December 22, 2018
Inova Diagnostics, Inc.
Roger Albesa
Supervisor, Research and Development
9900 Old Grove Road
San Diego, California 92131-1638

Re: K170993
Trade/Device Name: QUANTA Flash Calprotectin Reagents, QUANTA Flash Calprotectin Calibrators, QUANTA Flash Calprotectin Controls, QUANTA Flash Calprotectin Extraction Buffer
Regulation Number: 21 CFR 866.5180
Regulation Name: Fecal calprotectin immunological test system
Regulatory Class: Class II
Product Code: NXO, JIT
Dated: November 21, 2017
Received: November 22, 2017

Dear Roger Albesa:

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 Part 801 and Part 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.

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.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kelly Oliner

For,
Lea Carrington
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)*

Device Name

QUANTA Flash Calprotectin, QUANTA Flash Calprotectin Calibrators, QUANTA Flash Calprotectin Controls, QUANTA Flash Calprotectin Extraction Buffer

Indications for Use *(Describe)*

QUANTA Flash Calprotectin is a chemiluminescent immunoassay for the quantitative determination of fecal calprotectin in extracted human stool samples. Elevated levels of fecal calprotectin, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease), and in the differentiation of IBD from irritable bowel syndrome (IBS).

QUANTA Flash Calprotectin Calibrators are intended for use with the QUANTA Flash Calprotectin Reagents for the determination of fecal calprotectin levels in extracted stool samples. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash Calprotectin Controls are intended for use with the QUANTA Flash Calprotectin Reagents for quality control in the determination of fecal calprotectin levels in extracted stool samples.

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.

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® Calprotectin Reagents
QUANTA Flash® Calprotectin Calibrators
QUANTA Flash® Calprotectin Controls
QUANTA Flash® Calprotectin Extraction Buffer

Scientific contact:
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Quality Systems contact:
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Phone: 858-586-9900/1381
Fax: 858-863-0025
Email: relliot@inovadx.com

Device name (assay kit):

Proprietary name:	QUANTA Flash® Calprotectin Reagents
Common name:	Fecal Calprotectin Chemiluminescent Immunoassay
Classification name:	Calprotectin, Fecal

Regulation Description Fecal Calprotectin Immunological Test System

Regulation Medical Specialty Immunology

Review Panel Immunology

Product Code NXO

Regulation Number	866.5180	
Device Class	2	
Device name (Calibrators):	Proprietary name:	QUANTA Flash® Calprotectin Calibrators
	Common name:	Calprotectin 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® Calprotectin Controls
	Common name:	Calprotectin 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)	
Device name (Extract. Buffer):	Proprietary name:	QUANTA Flash® Calprotectin Extraction Buffer
	Common name:	Calprotectin Extraction Solution
	Classification name:	Calprotectin, Fecal
Regulation Description	Fecal Calprotectin Immunological Test System	
Regulation Medical Specialty	Immunology	
Product Code	NXO	
Regulation Number	866.5180	
Device Class	2	

Predicate device

Calprest NG (QUANTA Lite® Calprotectin Extended Range ELISA), 510(k) number: k160447. Date declared: November 10, 2016.

Device description

The principle of the assay is chemiluminescent microparticle immunoassay, a variation of solid phase immunoassay. The QUANTA Flash® Calprotectin 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® Calprotectin assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH® instrument.

Calprotectin-specific capture antibodies are coated on to paramagnetic beads, which are stored in the reagent cartridge under conditions that preserve the antibody in its reactive state. Prior to use in the BIO-FLASH® system, the reagent pack containing all the necessary assay reagents is mixed thoroughly by being inverted several times. The sealed reagent tubes are pierced with the reagent cartridge lid, and the reagent cartridge is loaded onto the instrument. Reagents are calibrated when the lot is first used. A patient extracted stool sample is prediluted by the BIO-FLASH® with sample buffer in a disposable plastic cuvette. Small amounts of the diluted patient extracted stool, the beads, and the 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 monoclonal anti-calprotectin 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 measured RLU is proportional to the amount of bound isoluminol conjugate, which is in turn proportional to the amount of calprotectin antigen captured by the antibodies (anti-calprotectin polyclonal antibodies in this case) on the beads. For quantitation, the QUANTA Flash® Calprotectin will utilize a predefined lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. The Master Curve is generated by Inova Diagnostics for each reagent pack lot with in-house Standards with assigned unit values (ng/mL). The RLU and assigned ng/mL values of the Standards are used to create a 4 parameter logistic curve. These four parameters are embedded in the reagent pack barcode. When the lot is used the first time, the Calibrators are run, and based on the results obtained on the Calibrators, an instrument specific Working Curve is created; The Working Curve is used to calculate units (ng/mL) based on RLU values obtained on each sample. The obtained ng/mL values will be converted to mg/kg by a calculation that takes into account the dilution of the samples. This unit conversion is calculated automatically by the software.

