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

April 11, 2016

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

Re: K152013
Trade/Device Name: QUANTA Flash® dsDNA
QUANTA Flash® dsDNA Calibrators
QUANTA Flash® dsDNA Controls
Regulation Number: 21 CFR 866.5100
Regulation Name: Antinuclear Antibodies Immunological Test System
Regulatory Class: II
Product Code: LSW, JIT, JJX
Dated: March 9, 2016
Received: March 11, 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)*
K152013

Device Name
QUANTA Flash® dsDNA
QUANTA Flash® dsDNA Calibrators
QUANTA Flash® dsDNA Controls

Indications for Use *(Describe)*
QUANTA Flash dsDNA is a chemiluminescent immunoassay for the quantitative determination of IgG anti-double stranded deoxyribonucleic acid (dsDNA) antibodies in human serum. The presence of anti-dsDNA antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus.

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

QUANTA Flash dsDNA Controls are intended for use with the QUANTA Flash dsDNA chemiluminescent immunoassay for quality control in the determination of IgG anti-dsDNA 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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510(k) Summary

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® dsDNA
QUANTA Flash® dsDNA Calibrators
QUANTA Flash® dsDNA Controls

Scientific contact: Gabriella Lakos, Director of Assay Development
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Fax: 858-863-0025/1381
email: relliott@inovadx.com

Preparation date: 06/26/2015

Device name (assay kit): Proprietary name: QUANTA Flash® dsDNA

	Common name:	Anti-dsDNA Chemiluminescent Immunoassay
	Classification name:	anti-dsDNA antibody, antigen and control
Regulation Description	Antinuclear antibody immunological test system	
Regulation Medical Specialty	Immunology	
Review Panel	Immunology	
Product Code	LSW, Anti-DNA Antibody, Antigen and Control	
Regulation Number	866.5100	
Device Class	2	

Device name (Calibrators):	Proprietary name:	QUANTA Flash® dsDNA Calibrators
	Common name:	dsDNA 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® dsDNA Controls
	Common name:	dsDNA Controls
	Classification name:	Single (specified) analyte controls (assayed and unassayed)
Regulation Description	Quality control material (assayed and unassayed)	
Regulation Medical Specialty	Clinical Chemistry	
Product Code	JJX	
Regulation Number	862.1660	
Device Class	1 (reserved)	

Predicate device: QUANTA Lite® dsDNA SC ELISA, 510(k) number: K993727

Device description

QUANTA Flash dsDNA is a chemiluminescent microparticle immunoassay for the quantitative determination of IgG anti-double stranded deoxyribonucleic acid (dsDNA) antibodies in human serum. The QUANTA Flash dsDNA 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 dsDNA assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

Synthetic dsDNA is coated onto paramagnetic beads, which are stored in the reagent cartridge in suspension. When the assay cartridge is ready to be used for the first time, the entire cartridge is inverted several times to thoroughly mix the reagents. The sealed reagent tubes are then pierced with the reagent cartridge lid. 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 1:10 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-dsDNA antibodies bound to the corresponding dsDNA on the beads.

For quantitation, the QUANTA Flash dsDNA 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 dsDNA Calibrators. The Master Curve is created during manufacturing by using in-house standards that are traceable to the First International Standard Preparation for dsDNA (WHO code: Wo/80). 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 international units (IU)/mL from the instrument signal (RLU) obtained for each sample.

The QUANTA Flash dsDNA kit contains the following materials:

One (1) QUANTA Flash dsDNA Reagent Cartridge, containing the following reagents for 50 determinations:

a. dsDNA antigen coated paramagnetic beads in a suspension.

b. Assay Buffer 3 – buffer containing protein stabilizers and preservatives.

c. Tracer IgG 2 – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.

The QUANTA Flash dsDNA Calibrators kit contains two vials of Calibrator 1 and two vials of Calibrator 2.

