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

October 23, 2015

INOVA DIAGNOSTICS, INC.
DR. GABRIELLA LAKOS
DIRECTOR, RESEARCH AND DEVELOPMENT
9900 OLD GROVE ROAD
SAN DIEGO, CA 92131-1638

Re: K143754
Trade/Device Name: Quanta Flash® CCP3
Quanta Flash® CCP3 Calibrators
Quanta Flash® CCP3 Controls
Regulation Number: 21 CFR 866.5775
Regulation Name: Rheumatoid Factor Immunological Test System
Regulatory Class: Class II
Product Code: NHX, JIT, JJX
Dated: August 19, 2015
Received: August 20, 2015

Dear Dr. Lakos:

This letter corrects our substantially equivalent letter of September 21, 2015.

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 regulation (21 CFR Part 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" (21CFR 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,

Leonthena R. Carrington -S

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)*
K143754

Device Name

QUANTA Flash® CCP3, QUANTA Flash® CCP3 Calibrators, QUANTA Flash® CCP3 Controls

Indications for Use *(Describe)*

QUANTA Flash CCP3 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-CCP3 antibodies in human serum. The presence of anti-CCP3 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of rheumatoid arthritis.

QUANTA Flash CCP3 Calibrators are intended for use with the QUANTA Flash CCP3 chemiluminescent immunoassay for the determination of IgG anti-CCP3 antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash CCP3 Controls are intended for use with the QUANTA Flash CCP3 chemiluminescent immunoassay for quality control in the determination of IgG anti-CCP3 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.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

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® CCP3
QUANTA Flash® CCP3 Calibrators
QUANTA Flash® CCP3 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® CCP3
Common name:	Anti-CCP3 Chemiluminescent Immunoassay
Classification name:	antibodies, anti-cyclic citrullinated peptide (ccp)

Regulation Description	Rheumatoid factor immunological test system
Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	NHX

Regulation Number	866.5775	
Device Class	2	
Device name (Calibrators):	Proprietary name: Common name: Classification name:	QUANTA Flash® CCP3 Calibrators CCP3 Calibrators 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: Common name: Classification name:	QUANTA Flash® CCP3 Controls CCP3 Controls 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® CCP3 IgG ELISA, 510(k) number: K052264

Device description

The QUANTA Flash CCP3 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 CCP3 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

Synthetic cyclic citrullinated peptide 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. 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-CCP3 antibodies bound to the corresponding beads.

For quantitation, the QUANTA Flash CCP3 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 CCP3 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 CCP3 kit contains the following materials:

One (1) QUANTA Flash CCP3 Reagent Cartridge
One (1) vial of Resuspension buffer
One (1) Transfer pipette

The QUANTA Flash CCP3 reagent cartridge contains the following reagents for 100 determinations:

a. CCP3 coated paramagnetic beads, lyophilized.
b. Assay buffer – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.
c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.

The QUANTA Flash CCP3 Calibrators kit contains two vials of Calibrator 1 and two vials of Calibrator 2:

QUANTA Flash CCP3 Calibrators:

- QUANTA Flash CCP3 Calibrator 1: Two (2) barcode labeled tubes containing 0.7 mL prediluted, ready to use reagent. Calibrators contain human antibodies to CCP3 in stabilizer

and preservative.

- QUANTA Flash CCP3 Calibrator 2: Two (2) barcode labeled tubes containing 0.7 mL prediluted, ready to use reagent. Calibrators contain human antibodies to CCP3 in stabilizer and preservative.

The QUANTA Flash CCP3 Controls kit contains two vials of Negative Control and two vials of Positive Control:

QUANTA Flash CCP3 Controls:

- QUANTA Flash CCP3 Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to CCP3 in stabilizer and preservative.
- QUANTA Flash CCP3 Positive Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to CCP3 in stabilizer and preservative.

Intended use(s)

QUANTA Flash CCP3 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-CCP3 antibodies in human serum. The presence of anti-CCP3 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of rheumatoid arthritis.

QUANTA Flash CCP3 Calibrators are intended for use with the QUANTA Flash CCP3 chemiluminescent immunoassay for the determination of IgG anti-CCP3 antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash CCP3 Controls are intended for use with the QUANTA Flash CCP3 chemiluminescent immunoassay for quality control in the determination of IgG anti-CCP3 antibodies in human serum.

Indications for use

Same as Intended use.