The QUANTA Flash Calprotectin Reagents kit contains the following materials:

One (1) QUANTA Flash Calprotectin Reagent Cartridge
One (1) bottle of QUANTA Flash Special Wash

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

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

The QUANTA Flash Calprotectin Calibrators kit contains two vials each of Calibrator 1, Calibrator 2, and Calibrator 3:
QUANTA Flash Calprotectin Calibrators:

- QUANTA Flash Calprotectin Calibrator 1: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain recombinant calprotectin antigen in stabilizers and preservatives.
- QUANTA Flash Calprotectin Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain recombinant calprotectin antigen in stabilizers and preservatives.
- QUANTA Flash Calprotectin Calibrator 3: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain recombinant calprotectin antigen in stabilizers and preservatives.

The QUANTA Flash Calprotectin Controls kit contains two vials of Low Control and two vials of High Control:
QUANTA Flash Calprotectin Controls:

- QUANTA Flash Calprotectin Low Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain recombinant calprotectin antigen in stabilizers and preservatives.
- QUANTA Flash Calprotectin High Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain recombinant calprotectin antigen in stabilizers, and preservatives.

The QUANTA Flash Calprotectin Extraction Buffer kit contains two bottles of Extraction Buffer (2.5 X):
QUANTA Flash Calprotectin Extraction Buffer:

- QUANTA Flash Calprotectin Extraction Buffer (2.5 X): Two (2) labeled bottles containing 125 mL, concentrated reagent.

Intended use(s)

QUANTA Flash Calprotectin is a chemiluminescent immunoassay for the quantitative determination of fecal calprotectin in extracted human stool samples. Elevated levels of fecal calprotectin, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease), and in the differentiation of IBD from irritable bowel syndrome (IBS).

QUANTA Flash Calprotectin Calibrators are intended for use with the QUANTA Flash Calprotectin Reagents for the determination of fecal calprotectin levels in extracted stool samples. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash Calprotectin Controls are intended for use with the QUANTA Flash Calprotectin Reagents for quality control in the determination of fecal calprotectin levels in extracted stool samples.

QUANTA Flash Calprotectin Extraction Buffer is intended for use with the QUANTA Flash Calprotectin Reagents as sample extraction solution.

Indications for use

Same as Intended use.

Substantial equivalence

The QUANTA Flash Calprotectin Reagents, the QUANTA Flash Calprotectin Calibrators, the QUANTA Flash Calprotectin Controls and the QUANTA Flash Calprotectin Extraction Buffer have the same intended use and assay principle as the predicate device.

Comparison to predicate device

QUANTA Flash Calprotectin Reagents

Similarities		
Item	**QUANTA Flash Calprotectin Reagents**	**QUANTA Lite Calprotectin Extended Range ELISA**
Intended use	QUANTA Flash Calprotectin is a chemiluminescent immunoassay for the quantitative determination of fecal calprotectin in extracted human stool samples. Elevated levels of fecal calprotectin, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease), and in the	QUANTA Lite Calprotectin Extended Range is a quantitative ELISA for detecting concentration of fecal calprotectin, which can be used as an in vitro diagnostic to aid in the diagnosis of Inflammatory Bowel Diseases (IBD), specifically Crohn's disease and ulcerative colitis, and to differentiate IBD from Irritable Bowel Syndrome (IBS)

Similarities		
Item	**QUANTA Flash Calprotectin Reagents**	**QUANTA Lite Calprotectin Extended Range ELISA**
	differentiation of IBD from irritable bowel syndrome (IBS).	in conjunction with other clinical and laboratory findings.
Assay methodology	Solid phase (heterogeneous) immunoassay	Solid phase (heterogeneous) immunoassay
Antigen	Rabbit polyclonal anti-calprotectin antibody	Rabbit polyclonal anti-calprotectin antibody
Shelf life	One year	One year
Sample type	Extracted Human Stool	Extracted Human Stool
Units	mg/kg (milligram of calprotectin per kilogram of stool)	mg/kg (milligram of calprotectin per kilogram of stool)