- QUANTA Flash dsDNA Calibrator 1: Two (2) barcode labeled tubes containing 0.7 mL prediluted, ready to use reagent. Calibrators contain human antibodies to dsDNA in stabilizers and preservatives.
- QUANTA Flash dsDNA Calibrator 2: Two (2) barcode labeled tubes containing 0.7 mL prediluted, ready to use reagent. Calibrators contain human antibodies to dsDNA in stabilizers and preservatives.

The QUANTA Flash dsDNA Controls kit contains two vials of Negative Control and two vials of Positive Control.

- QUANTA Flash dsDNA Low Control: Two (2) barcode labeled tubes containing 0.7 mL, ready to use reagent. Controls contain human antibodies to dsDNA in stabilizers and preservatives.
- QUANTA Flash dsDNA High Control: Two (2) barcode labeled tubes containing 0.7 mL, ready to use reagent. Controls contain human antibodies to dsDNA in stabilizers and preservatives.

Intended use(s)

QUANTA Flash dsDNA is a chemiluminescent immunoassay for the quantitative determination of IgG anti-double stranded deoxyribonucleic acid (dsDNA) antibodies in human serum. The presence of anti-dsDNA antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus.

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

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

Substantial equivalence

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

Comparison to predicate device

QUANTA Flash dsDNA reagent kit

Similarities		
Item	**QUANTA Flash dsDNA**	**Predicate Device**
Intended use	QUANTA Flash dsDNA is a chemiluminescent immunoassay for the quantitative determination of IgG anti-double stranded deoxyribonucleic acid (dsDNA) antibodies in human serum. The presence of anti-dsDNA antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus (SLE).	This assay is intended for the in-vitro measurement of specific IgG autoantibodies against double stranded deoxyribonucleic acid (dsDNA) present in human serum, as an aid to the diagnosis of systemic lupus erythematosus (SLE), in conjunction with other clinical findings.
Assay methodology	Solid phase (heterogenous) immunoassay	Solid phase (heterogenous) immunoassay
Traceability	Traceable to the First International Standard Preparation for dsDNA (WHO code: Wo/80)	Traceable to the First International Standard Preparation for dsDNA (WHO code: Wo/80)
Sample type	Serum	Serum
Shelf life	One year	One year

Differences		
Item	**QUANTA Flash dsDNA**	**Predicate Device**
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well plate
Antigen	Synthetic dsDNA	Calf thymus dsDNA
Conjugate	Isoluminol conjugated anti-human IgG	HRP conjugated anti-human IgG
Calibration	Lot specific Master Curve + two Calibrators (Sold separately)	Five lot specific calibrators (Included in the kit)
Interpretation	< 27 IU/mL Negative result 27-35 IU/mL Indeterminate > 35 IU/mL Positive result	< 30 IU/mL Negative result 30-75 IU/mL Borderline > 75 IU/mL Positive result
Measuring Range	AMR = 9.8 – 666.9 IU/mL Reportable range = up to 6669.0	12.3 – 1000.0 IU/mL

	IU/mL	

QUANTA Flash dsDNA Calibrators

Item	**QUANTA Flash dsDNA Calibrators**	**Predicate Device**
Intended use	QUANTA Flash dsDNA Calibrators are intended for use with the QUANTA Flash dsDNA chemiluminescent immunoassay for the determination of IgG anti-dsDNA antibodies 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-dsDNA antibodies	Anti-dsDNA antibodies
Method	QUANTA Flash dsDNA chemiluminescent immunoassay	QUANTA Lite dsDNA SC ELISA
Unit	IU/mL	IU/mL
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 ^{o}C	2-8 ^{o}C
Shelf life	One year	One year

QUANTA Flash dsDNA Controls

Item	**QUANTA Flash dsDNA Controls**	**Predicate Device**
Intended use	QUANTA Flash dsDNA Controls are intended for use with the QUANTA Flash dsDNA chemiluminescent immunoassay for quality control in the determination of IgG anti-dsDNA antibodies in human serum.	No separate intended use; controls are part of the kit.
Analyte	Anti-dsDNA antibodies	Anti-dsDNA antibodies
Method	QUANTA Flash dsDNA chemiluminescent immunoassay	QUANTA Lite dsDNA SC ELISA
Unit	IU/mL	IU/mL

Matrix	Human serum, stabilizers, and preservative	Human serum, stabilizers, and preservative
Physico-chemical characteristics	Liquid, ready to use	Liquid, prediluted, ready to use
Levels	2 (low and high)	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 dsDNA Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-dsDNA 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 IU/mL 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.