Substantial equivalence

The QUANTA Flash CCP3 Reagent, the QUANTA Flash CCP3 Calibrators and the QUANTA Flash CCP3 Controls have the same intended use and assay principle as the predicate device.

Comparison to predicate device

QUANTA Flash CCP3 reagent kit

Similarities		
Item	**QUANTA Flash CCP3**	**Predicate Device**
Intended use	Semi-quantitative determination of anti-CCP3 antibodies in human serum	Semi-quantitative detection of anti-CCP3 antibodies in human serum, citrated and EDTA plasma
Assay methodology	Solid phase (heterogeneous) immunoassay	Solid phase (heterogeneous) immunoassay
Antigen	Synthetic peptide	Synthetic peptide
Shelf life	One year	One year

Differences		
Item	**QUANTA Flash CCP3**	**Predicate Device**
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Sample type	Serum	Serum; citrated or EDTA plasma
Solid phase	Paramagnetic microparticles (beads)	96-well polystyrene plate
Conjugate	Isoluminol conjugated anti-human IgG	HRP conjugated anti-human IgG
Calibration	Lot specific Master Curve + two calibrators (sold separately)	CCP3 ELISA Low Positive, and Calibrators A through E (Included in the kit)

QUANTA Flash CCP3 Calibrators

Item	**QUANTA Flash CCP3 Calibrators**	**Predicate Device**
Intended use	For use with the QUANTA Flash CCP3 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-CCP3 antibodies	Anti-CCP3 antibodies
Method	QUANTA Flash CCP3 chemiluminescent immunoassay	QUANTA Lite CCP3 IgG ELISA
Matrix	Human serum, stabilizer, and preservative	Human serum, buffer, protein stabilizer, and preservative
Unit	CU (Chemiluminescent units) (arbitrary) U/mL (based on IUIS – CDC reference reagent)	units (arbitrary)
Physico-chemical	Liquid, prediluted, ready to use	Liquid, prediluted, ready to use

Item	QUANTA Flash CCP3 Calibrators	Predicate Device
characteristics		
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

QUANTA Flash CCP3 Controls

Item	QUANTA Flash CCP3 Controls	Predicate Device
Intended use	For use with the QUANTA Flash CCP3 reagents for quality control in the determination of IgG anti-CCP3 autoantibodies in human serum.	No separate intended use; controls are part of the kit.
Analyte	Anti-CCP3 antibodies	Anti-CCP3 antibodies
Method	QUANTA Flash CCP3 chemiluminescent immunoassay	QUANTA Lite CCP3 IgG ELISA
Matrix	Human serum, stabilizers, and preservative	Human serum, buffer, protein stabilizer, and preservative
Unit	CU (Chemiluminescent units) (arbitrary) U/mL (based on IUIS – CDC reference reagent)	units (arbitrary)
Physico-chemical characteristics	Liquid, ready to use	Liquid, prediluted, ready to use
Levels	2 (negative and positive)	2 (ELISA negative and High positive)
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

Analytical performance characteristics

Quantitation and units of measure

For quantitation, the QUANTA Flash CCP3 assay utilizes a lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. The Master Curve for QUANTA Flash CCP3 consists of 7 Standards. These Master Curve Standards are used to create the lot specific Master Curve during the manufacturing procedure.

List of CCP3 Standards:

Material	Assigned Value
CCP3 Master Curve Standard 1	4.6 CU
CCP3 Master Curve Standard 2	14.6 CU
CCP3 Master Curve Standard 3	42.2 CU
CCP3 Master Curve Standard 4	124.3 CU
CCP3 Master Curve Standard 5	313.7 CU

CCP3 Master Curve Standard 6	996.1 CU
CCP3 Master Curve Standard 7	2776.8 CU

To establish traceability to a reference material, the IUIS-Centers for Disease Control and Prevention (CDC) reference reagent for anti-citrullinated peptide/protein antibodies (ACPA) (P/N IS2723 L/N 08-0202) was tested, and the value of 379.5 CU was obtained. The reference reagent has an assigned value of 100 U/mL.
Using the 100 U/mL assigned value of the reference material, the correlation between CU and U/mL values was established. Based on the obtained correlation, the BIO-FLASH software is able to automatically convert anti-CCP3 CU values into U/mL values. The 20 CU cutoff value equals 5.3 U/mL.

Value assignment and traceability of Calibrators and Controls

The QUANTA Flash CCP3 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-CCP3 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 CCP3 assay.