Differences		
Item	**QUANTA Flash Calprotectin Reagents**	**QUANTA Lite Calprotectin Extended Range ELISA**
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well polystyrene plate
Conjugate	Isoluminol conjugated monoclonal anti-calprotectin antibody	HRP conjugated monoclonal anti-calprotectin antibody
Analytical Measuring Range	16.1 – 3,500.0 mg/kg	27.1 – 3,000.0 mg/kg
Calibration	Lot specific Master Curve + three calibrators (sold separately)	Six lot specific calibrators

QUANTA Flash Calprotectin Calibrators

Item	**QUANTA Flash Calprotectin Calibrators**	**QUANTA Lite Calprotectin Extended Range ELISA**
Intended use	QUANTA Flash Calprotectin Calibrators are intended for use with the QUANTA Flash Calprotectin Reagents for the determination of fecal calprotectin levels in extracted stool samples. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.	No separate intended use; calibrator is part of the kit.
Analyte	Recombinant calprotectin antigen (rAg)	Recombinant calprotectin antigen (rAg)
Method	QUANTA Flash Calprotectin chemiluminescent immunoassay	QUANTA Lite Calprotectin Extended Range ELISA
Matrix	Calprotectin rAg, stabilizer, and preservative	Calprotectin rAg, stabilizer, and preservative

Item	QUANTA Flash Calprotectin Calibrators	QUANTA Lite Calprotectin Extended Range ELISA
Units	ng/mL	ng/mL
Physico-chemical characteristics	Liquid, prediluted, ready to use	Liquid, prediluted, ready to use
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

QUANTA Flash Calprotectin Controls

Item	QUANTA Flash Calprotectin Controls	QUANTA Lite Calprotectin Extended Range ELISA
Intended use	QUANTA Flash Calprotectin Controls are intended for use with the QUANTA Flash Calprotectin Reagents for quality control in the determination of fecal calprotectin levels in extracted stool samples.	No separate intended use; controls are part of the kit.
Analyte	Recombinant calprotectin antigen (rAg)	Recombinant calprotectin antigen (rAg)
Method	QUANTA Flash Calprotectin chemiluminescent immunoassay	QUANTA Lite Calprotectin Extended Range ELISA
Matrix	Calprotectin rAg, stabilizer, and preservative	Calprotectin rAg, stabilizer, and preservative
Units	ng/mL	ng/mL
Physico-chemical characteristics	Liquid, ready to use	Liquid, prediluted, ready to use
Levels	2 (low and high)	2 (1 and 2)
Storage	2-8 °C	2-8 °C
Shelf life	One year	One year

QUANTA Flash Calprotectin Extraction Buffer

Item	QUANTA Flash Calprotectin Extraction Buffer	QUANTA Lite Calprotectin Extended Range ELISA
Intended use	QUANTA Flash Calprotectin Extraction Buffer is intended for use with the QUANTA Flash Calprotectin Reagents as sample extraction solution.	No separate intended use; controls are part of the kit.
Method	QUANTA Flash Calprotectin chemiluminescent immunoassay	QUANTA Lite Calprotectin Extended Range ELISA
Concentration	2.5X (1X working concentration)	2.5X (1X working concentration)
Physico-chemical characteristics	Liquid, ready to use	Liquid, ready to use
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 Calprotectin assay utilizes a lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. The Master Curve for QUANTA Flash Calprotectin consists of 7 Standards. These Master Curve Standards are used to create the lot specific Master Curve during the manufacturing procedure.

List of Calprotectin Standards:

Material	Assigned Value
Calprotectin Master Curve Standard 1	0.0 ng/mL
Calprotectin Master Curve Standard 2	10.9 ng/mL
Calprotectin Master Curve Standard 3	43.5 ng/mL
Calprotectin Master Curve Standard 4	173.9 ng/mL
Calprotectin Master Curve Standard 5	695.6 ng/mL
Calprotectin Master Curve Standard 6	1391.3 ng/mL
Calprotectin Master Curve Standard 7	3478.3 ng/mL

Value assignment and traceability of Calibrators and Controls

The QUANTA Flash Calprotectin Calibrators and Controls are manufactured by diluting recombinant calprotectin antigen in a buffer with stabilizers and preservatives. The recombinant calprotectin antigen is obtained from commercial sources.
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 5 to obtain a minimum of 10 data points 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 Calprotectin assay.