The Master Curve Standards are traceable to the First International Standard Preparation for dsDNA (WHO code: Wo/80). Based upon this standardization, results are reported in International Units (IU)/mL. Calibrator and Control values are directly traceable to in-house Standards that are used to create the Master Curves for the QUANTA Flash dsDNA assay.

List of dsDNA Standards, Calibrators and Controls:

Material	Assigned Value (IU/mL)
dsDNA Master Curve Standard 1	9.8
dsDNA Master Curve Standard 2	19.4
dsDNA Master Curve Standard 3	49.6
dsDNA Master Curve Standard 4	90.7
dsDNA Master Curve Standard 5	188.7
dsDNA Master Curve Standard 6	375.6
dsDNA Master Curve Standard 7	666.9

Material	Manufacturing Target Value (IU/mL)	Manufacturing Target Range (IU/mL)
dsDNA Calibrator 1	19	17 - 21

dsDNA Calibrator 2	189	169 - 209
dsDNA Low Control	22	19 - 25
dsDNA High Control	90	81 – 99

Analytical performance characteristics

Precision

The precision of the QUANTA Flash dsDNA assay was evaluated on 9 samples containing various concentrations of dsDNA 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 or 21 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%.

QUANTA Flash® dsDNA			**Within-Run (repeatability)**		**Between-Run**		**Between-Day**		**Total**	
Sample ID	**N**	**Mean (IU/mL)**	**SD (IU/mL)**	**CV (%)**	**SD (IU/mL)**	**CV (%)**	**SD (IU/mL)**	**CV (%)**	**SD (IU/mL)**	**CV (%)**
1	80	14.1	0.5	3.3	0.3	2.1	0.3	2.1	0.6	4.4
2	84	27.3	1.5	5.4	1.1	3.9	0.8	2.9	2.0	7.3
3	80	35.6	0.8	2.3	0.8	2.2	0.6	1.7	1.3	3.6
4	84	49.0	2.4	4.9	2.2	4.5	1.2	2.5	3.5	7.1
5	84	86.4	4.0	4.7	3.5	4.0	3.1	3.5	6.1	7.1
6	84	132.4	6.8	5.1	6.6	4.9	5.3	4.0	10.8	8.2
7	84	137.6	6.5	4.7	2.9	2.1	5.5	4.0	9.0	6.6
8	84	344.8	23.1	6.7	2.4	0.7	8.0	2.3	24.6	7.1
9	84	402.8	27.9	6.9	0.0	0.0	14.1	3.5	31.2	7.8

Reproducibility

Reproducibility between sites (instruments)

Studies were performed in two sets.

In the first set, three samples were tested at three different testing sites in quadruplicates, two times a day, for 10 days, to generate 80 data points per testing site, 240 data points total for each sample, using the same reagent lot.

In the second set, two additional samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, at three different testing sites in five replicates for 5 days, to generate 25 data points per testing site, 75 data points total for each sample, using the same reagent lot.

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, 10%.

			Between Site Imprecision	
Sample ID	Mean (IU/mL)	N	SD (IU/mL)	CV (%)
1	32.6	240	2.0	6.1
2	52.9	240	1.3	2.5
3	142.7	240	3.9	2.8
4	13.2	75	0.0	0.0
5	466.1	75	24.8	5.3

Reproducibility between lots

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

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%.