CCP3 Calibrators and Controls with target manufacturing values:

Material	Manufacturing Target Value	Manufacturing Target Range
CCP3 Calibrator 1	14 CU	10 – 16 CU
CCP3 Calibrator 2	300 CU	270 – 330 CU
CCP3 Negative Control	10 CU	8 – 12 CU
CCP3 Positive Control	50 CU	40 – 60 CU

Precision

The precision of the QUANTA Flash CCP3 assay was evaluated on 8 samples containing various concentrations of CCP3 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 CCP3			Within Run		Between Runs		Between Days		Total	
Sample ID	Number of replicates	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
1	80	11.7	0.7	5.8	0.0	0.0	0.4	3.5	0.8	6.8
2	80	21.3	1.0	4.6	0.7	3.2	0.7	3.5	1.4	6.6
3	80	21.4	0.8	3.6	0.6	2.8	0.9	4.1	1.3	6.1
4	80	23.0	1.0	4.4	0.0	0.0	0.6	2.8	1.2	5.2
5	80	55.9	1.4	2.6	1.1	1.9	2.1	3.8	2.8	5.0
6	80	195.1	8.8	4.5	6.0	3.1	1.3	0.6	10.7	5.5
7	80	1155.3	56.2	4.9	20.3	1.8	32.6	2.8	68.0	5.9
8	80	2210.3	119.9	5.4	42.3	1.9	36.6	1.7	132.3	6.0

Reproducibility

Three samples were tested at three different sites. Samples were run in quadruplicates, two times a day, for 5 days, to generate 40 data points per sample, per site. An additional 5 samples were tested at three different sites. Samples were run in replicates of 5, 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 to calculate between site precision.
Acceptance criteria: Total %CV: < 10%
Results are summarized in the Table below.

QUANTA Flash CCP3			Between Site Precision (Reproducibility)	
Sample ID	Number of replicates	Mean (CU)	SD (CU)	CV (%)
1	120	9.3	0.0	0.0
2	120	35.1	1.1	3.0
3	120	207.9	4.1	2.0
4	75	16.0	0.5	3.2
5	75	24.4	0.5	1.9
6	75	767.5	11.5	1.5
7	75	1447.6	38.2	2.6
8	75	2274.4	0.0	0.0

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

The LoD of the QUANTA Flash CCP3 assay is 478 RLU, which is below the analytical measuring range of the assay. It was determined by using 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 120 determinations, with 60 measurements on blank samples and 60 measurements of low level samples, per reagent lot. The LoB is 410 RLU.
These values are below the value of the lowest QUANTA Flash CCP3 Master Curve standard, i.e. below the Analytical Measuring Range.

Analytical Measuring Range (AMR)

QUANTA Flash CCP3: 4.6 CU – 2776.8 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 >2776.8 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 2776.8 CU, the highest value that can be reported is 55536 CU.

High concentration hook effect

To assess hook effect, measurement signal (relative light units, RLU) was examined for four high positive samples (results above the AMR) before and after automatic or manual dilution. All sera produced significantly higher RLU values (above the AMR) when used "as is" compared to the manually or automatically diluted ones (that were within the AMR), thereby confirming that high positive specimens above the analytical measuring range do not show hook effect up to 113191 CU in the CCP3 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. Five serum samples with various CCP3 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 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
1	358.3 to 2772.3	1.00 (1.00 to 1.00)	1.00
2	190.7 to 2030.0	0.99 (0.96 to 1.03)	0.99
3	82.5 to 705.4	1.00 (0.95 to 1.04)	0.99
4	14.0 to 134.5	0.94 (0.88 to 1.00)	0.98
5	6.6 to 34.9	0.97 (0.93 to 1.02)	0.99

The combined data yielded the following results with linear regression:

Sample	Test Range (CU)	Slope (95% CI)	R^2
All	6.6 to 2772.3	1.07 (1.05 to 1.10)	0.99

These data demonstrate the linearity of the analytical measuring range (4.6 CU - 2776.8 CU) of the QUANTA Flash CCP3 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: 10.1 CU; low positive: 40.5 CU; positive: 939.8 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 CCP3 assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluents (10% of total). Acceptance criteria for the interference studies were 85% - 115% recovery, or ± 4 CU difference, whichever is greater.