Calprotectin Calibrators and Controls with target manufacturing values:

Material	Manufacturing Target Value	Manufacturing Target Range
Calprotectin Calibrator 1	40 ng/mL	32 – 48 ng/mL
Calprotectin Calibrator 2	800 ng/mL	640 – 960 ng/mL
Calprotectin Calibrator 3	2400 ng/mL	2160 – 2640 ng/mL
Calprotectin Low Control	40 ng/mL	32 – 48 ng/mL
Calprotectin High Control	200 ng/mL	180 – 220 ng/mL

Precision

The precision of the QUANTA Flash Calprotectin assay was evaluated on 8 samples containing various concentrations of calprotectin antigen in accordance with CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Procedures - Approved Guideline. Samples were run in duplicates, twice a day, for 20 days.
Data were analyzed with the Analyse-it for Excel method evaluation software, and repeatability (within-run), between run, between day and within-laboratory precision (total precision) were calculated.
Acceptance criteria: Total %CV: < 12%
Results are summarized in the Table below.

QUANTA Flash Calprotectin			Repeatability		Between-Run		Between-Day		Within-Laboratory Precision	
Sample ID	N	Mean (mg/kg)	SD (mg/kg)	CV (%)	SD (mg/kg)	CV (%)	SD (mg/kg)	CV (%)	SD (mg/kg)	CV (%)
1	80	51.1	2.1	4.0%	1.9	3.6%	0.5	1.0%	2.8	5.5%
2	80	72.2	2.1	3.0%	1.5	2.0%	0.7	0.9%	2.7	3.7%
3	80	108.1	2.5	2.3%	2.5	2.3%	0.0	0.0%	3.6	3.3%
4	80	104.2	2.4	2.3%	1.4	1.3%	1.6	1.5%	3.2	3.1%
5	80	196.0	5.8	2.9%	4.5	2.3%	1.1	0.5%	7.4	3.8%
6	80	639.5	18.3	2.9%	11.1	1.7%	4.3	0.7%	21.9	3.4%
7	80	1086.9	34.2	3.1%	27.0	2.5%	0.0	0.0%	43.6	4.0%
8	80	1828.0	58.2	3.2%	33.8	1.8%	44.9	2.5%	80.8	4.4%
9	80	43.1	1.6	3.8%	1.7	4.0%	1.2	2.9%	2.7	6.2%
10	80	3036.3	90.2	3.0%	120.3	4.0%	46.2	1.5%	157.3	5.2%

Reproducibility Studies

Reproducibility between sites (instruments)

Eight samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, 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: < 15%

Results are summarized in the Table below.

			Within-Run		Between-Day		Within-Site		Between-Site		Total Imprecision	
Sample ID	**N**	**Mean (mg/kg)**	**SD (mg/kg)**	**CV (%)**	**SD (mg/kg)**	**CV (%)**	**SD (mg/kg)**	**CV (%)**	**SD (mg/kg)**	**CV (%)**	**SD (mg/kg)**	**CV (%)**
Sample 1	75	46.9	1.3	2.7%	2.6	5.5%	2.9	6.1%	0.0	0.0%	2.9	6.1%
Sample 2	75	63.6	1.7	2.6%	6.4	10.1%	6.6	10.4%	0.0	0.0%	6.6	10.4%
Sample 3	75	93.4	2.0	2.1%	9.1	9.8%	9.3	10.0%	0.0	0.0%	9.3	10.0%
Sample 4	75	89.4	1.9	2.1%	9.5	10.6%	9.7	10.8%	0.0	0.0%	9.7	10.8%
Sample 5	75	171.3	3.3	1.9%	13.4	7.8%	13.8	8.1%	0.0	0.0%	13.8	8.1%
Sample 6	75	649.5	15.4	2.4%	13.6	2.1%	20.5	3.2%	0.0	0.0%	20.5	3.2%
Sample 7	75	1127.5	27.4	2.4%	55.5	4.9%	61.9	5.5%	0.0	0.0%	61.9	5.5%
Sample 8	75	1967.7	56.8	2.9%	65.5	3.3%	86.7	4.4%	32.6	1.7%	92.7	4.7%

Reproducibility between lots

Eight samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, using three different lots. Samples were run in replicates of 5, once a day, for 5 days, to generate 25 data points per sample, per lot. Data were analyzed with the Analyse-it for Excel method evaluation software to calculate between lot precision.