			Between Lot Imprecision	
Sample ID	Mean (IU/mL)	N	SD (IU/mL)	CV (%)
1	13.9	75	0.7	5.2
2	25.7	75	0.2	0.7
3	33.1	75	2.5	7.5
4	85.9	75	4.5	5.3
5	349.3	75	24.7	7.1

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

The LoD of the QUANTA Flash dsDNA assay is 786 RLU (corresponding to 3 IU/mL. It was determined on two reagent lots, consistent with CLSI EP17-A2 guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 134 determinations, with 72 measurements on blank samples, and 60 measurements of low level samples, per reagent lot. The LoB is 520 RLU, , which corresponds to 2.2 IU/mL. The Limit of Quantitation (LoQ) is 1805 RLU (6.6 IU/mL). It was

determined consistent with the guidelines in CLSI document EP17-A2, based on 120 determinations, and a Total Error goal of 20%. The LoB, the LoD and the LoQ are below the AMR (below the lowest standard).

For the LoB study, 4 different aliquots of System Rinse were tested in triplicates over 6 days, on two reagent lots (72 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, five low level samples were used, with RLU values between the LoB and approximately 4 times of the LoB. Samples were tested in 4 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.

For the LoQ study, total imprecision was calculated for each sample that was used in the LoD study on each lot, and compared to the 20% Total Error goal. For each reagent lot, the sample with the lowest concentration that met accuracy specifications was taken as the LoQ for that lot. The greater LoQ across the lots was taken as the LoQ of the measurement procedure.

Analytical Measuring Range (AMR)

9.8 IU/mL – 666.9 IU/mL

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 re-test any sample that has a result of result >666.9 IU/mL by further diluting it by a factor specified in the assay definition file (10 fold), thereby bringing the measured value within the AMR. The final result will be calculated by the software by taking into account the additional dilution factor. As the highest value that can be measured is 666.9 IU/mL, the highest value that can be reported is 6669.0 IU/mL.

To validate the Auto-rerun function, eight 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 the same way as it happens in the Auto-rerun function (10 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 between 84% and 106% (average 96%) and are within the ± 20% acceptance limit.

High concentration hook effect

Not applicable.

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 dsDNA antibody concentrations were diluted with stripped 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 90.8% to 113.0%. 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% (or ± 7 IU/mL) recovery, 0.9-1.1 slope and ≥0.95 R^2. Linear regression results are shown in the Table below.

Sample ID	Test Range (IU/mL)	Slope (95% CI)	R^2
1	20.0 to 200.2	1.00 (0.98 to 1.02)	1.00
3	54.9 to 549.2	1.02 (0.98 to 1.06)	0.99
4	71.6 to 644.5	1.08 (1.02 to 1.15)	0.99
5	11.2 to 56.1	0.97 (0.93 to 1.01)	0.99
7	65.1 to 585.8	1.00 (0.97 to 1.03)	1.00
All Samples	11.2 to 644.5	1.03 (1.02 to 1.05)	0.99

Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Six specimens were tested (24.3 IU/mL, 30.9 IU/mL, 37.9 IU/mL, 41.5 IU/mL, 132.3 IU/mL and 375.6 IU/mL). 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 dsDNA assay. Moreover, 6 additional samples (21.4, 32.0, 40.3, 43.9, 140.3, and 364.0 IU/mL) 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 positive samples, or ± 7 IU/mL difference for indeterminate and negative samples. 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
Triglicerydes	1000 mg/dL	500 mg/dL	250 mg/dL
Cholesterol	224.3 mg/dL	112.1 mg/mL	56 mg/mL
RF IgM	947.1 IU/mL	568.2 IU/mL	189.4 IU/mL

No interference was detected with bilirubin up to 10 mg/dL (recovery: 86% to 102%), hemoglobin up to 200 mg/dL (recovery: 88% to 107%), triglycerides up to 1000 mg/dL (recovery: 91% to 109%), cholesterol up to 224.3 mg/dL (recovery: 91% to 109%), and RF IgM up to 947.1 IU/mL (recovery: 94% to 114%, or 3.6 to 3.7 IU/mL).