No interference was detected with bilirubin up to 10 mg/dL (recovery: 101% to 102%), hemoglobin up to 200 mg/dL (recovery: 97% to 108%), triglycerides up to 1000 mg/dL (recovery: 98% to 111%), and cholesterol up to 224.3 mg/dL (recovery: 98% to 111%). Interference of rheumatoid factor was not tested.

Cross-reactivity

To test potential cross-reactivity with autoantibodies and infection-induced antibodies, results obtained on 234 of the total 376 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 positive infectious disease serology. The composition of the cohort and the anti-CCP3 positivity rate is shown in the Table below:

Diagnosis	Number of samples	# pos	% pos
Systemic Lupus Erythematosus	53	2	3.8%
HBV	22	0	0.0%
HCV	13	0	0.0%
Limited Systemic Sclerosis	8	0	0.0%
Mixed Connective Tissue Disease	7	0	0.0%
Primary Biliary Cirrhosis	7	1	14.3%
Vasculitis	6	0	0.0%
Wegener's Granulomatosis	1	0	0.0%
Parvo virus infection	12	0	0.0%
Salmonella infection	10	0	0.0%
Lyme disease	25	0	0.0%
Ulcerative colitis (UC)	11	0	0.0%
Autoimmune hepatitis (AIH)	19	1	5.3%
Celiac disease (CD)	20	1	5.0%
Sjögren's syndrome	20	0	0.0%
Total	**234**	**5**	**2.1%**

Based on the results, the QUANTA Flash CCP3 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: 141004, 141005 and 151006. 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 CCP3			Between Lot Precision	
Sample ID	Mean (CU)	N	SD (CU)	CV (%)
1	16.2	75	0.6	3.9
2	24.9	75	0.7	2.8
3	77.5	75	2.6	3.4
4	341.1	75	10.2	3.0
5	1528.7	75	61.2	4.0

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 CCP3 to establish initial stability claim: the beads, the resuspension buffer, 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 and Resuspension Buffer:
With regression analysis, the lower 95% CI interval of the regression line is ≥85% and the upper 95% CI interval is at ≤115% at day 14, and no individual data point has ≤75% or ≥125% recovery at day 14.

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

Beads
Testing was performed on three lots of CCP3 coupled beads using up to 9 characterized samples with various reactivity levels, along with the negative and positive controls.

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

Resuspension Buffer

Testing was performed on three lots of resuspension buffer using up to 6 characterized samples with various reactivity levels, along with the negative and positive controls.

All three lots of resuspension buffer retained between 85% and 115% reactivity (considering the 95% CI) 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 CCP3 Calibrators and Controls. All Calibrators and Controls maintained between 90% and 110% reactivity (considering the 95% CI) when sored 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 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.

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

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 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 CCP3 reagent cartridge, three lots of cartridges were tested with up to 6 serum specimens (with different reactivity levels) along with the Negative and Positive Controls. The specimens were tested periodically up to 90 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 proceeding 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.

The onboard stability results of the three lots are as follows:

RP0001: 64 days

131001: 83 days

141002: 63 days

Using these criteria, the in-use (onboard) stability of CCP3 reagent cartridge was set at 60 days.

Real time stability

Real time stability testing has been scheduled to be performed 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 6 months for reagent cartridge, and up to 9 months on Calibrators and Controls.

For reagent cartridge, QC panel samples were tested in duplicates or triplicates 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 tested in triplicates on a calibrated cartridge at each time point. 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%.

Controls were tested in triplicates on a calibrated cartridge at each time point. Individual values, and averages of the triplicates 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 limits.

Cut-off, reference range

QUANTA Flash CCP3: Negative <20 CU
Positive ≥20 CU

The reference population for establishing the reference interval for the CCP3 assay consisted of 210 subjects:

Sample Group	N
Apparently healthy blood donors	170
Viral hepatitis positive samples	20
Autoimmune thyroid disease	20

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 (Saphiro-Wilk $p<0.0001$), so the non-parametric percentile method was used. One high positive sample was excluded from the calculations as an outlier. This sample was confirmed to be true positive with the predicate device. The 99th percentile of the remaining obtained values was calculated as 11294 RLU.
The cutoff was set to 12000 RLU. One hepatitis C patient tested positive at this cutoff level, along with the one apparently healthy blood donor outlier.