Acceptance criteria: Total %CV: < 15%

Results are summarized in the Table below.

			Within-Run		Between-Day		Within-Lot		Between-Lot		Total Imprecision	
Sample ID	**N**	**Mean (mg/kg)**	**SD (mg/kg)**	**CV (%)**	**SD (mg/kg)**	**CV (%)**	**SD (mg/kg)**	**CV (%)**	**SD (mg/kg)**	**CV (%)**	**SD (mg/kg)**	**CV (%)**
Sample 1	75	54.1	2.4	4.5%	1.8	3.4%	3.0	5.6%	4.2	7.8%	5.2	9.7%
Sample 2	75	76.3	2.2	2.9%	1.6	2.0%	2.7	3.6%	6.7	8.7%	7.2	9.4%
Sample 3	75	113.6	3.0	2.6%	2.6	2.3%	4.0	3.5%	11.5	10.1%	12.1	10.7%
Sample 4	75	109.7	3.8	3.5%	0.0	0.0%	3.8	3.5%	13.6	12.4%	14.1	12.9%
Sample 5	75	203.7	6.0	2.9%	3.4	1.7%	6.9	3.4%	18.8	9.2%	20.0	9.8%
Sample 6	75	674.8	20.0	3.0%	19.9	2.9%	28.2	4.2%	14.6	2.2%	31.7	4.7%
Sample 7	75	1145.4	37.6	3.3%	42.4	3.7%	56.7	4.9%	21.9	1.9%	60.7	5.3%
Sample 8	75	1955.4	75.6	3.9%	54.0	2.8%	92.9	4.8%	33.4	1.7%	98.7	5.0%
Sample 9	75	45.5	0.6	1.4%	0.7	1.5%	0.9	2.0%	3.6	8.0%	3.8	8.3%
Sample 10	75	2933.2	99.6	3.4%	137.7	4.7%	169.9	5.8%	174.4	5.9%	243.5	8.3%

Reproducibility between operators (Extraction reproducibility)

Five samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, using three different operators. Samples were extracted every day by each operator independently. Samples were run in replicates of 5, once a day, for 5 days, to generate 25 data points per sample, per operator. Data were analyzed with the Analyse-it for Excel method evaluation software to calculate between operator precision.

Acceptance criteria: Total %CV: < 15%
Results are summarized in the Table below.

QUANTA Flash Calprotectin			Between Operator Reproducibility	
Sample ID	**Number of replicates**	**Mean (mg/kg)**	**SD (mg/kg)**	**CV (%)**
Sample 1	75	59.0	4.3	7.2%
Sample 2	75	108.1	9.0	8.3%
Sample 3	75	131.4	6.0	4.5%
Sample 4	75	220.9	21.6	9.8%
Sample 5	75	1465.6	95.7	6.5%

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

The LoD of the QUANTA Flash Calprotectin assay is 2.4 mg/kg, 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 240 determinations, with 60 measurements on blank samples and 60 measurements of low level samples, per reagent lot. The LoB is 0.0 mg/kg (513 RLU).

Two low level samples were tested in replicates of five on two reagent lots, once per day, for 3 days, obtaining 30 data points per sample to generate data used to calculate the LoQ for the QUANTA Flash Calprotectin assay. The LoQ was determined by calculating the total imprecision of each sample.
Acceptance criteria: Total imprecision CV% <20%.
The results obtained are summarized in the table below:

LoQ Precision			Total Imprecision	
Sample ID	N	Mean (mg/kg)	SD (mg/kg)	CV%
Sample 1	30	17.3	2.2	12.6%
Sample 2	30	14.1	2.1	14.7%

The LoQ for the assay has been found to be at 14.1 mg/kg, which has been set as the lower limit of the analytical measuring range.