Cross-reactivity

To test potential cross-reactivity with autoantibodies and infection-induced antibodies, 465 patient samples with various antibodies to autoimmune or infectious disease markers were tested. Altogether, 26 samples (5.6%) tested positive, proving the lack of cross-reactivity with autoimmune and infection-induced antibodies:

Patient Group	N	Positive	Indeterminate
Primary anti-phospholipid syndrome (PAPS)	20	3	2
Sjogren's syndrome (SS)	50	3	1
Celiac disease (CD)	20	0	3
Systemic sclerosis (SSc)	40	3	4
Idiopathic Inflammatory Myopathy (IIM)	20	0	2
MCTD	20	3	3
Crohn's disease	20	1	0
Graves Disease	21	0	1
Hashimoto thyroiditis	61	3	4
Hepatitis B	20	0	1
Hepatitis C	18	0	1
Syphilis	20	1	0
Rheumatoid arthritis (RA)	101	8	6
Vasculitis	34	1	4
Total	**465**	**26**	**32**
Percent		**5.6%**	**6.9%**

Sample stability

Thirteen samples, encompassing negative, equivocal and low to high positive samples were tested in duplicates for up to 17 days at 2-8°C, up to 48 hours at room temperature, moreover, after repeated freeze/thaw cycles up to 6 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 10 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 dsDNA to establish initial stability claim:

- dsDNA beads
- Assay Buffer
- Tracer IgG
- 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 ± 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 were:

-Microparticles (beads), Assay Buffer, and Tracer IgG:

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 performed.

Real time stability testing was performed on Calibrators, Controls and reagent cartridge at regular time points to support the one year expiration.

We are providing real time stability data for Calibrators up to 16 months, Controls up to 16 months, and reagent cartridge up to 21 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 with two different protocols, due to internal procedure change during the study. In the first part of the study Calibrators were used to calibrate a reagent cartridge and a panel of QC samples was tested.
- Acceptance criteria: results of the samples should fall within their respective QC ranges

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

All results were within the acceptance limits.

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, then 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 20 runs, over 9 days. 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 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 20 runs, resulting in the %CV values of 5.2 % and 7.7 % for the Low and High control, respectively.

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 dsDNA reagent cartridge, four serum specimens (with different reactivity levels) along with the Low and High Controls were tested. The specimens were tested periodically for up to 63 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 ≥2% of the recovery data (whichever is greater) is ≤ 75% or ≥ 125% recovery.

The onboard study was ended at 63 days, prior to fulfilling either limit described above, because a claim of only 60 days was desired. The in-use (onboard) stability of the dsDNA reagent cartridge was therefore set at 60 days.

Cut-off (reference range) establishment and verification

The reference population for establishing and verifying the reference interval for the dsDNA assay consisted of 171 subjects:

Apparently healthy blood donors	121
Viral hepatitis positive samples	7
Vasculitis	20
Rheumatoid arthritis	19
HIV positive samples	4

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. One vasculitis sample produced a 76.4 IU/mL value, which was considered as an outlier, and was excluded from the calculations. Using Analyse-it for Excel, the upper 95th percentile Reference Interval limit of

the remaining 170 samples was calculated as 26.9 IU/mL. Additionally, the 99th percentile Reference Interval limit of the 170 samples was calculated as 35.8 IU/mL. Therefore 27 IU was set as the cut-off, and 27-35 IU/mL was set as equivocal/indeterminate range. Samples with > 35 IU/mL are positive.

If a result falls in the indeterminate range, it means that the probability of SLE is low, but it is still higher than in the normal population. Forty-one out of the 644 SLE patients (6.4%) that were tested during the validation studies had their results fall in the indeterminate range. The frequency of indeterminate anti-dsDNA results in the tested normal population (n=300) was 2.0%. As several studies have documented a close relationship between antibody titer and disease activity, indeterminate anti-dsDNA results are more likely to occur in inactive disease compared to newly diagnosed SLE.