Clinical performance characteristics

Clinical sensitivity, specificity

A cohort of characterized samples, none of which were used for establishing the reference range, was used to validate the clinical performance of the QUANTA Flash CCP3. A total of 728 characterized samples were included in the Validation Set for the QUANTA Flash CCP3. All samples were run on the QUANTA Flash CCP3. The distribution of the cohort and the CCP3 positivity rate is in the Table below:

Patient group	**N**	**Number positive**	**% positive**
Ankylosing Spondylitis	13	0	0.0%
Osteoarthritis	49	2	4.1%
Polymyalgia Rheumatica	20	2	10.0%
Psoriasis Arthritis	14	1	7.1%

Patient group	N	Number positive	% positive
Systemic Lupus Erythematosus	53	2	3.8%
HBV	22	0	0.0%
HCV	13	0	0.0%
Parvo virus infection	12	0	0.0%
Salmonella infection	10	0	0.0%
Lyme disease	25	0	0.0%
Ulcerative colitis (UC)	11	0	0.0%
Autoimmune hepatitis (AIH)	19	1	5.3%
Celiac disease (CD)	20	1	5.0%
Sjögren's syndrome	20	0	0.0%
Other diseases*	75	4	5.3%
Total controls	**376**	**13**	**3.5%**
Rheumatoid arthritis (RA)	352	249	70.7%
Total	**728**	-	-

*List of other diseases	n
Arthritis with Colitis ulcerosa	1
Arthritis with Crohn' disease	1
Colon Carcinoma	1
Connective Tissue Disease	2
Cryoglobulinemia	1
Degenerative Spine Disease	6
Fibromyalgia	6
Fibromyalgia, Connective Tissue Disease	1
Fibromyalgia, Osteoarthrosis	1
Gout	3
Juvenile Rheumatoid Arthritis	1
Limited Systemic Sclerosis	8
Mixed Connective Tissue Disease	7
Monoarthritis	2
Monoclonal Gammopathy of Unknown Significance (MGUS)	2
Multiple Sclerosis	1
Osteoarthrosis, MGUS	1
Osteoarthrosis, Osteoarthritis	1
Periarthropathia Humero-Scapularis	1
Polymyalgia Rheumatica, Arteriitis temporalis	1
Polymyositis	2

***List of other diseases**	**n**
Primary Biliary Cirrhosis	7
Raynaud-Syndrome	2
Rosacea	1
Sakroiliitis	1
Scoliosis	1
Spondylosis	1
Undifferentiated Connective Tissue Disease	5
Vasculitis	6
Wegener's Granulomatosis	1

The results were analyzed to calculate sensitivity and specificity for RA (n=352), using all 376 controls.

Clinical sensitivity and specificity of the QUANTA Flash CCP3 in RA

N=728		Diagnosis			Analysis (95% confidence)
		RA	Controls	Total	
QUANTA Flash CCP3	Positive	249	13	262	Sensitivity = 70.7% (65.8-75.2%)
	Negative	103	363	466	Specificity = 96.5% (94.2-98.0%)
	Total	352	376	728	

Expected values

The expected value in the normal population is “negative”. Anti-CCP3 antibody levels were analyzed in a cohort of 146 apparently healthy blood donors (66 females and 80 males, ages 17 to 60 years, with an average and median age of 34 years) using the QUANTA Flash CCP3. This patient population was different from the one that was used to establish the cutoff, and was only used to assess expected values. With the cut-off of 20 CU, one sample was positive on the QUANTA Flash CCP3. The mean concentration was 5.5 CU, and the values ranged from <4.6 to 31.1 CU.

Comparison with predicate device

Samples for method comparison analysis included all 728 samples from the clinical validation study. These samples were tested on both the QUANTA Flash CCP3 and on the predicate ELISA. The data are presented in two ways; first using all samples, and next using only samples within the AMR:

Method Comparison, all samples:

Method Comparison (N=728)		CCP3 ELISA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Flash®	Negative	447	19	466	Pos. Agree = 92.7% (89.0 – 95.3%)

CCP3 CIA	Positive	19	243	262	Neg. Agree = 95.9% (93.7 – 97.4%)
	Total	466	262	728	Total Agree = 94.8% (92.9 – 96.2%)

Method Comparison, samples with the AMR:

Method Comparison (N=420)		CCP3 ELISA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
QUANTA Flash® CCP3 CIA	Negative	177	13	190	Pos. Agree = 94.2% (90.3 – 96.6%)
	Positive	19	211	230	Neg. Agree = 90.3% (85.4 – 93.7%)
	Total	196	224	420	Total Agree = 92.4% (89.4 – 94.6%)