Even though the LoQ has been found to be at 14.1 mg/kg, the AMR of the QUANTA Flash Calprotectin will start at 16.1 mg/kg.

Analytical Measuring Range (AMR)

QUANTA Flash Calprotectin: 16.1 mg/kg – 3,500.0 mg/kg

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 >3,500.0 mg/kg after further diluting it by 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 directly measured is 3,500.0 mg/kg, the highest value that can be reported is 35,000.0 mg/kg.

High concentration hook effect

To assess hook effect, measurement signal in 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 AMR do not show hook effect up to 21,753.6 mg/kg (theoretical value calculated using the highest value in the AMR and its dilution factor) in the QUANTA Flash Calprotectin assay.

Linearity

The linearity of the AMR of the QUANTA Flash Calprotectin was evaluated by a study according to CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. The linearity was evaluated in two ways: using extracted stool samples and using recombinant antigen samples. Three extracted stool samples with various calprotectin antigen concentrations were combined with another extracted stool sample containing low levels of calprotectin antigen in 10% increments (from 0% to 90% of low sample) to obtain values that cover the entire AMR. Additionally, three samples made by diluting recombinant antigen in buffer containing stabilizers and preservatives (same buffer used to make controls and calibrators) containing various calprotectin recombinant antigen concentrations were diluted in buffer in 10% increments (from 0% to 90% of buffer) to obtain values that cover the entire AMR. The dilutions were assayed in duplicates. Results were analyzed according to the guideline performing regression analysis and identifying the best fitting polynomial.
Acceptance criteria:
- Best fitting polynomial is a linear one, otherwise, the difference between the best-fitting nonlinear and linear polynomial is less than 15% (allowable nonlinearity).

All samples (3 stool samples and 3 recombinant antigen samples) have been found that the best fitting polynomial is a linear one except rAg Sample 2, where the best fitting polynomial found was a second order polynomial. The nonlinearity for rAg sample 2 ranged from -13.0% to 2.1%, fulfilling the acceptance criteria.

Moreover, a regression analysis has been performed in all individual samples and in combination of them, obtaining the results summarized in the tables below:

Stool Sample	Test Range (mg/kg)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	4102.8 to 410.3	1.02 (0.97 to 1.06)	16.1 (-90.1 to 122.3)	1.00	102.6%
2	890.3 to 89.0	0.95 (0.90 to 1.00)	11.9 (-14.2 to 38.0)	1.00	100.0%
3	155.6 to 15.6	1.12 (0.98 to 126)	-5.5 (-19.0 to 8.0)	0.98	100.8%
Combined	4102.8 to 15.6	1.02 (1.01 to 1.03)	-5.8 (-25.6 to 14.1)	1.00	101.1%

All three extracted stool samples showed dilution linearity individually and in combination.

rAg Sample	Test Range (ng/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	3376.4 to 337.6	1.03 (0.97 to 1.09)	-85.9 (-209.5 to 37.6)	1.00	96.8%
2	618.9 to 61.9	0.98 (0.95 to 1.02)	15.9 (3.0 to 28.7)	1.00	104.7%
3	115.1 to 11.5	0.99 (0.93 to 1.05)	4.0 (-0.6 to 8.5)	0.99	108.7%
Combined	3376.4 to 11.5	1.00 (0.98 to 1.02)	-2.9 (-26.0 to 20.2)	1.00	103.4%

These data demonstrate the linearity of the analytical measuring range (14.0 ng/mL – 3,043.5 ng/mL / 16.1 mg/kg – 3,500.0 mg/kg) of the QUANTA Flash Calprotectin assay.

Recovery

The recovery of the QUANTA Flash Calprotectin assay has been evaluated using seven extracted stool samples containing various concentrations of calprotectin antigen covering the AMR of the assay. Samples were spiked with calibrator material and then tested to calculate recovery results. For the samples with calprotectin concentrations lower than 200 mg/kg, Calibrator 2 was used as spiking material, while for samples with concentrations higher than 200 mg/kg, Calibrator 3 was used. Each sample was mixed with their correspondent calibrator material in a proportion 9:1. Each sample, calibrator and spiked sample was tested in duplicate and recovery values were calculated.

