hours at room temperature (RT), moreover, after repeated freeze/thaw cycles up to 5 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 14 days at 2-8 ^{o}C, and can be subjected to up to 3 freeze/thaw cycles (when samples are stored at or below -20 ^{o}C).

Reagent Stability

Shelf life

To establish the initial claim for shelf life, accelerated stability studies were performed for 4 weeks at 37°C ± 3°C, where one week is equal to six months at 5 ± 3°C.

Accelerated stability testing was performed on each of the following sealed components of the QUANTA Flash Jo-1 to establish initial stability claim: the beads, the two Calibrators, and the Negative and Positive Controls. Each week a new sealed component was placed in the incubator, and all components were tested at the end of the experiment together with the one that was stored at 5 ± 3°C. The recovery of the measured values was calculated for each time point (compared to those obtained with 5 ± 3°C stored reagent). All calculations were performed by comparing results of sealed components stored at 5 ± 3°C (control) to those stored at 37 ± 3°C (test) for 1, 2, 3, and 4 weeks, where one week is equal to six months at 5 ± 3°C. Linear regression analysis was performed between recovery values and the number of days.

Acceptance criteria for one year preliminary expiration dating:

- Beads:

With regression analysis, the lower and upper 95% Cl interval of the regression line is between 85% and 115% recovery at day 14, and no individual data point has ≤75% or ≥125% recovery at day 14.

- Controls and Calibrators:

With regression analysis, the lower and upper 95% Cl interval of the regression line is between 90% and 110% recovery at day 14, and no individual data point has ≤80% or ≥120% recovery at day 14.

Beads

Testing was performed on three lots of Jo-1 coupled beads using up to 13 characterized samples with various reactivity levels.

All three lots of beads retained between 85% and 115% reactivity (considering the 95% CI of the regression line) after two weeks at 37 ± 3°C, and therefore pass the acceptance criteria for one year expiration date.

Calibrators and Controls

Testing was performed on three lots of Jo-1 Calibrators and Controls. All Calibrators and Controls maintained between 90% and 110% reactivity (considering the 95% CI of the regression line) when stored at 37 ± 3°C for 2 weeks, and therefore pass the acceptance criteria for one year expiration dating.

In-use (onboard) stability

Calibrators

Onboard stability claim: 4 calibrations, or 8 hours onboard

During assessment of on-board stability, Calibrators were placed uncapped, onboard the instrument, and calibration was performed altogether five times over 9.5 hours. Controls and a panel of characterized patient specimens were run on each calibration curve.

Calibrators are considered stable if all five calibrations performed in the 9.5 hour period are successful, and average Calibrator RLU recovery values are between 90% and 110% compared to the first use.

A total of 5 successful calibrations were performed over a 9.5 hour period. Calibrator RLU values remained within the 90-110% range. Moreover, all Controls and patient panel samples ran within their expected range. This supports the claim that calibrators can be used for up to 4 calibrations over an 8 hour period.

Controls

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

During assessing on-board stability, 2 vials of each Control were assayed twice a day for a total of 21 runs. The first run was used to establish baseline value, by running each vial in duplicate, and then additional 20 runs were performed, by running each vial in singleton. 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 5 ± 3°C.

Percent recovery of each value was calculated compared to the baseline value. Controls are considered stable when all values run within their established range, and the linear regression line obtained by plotting %recovery values against the number of runs stays between 85% and 115% at run 15.

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

Reagent Cartridge

To establish the in-use stability of the QUANTA Flash Jo-1 reagent cartridge, two lots of reagent were tested with 4 serum specimens (with different reactivity levels) along with the Negative and Positive Controls. The specimens were tested periodically up to 92 days. Percent recoveries were calculated compared to the day zero average values, and linear regression analysis was performed by plotting %recovery against the number of days. The claim was established using the following criteria (using the one that is fulfilled first):

- The stability claim is established at the actual measurement day preceding the day when the 95% confidence interval of the regression line reaches 85% or 115% recovery, or
- At the actual measurement day preceding the day when 2 data points or ≥2% of the recovery data (whichever is greater) is ≤ 75% or ≥ 125% recovery.

As none of these endpoints were reached during the duration of the studies, the in-use (onboard) stability of two lots are as follows:

111001: 74 days

111002: 71 days

The in onboard stability of Jo-1 reagent cartridge was set at 71 days.

Real time stability

Real time stability testing has been scheduled to be performed approximately every three months on the reagent cartridge, Calibrators and Controls, to verify the one year expiration that was assigned based on accelerated stability studies. At the time of the submission, results were available up to 12 months for reagent cartridge, up to 15 months on Calibrators and up to 16 months on Controls.

For reagent cartridge, QC panel samples were tested in singleton at each time point. The QC panel is a group of characterized patient samples with target values, used by the QC Department for reagent release and QC.

- Acceptance criteria: results should fall within their respective QC ranges.

Calibrators were used to calibrate a cartridge at each time point. After calibration, the QC panel samples were tested in singleton at each time point.

- Acceptance criteria: results should fall within their respective QC ranges.

Controls were tested in singleton on a calibrated cartridge at each time point. Individual values were compared to the values that were assigned to the Controls at release.

- Acceptance criteria: results should fall within their acceptable ranges as were established at the release of the Controls.