To verify the reference range, a second cohort of samples, consisting of 210 samples was tested. This cohort had similar composition as the first reference cohort:

Diagnosis	Number	Number of equivocal	Number of positive
Apparently healthy blood donor	120	1	2
HBV positive samples	5	0	1
HCV positive samples	5	0	0
Syphilis	5	0	1
HIV positive samples	5	0	0
Sjogren's	10	0	1
Rheumatoid arthritis	50	0	4
anti-MPO positive	10	0	0
Total	**210**	**1**	**10**

There were 11 samples above the cutoff in this cohort, resulting in 94.8% specificity. These results verify the reference range that was set based on the first cohort.

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 dsDNA. A total of 1151 samples were included in the Validation Set for the QUANTA Flash dsDNA.

Distribution of the cohort used in the QUANTA Flash dsDNA validation study:

		QUANTA Flash dsDNA, n pos	
Patient Group	**N**	**ind=pos**	**ind=neg**
Primary anti-phospholipid syndrome (PAPS)	20	5	3
Sjögren's syndrome (SS)	50	4	3
Celiac disease (CD)	20	3	0
Systemic sclerosis (SSc)	40	7	3

		QUANTA Flash dsDNA, n pos	
Patient Group	**N**	**ind=pos**	**ind=neg**
Idiopathic Inflammatory Myopathy (IIM)	20	2	0
MCTD	20	6	3
Crohn's disease	20	1	1
Graves Disease	21	1	0
Hashimoto thyroiditis	61	7	3
Hepatitis B	20	1	0
Hepatitis C	18	1	0
Syphilis	20	1	1
Rheumatoid arthritis (RA)	101	14	8
Vasculitis	34	5	1
Healthy controls *	20	0	0
Drug induced lupus (DIL) *	22	2	2
Total controls	**507**	**60**	**28**
Systemic lupus erythematosus (SLE)	644	316	275
Total	**1151**	**376**	**303**

* Not included in the clinical sensitivity and specificity calculations

Clinical sensitivity and specificity for SLE is calculated in the tables below. Calculations were done in two ways; with equivocal results as negative, and as positive. The drug induced lupus group and the healthy controls group were excluded from sensitivity and specificity calculations.

Clinical Analysis (N=1109) Indeterminate = Negative		Diagnosis			Analysis (95% confidence)
		SLE	Controls	Total	
QUANTA Flash® dsDNA	Positive	275	26	301	Sensitivity: 42.7 % (38.9-46.6%)
	Negative	369	439	808	Specificity: 94.4 % (91.9 - 96.3%)
	Total	644	465	1109	

Clinical Analysis (N=1109) Indeterminate = Positive		Diagnosis			Analysis (95% confidence)
		SLE	Not SLE	Total	
QUANTA Flash® dsDNA	Positive	316	58	374	Sensitivity: 49.1 % (45.2-52.9%)
	Negative	328	407	735	Specificity: 87.5 % (84.2-90.2%)
	Total	664	465	1109	

For comparison, clinical sensitivity and specificity were calculated on the results obtained with the predicate device on the same population. The summary of the results is shown below:

Clinical Performance (N=1109)	QUANTA Flash dsDNA	QUANTA Lite dsDNA SC
Indeterminate as positive		
Sensitivity % (95% CI)	49.1 (45.2-52.9)	57.1 (53.3 - 60.9)
Specificity % (95% CI)	87.5 (84.2-90.2)	86.9 (83.5 - 89.7)
Indeterminate as negative		
Sensitivity % (95% CI)	42.7 (38.9-46.6)	39.3 (35.6 - 43.1)
Specificity % (95% CI)	94.4 (91.9 - 96.3)	96.8 (94.7 - 98.0)

Out of the 644 SLE patients, 25 were known to have active lupus nephritis. The clinical sensitivity of the QUANTA Flash dsDNA on this population was 80%, as 20 samples were positive out of the 25. The predicate device detected 20 and 21 samples (with indeterminate results considered as negative or positive, respectively):

Sensitivity in lupus nephritis:	QUANTA Flash dsDNA	QUANTA Lite dsDNA SC
Indeterminate as negative	20/25 (80%)	20/25 (80%)
Indeterminate as positive	20/25 (80%)	21/25 (84%)

Expected values

The expected value in the normal population is “negative”. Anti-dsDNA antibody levels were analyzed in a cohort of 300 apparently healthy blood donors (131 females and 169 males, ages 19 to 68 years, with an average and median age of 44 years) using the QUANTA Flash dsDNA. This patient population was different from the one that was used to establish and verify the cutoff. With a cut-off of 27 IU/mL and indeterminate range of 27-35 IU/mL, six samples (2.0%) were in the indeterminate range, and four (1.3%) of the samples were positive with the QUANTA Flash dsDNA. The mean concentration was 11.6 IU/mL, and the values ranged from <9.8 to 81.9 IU/mL.