Acceptance criteria: Percent Recovery must be between 88% and 112%

All recovery results fulfilled the acceptance criteria and ranged from 94.7% to 109.8% and are summarized in the table below:

Sample	Baseline (mg/kg)	Calibrator Value (mg/kg)	Theoretical Value 90% sample + 10% Calibrator (mg/kg)	Observed Value 90% sample + 10% Calibrator (mg/kg)	Recovery %
Sample 1	49.6	740.6	118.7	128.3	108.1%
Sample 2	83.1	740.6	148.8	141.9	95.4%
Sample 3	155.3	740.6	213.8	212.7	99.5%
Sample 4	126.9	740.6	188.2	202.3	107.5%
Sample 5	252.3	2624.4	489.5	518.4	105.9%
Sample 6	833.2	2624.4	1012.3	1112.0	109.8%
Sample 7	2501.6	2624.4	2513.9	2380.0	94.7%

Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Six human stool specimens, one high positive (1365.1 mg/kg), one moderately positive (664.7 mg/kg), one low positive (215.1 mg/kg), one near the cutoff (123.0 mg/kg), one in the indeterminate rage (62.2 mg/kg) and one negative (22.1 mg/kg), samples were tested. Interfering substances (Hemoglobin, mesalamine, prednisone, vancomycin, gamma-tocopherol, tacrolimus, beta-carotene, ciprofloxacin, cholecalciferol, lansoprazole, and ascorbic acid, along with bacterial cultures: Yersinia ruckeri, Klebsiella pneumoniae, Shigella sonnei, Salmonella enterica and Escherichia coli) were spiked into every specimen in 10% of total specimen volume, and the resulting samples were assessed in triplicates with the QUANTA Flash Calprotectin assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluents (10% of total sample volume). Acceptance criteria for the interference studies were 85% - 115% recovery, or ± 15% of the low indeterminate range (±7.5 mg/kg) difference, whichever is greater.

No interference was detected in the results of the QUANTA Flash® Calprotectin at the concentrations tested (hemoglobin 5.56mg/50mg stool, mesalamine 1.33mg/50mg stool, prednisone 0.01mg/50mg stool, vancomycin 0.67mg/50mg stool, gamma-tocopherol 0.0010mg/50mg stool, tacrolimus 0.07mg/50mg stool, beta-carotene 0.0048mg/50mg stool, ciproflaxin 0.50mg/50mg stool, cholecalciferol 0.275ng/50mg stool, lansoprazole 0.02mg/50 mg stool, ascorbic acid 0.05mg/50mg stool respectively for drugs and nutrients and $1.5x10^7$ cfu/mL for each individual bacterial cultures).

Sample Stability and Handling

Eight extracted human stool samples, encompassing negative (n=1), in the indeterminate range (n=2), around the cut-off (n=1), and positive samples (n=4) were tested in duplicates for up to 24 days while stored at 2-8°C, up to 73 hours while stored at room temperature, and after repeated freeze/thaw cycles up to 5 cycles.

Additionally, three extracted stool samples in the indeterminate range (n=1), around the cut-off (n=1) and high positive (n=1) were tested in triplicates for up to 91 days while frozen at -20±4℃.

Results were compared to those obtained on control samples (time zero / zero cycles).

Acceptance criteria: 80-120% average recovery.

All samples fulfilled the acceptance criteria at each time point for each condition. Based on these result, we recommend that extracted samples are stored up to 72 hours at room temperature, up to 21 days at 2-8°C, up to 3 months frozen at -20±5℃ and can be subjected to up to 4 freeze/thaw cycles.

Reagent Stability

Shelf life

To establish the initial claim for shelf life, accelerated stability studies were performed for 3 weeks at 37 °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 Calprotectin to establish initial stability claim:

- Calprotectin beads (3 Lots)
- Calprotectin tracer (3 Lots)
- Calibrators 1, 2 and 3 (3 Lots)
- Low and High controls (3 Lots)
- Extraction Buffer (3 Lots)
- Special Wash (3 Lots)

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 and 3 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:

With regression analysis, the lower and upper 95% CI interval of the regression line is between 80% and 120% recovery at day 14.

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

In-use (onboard) stability

Calibrators

Onboard stability claim: 4 calibrations, or 8 hours onboard.

During assessing onboard stability, Calibrators were placed uncapped, onboard the instrument, and calibration was performed altogether five times over 9 hours. Controls and a panel of characterized patient specimens were run on each calibration curve.