All results to date were within the acceptance limit, therefore one year expiration dating has been verified through real-time studies.

Cut-off, reference range

QUANTA Flash Jo-1: Negative <20 CU
Positive ≥20 CU

The reference population for establishing the reference interval for the Jo-1 assay consisted of 207 subjects:

Sample Group	N
Systemic Sclerosis	31
Systemic Lupus Erythematosus	30
Crohn's disease	21
Multiple Sclerosis	19
Hepatitis C Virus	19
Ulcerative Colitis	18
Psoriatic Arthritis	13
Syphilis	10
Healthy Individuals	10
Polymyalgia Rheumatica	9
Rheumatoid Arthritis	8
Spondylarthritis	5
Sjogren's Syndrome	3
Other*	11

*The Other samples group includes: Monoclonal Gammopathy (2), Primary Biliary Cirrhosis (2), Mixed Connective Tissue Disease (2), Wegener's Granulomatosis (2), CREST (1), Gout (1) and Psoriatic arthritis (1)

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 EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition. The Analyse-it for Excel software was used to make the calculations. The distribution of the results was non-normal (Shapiro-Wilk $p<0.0001$), so the non-parametric percentile method was used. The 99th percentile of the remaining obtained values was calculated as 9151 RLU.
The cutoff was set to 10000 RLU. One Systemic Sclerosis patient tested positive at this cutoff level.

Clinical performance characteristics

Clinical sensitivity, specificity

A cohort of 487 characterized samples, none of which were used for establishing the reference range, was used to validate the clinical performance of the QUANTA Flash Jo-1. All samples were run on the QUANTA Flash Jo-1. The distribution of the cohort and the Jo-1 positivity rate is in the Table below:

Patient group	**N**	**Number positive**	**% positive**
Systemic Sclerosis	44	0	0.0%
Rheumatoid Arthritis	59	1	1.7%
Systemic Lupus Erythematosus	41	0	0.0%
Septicacaemia	19	0	0.0%
Mixed Connective Tissue Disease	103	1	0.9%
Sjögren's syndrome	15	0	0.0%
Total controls	**281**	**2**	**0.7%**
Idiopathic Inflammatory Myopathy (IIM)	206	24	11.7%
Total	**487**	**-**	**-**

The composition if the IIM cohort is shown below.

Idiopathic Inflammatory Myopathy (IIM)	**N**	**Number positive**	**% positive**
Dermatomyositis	95	11	11.7%
Polymyositis	71	7	9.9%
Juvenile Dermatomyositis	7	1	14.3%
Others (IMNM, OM, UM)*	5	1	20.0%
IIM not further specified	28	4	14.3%
Total	**206**	**24**	**11.7%**

*IMNM – immune mediated necrotizing myopathy, OM – overlap myositis, UM – undifferentiated myopathies

The results were analyzed to calculate sensitivity and specificity for IIM (n=206), using all 281 controls.

Clinical sensitivity and specificity of the QUANTA Flash Jo-1 in IIM

Clinical Sensitivity and Specificity (N=487)		QUANTA Flash Jo-1			Analysis (95% confidence)
		Positive	Negative	Total	
Diagnosis	IIM	24	182	206	Sensitivity = 11.7% (8.0 – 16.7%)
	Controls	2	279	281	Specificity = 99.3% (97.4 – 99.8%)
	Total	26	433	487	

Expected values

The expected value in the normal population is "negative". Anti-Jo-1 antibody levels were analyzed in a cohort of 400 apparently healthy blood donors (246 females and 154 males, ages 17 to 60 years, with an average or 34.7 years and median age of 34 years) using the QUANTA Flash Jo-1. This patient population was different from the one that was used to establish the cutoff, and was only used to assess expected values. None of the samples tested positive in this cohort. The mean concentration was 2.3 CU, and the values ranged from <2.2 to 16.3 CU.

Comparison with predicate device

Samples for method comparison analysis included 425 samples from the clinical validation study, along with 26 additional samples. These additional samples were contrived by diluting Jo-1 positive samples with negative serum. Altogether 21 samples were used to create the additional 26 samples. These samples were tested on both the QUANTA Flash Jo-1 and on the predicate assay, FIDIS Connective 10. The predicate device has a borderline range, 30-40 AU/mL. A total of 105 samples were within the analytical measuring range of the assay. The data are presented in two ways; with predicate borderline samples as negative in the first table, then as positive in the following table:

Method Comparison, samples within AMR:

Method Comparison (N=105) Predicate borderline as negative		QUANTA Flash Jo-1			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate Device	Negative	72	11	83	Neg. Agree = 86.7% (77.8 – 92.4%)
	Positive	2	20	22	Pos. Agree = 90.9% (72.2 – 97.5%)
	Total	74	31	105	Total Agree = 87.6% (80.0 – 92.6%)

Method Comparison (N=105) Predicate borderline as positive		QUANTA Flash Jo-1			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate Device	Negative	70	6	76	Neg. Agree = 92.1% (83.8 – 96.3%)
	Positive	4	25	29	Pos. Agree = 86.2% (69.4 – 94.5%)
	Total	74	31	105	Total Agree = 90.5% (83.4 – 94.7%)