Comparison with the predicate device

All 1151 samples from the Validation Set study were tested on both the QUANTA Flash dsDNA and on the predicate ELISA.

Out of the 1151 samples, results were within the AMR of both the QUANTA Flash assay and of the predicate ELISA for 481 samples. The comparison is shown in the tables below. Data were analyzed with equivocal results treated as positive on both assays, and then as negative on both assays.

Agreement on samples within the AMR is shown below:

Method Comparison - Within AMR (N=481) Indeterminate = negative		QUANTA Flash® dsDNA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Lite dsDNA SC ELISA	Negative	200	53	253	Pos. Agrmnt = 80.3% (74.6 – 84.9%)
	Positive	45	183	228	Neg. Agrmnt = 79.1% (73.6 – 83.6%)
	Total	245	236	481	Total Agrmnt = 79.6% (75.8 – 83.0%)

Method Comparison - Within AMR (N=481) Indeterminate = positive		QUANTA Flash® dsDNA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Lite dsDNA SC ELISA	Negative	77	33	110	Pos. Agrmnt = 68.5% (63.6 – 73.0%)
	Positive	117	254	371	Neg. Agrmnt = 70.0% (60.9 – 77.8%)
	Total	194	287	481	Total Agrmnt = 68.8% (64.5 – 72.8%)

Method comparison was also performed as a three-way comparison, with negative, indeterminate and positive results:

QUANTA Lite dsDNA SC ELISA	QUANTA Flash® dsDNA			
	Negative	Borderline	Positive	Total
Negative	77	14	19 (12 SLE)	110
Borderline	81	28	34	143
Positive	36 (30 SLE)	9	183	228
Total	194	51	236	481

Moreover, 333 samples had results below the AMR of the QUANTA Flash assay (< 9.8 IU/mL), and 30 samples had results above the AMR (> 666.9 IU/mL). The agreement between QUANTA Flash and the predicate device was 98.8% for the samples below the AMR (4 out of 333 were positive with the QUANTA Lite assay), and it was 100% for samples above the AMR (all samples were positive with the predicate device).

Additionally, agreement was also calculated on samples that were around the cut-off with the QUANTA Flash dsDNA (25-40 IU/mL). There were 124 samples in this category. The agreement between QUANTA Flash and the predicate device was 69.4% and 55.6 % when indeterminate results were considered as negative and positive, respectively, as shown below:

Method Comparison - Within AMR (N=124) Indeterminate = negative		QUANTA Flash® dsDNA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Lite dsDNA SC ELISA	Negative	83	27 (21 SLE)	110	Pos. Agrmnt = 21.4% (7.6 – 47.6%)
	Positive	11 (8 SLE)	3	14	Neg. Agrmnt = 75.5% (66.6 – 82.5%)
	Total	94	30	124	Total Agrmnt = 69.4% (60.8 – 76.8%)

Method Comparison - Within AMR (N=124) Indeterminate = positive		QUANTA Flash® dsDNA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Lite dsDNA SC ELISA	Negative	15	49 (24 SLE)	64	Pos. Agrmnt = 90.0% (79.9 – 95.3%)
	Positive	6 (5 SLE)	54	60	Neg. Agrmnt = 23.4% (14.7 – 35.1%)
	Total	21	103	124	Total Agrmnt = 55.6% (46.9 – 64.1%)

The results show that the majority of the samples that were positive with QUANTA Flash but negative with the predicate device were samples from SLE patients.