Calibrators are considered stable if all four calibrations performed in the 8 hour period are successful, mean Calibrator RLU recovery values for the first 4 calibrations are between 90% and 110% compared to the first use, and Control/patient panel ng/mL recovery values are between 85% and 115% of those obtained on the first calibration curve.

The first four calibrations performed in the 8 hour period were considered valid by the software. The calibrators yielded average RLU recovery values ranging from 100.0% to 107.1%. The Control/patient panel ng/mL recovery ranged from 89.1% 105.2%. 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 once a day during 20 days for a total of 20 runs. The first run was used to establish baseline value, by running each vial in duplicate, and then additional 19 runs were performed, by running each vial in singleton. During runs, the Controls were left uncapped, onboard the instrument for 15±1 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 percent 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 Calprotectin reagent cartridge, one lot of reagent cartridge was tested with 4 extracted stool specimens (with different reactivity levels) along with the 2 controls made of recombinant antigen for a total of 6 samples. The specimens were tested periodically for of 97 days. Percent recoveries were calculated compared to the day zero average values, and linear regression analysis was performed by plotting percent 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% of the recovery data, (3 data points) is ≤75% or ≥125% recovery.

The onboard stability results are as follows:

Lot 160002: 97 days

Using these criteria, the in-use (onboard) stability of the QUANTA Flash Calprotectin reagent cartridge was set at 90 days.

Extraction Buffer

To establish the in-use stability of the QUANTA Flash Calprotectin Extraction Buffer, a bottle of buffer was diluted to a final concentration of 1X and kept at 2-8℃. After 91 days, another bottle of buffer of the same lot was diluted to 1X. Each 1X Calprotectin Extraction Buffer was used to extract 22 stool samples spanning the analytical measuring range (AMR) of the assay. All samples were run in duplicate on calibrated QUANTA Flash® Calprotectin Reagents.

A scatter plot with a linear fit was created by plotting the mean values obtained with the Day 91 1X Calprotectin Extraction Buffer against those obtained with the Day 0 1X Calprotectin Extraction Buffer, using the Weighted Least Squares function.

Acceptance criteria:

- r≥0.975
- Intercept of the regression line: ±15% of cut-off (±18mg/kg)
- Slope of the regression line: 0.9-1.1
- Weighted S y/x: ≤0.5
- Predicted bias at cut-off: ≤15% (18mg/kg)
- 95% CI of the bias: does not exceed medically significant difference, 20% of cut-off (24mg/kg)

All acceptance criteria were met at 91 days, with weighted r value of 0.999, intercept of the regression line at 3.87 mg/kg, slope of the regression line equal 0.9877, weighted S y/x of 0.10, predicted bias at the cut-off of 2.39 mg/kg and the 95% CI of the bias at -2.81-7.59 mg/kg.

The Calprotectin Extraction Buffer diluted at 1X is stable for 90 days when stored at 2-8°C.

Special Wash

To establish the onboard stability of the QUANTA Flash Special Wash, a bottle of Special Wash was placed uncapped, onboard the instrument for 32 continuous days. During this time, four samples, including three extracted stool samples and one sample made of recombinant antigen spanning the analytical measuring range of the assay, were tested in triplicates at different time points on the QUANTA Flash Calprotectin assay. Upon completion, the Special Wash bottle was taken off the instrument and kept capped, at room temperature until day 91 from the original opening date, when it was placed again onboard the instrument, uncapped, and tested again.

Percent recovery was calculated for each replicate of each sample against the data from day 0 testing. Percent recovery values were plotted against the number of days of the Special Wash onboard the instrument, and linear regression analysis with 95% confidence interval (CI) was performed.

Acceptance 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% of the recovery data, (2 data points) is ≤75% or ≥125% recovery.

All acceptance criteria were met at 91 days, the 95% confidence interval of the regression line for the QUANTA Flash Special Wash is between 97.9% and 105.5%. The results support the QUANTA Flash Special wash onboard (in-use) stability claim of 30 days uncapped continuous use, or 720 hours distributed over 90 days onboard of the instrument.

Real time stability

Real time stability testing has been scheduled to be performed every three or six months on the reagent cartridge, Calibrators, Controls and Extraction Buffer kits, 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, Calibrators and Controls. Data is not available for the Extraction Buffer at the time of the submission.

For reagent cartridge, a negative sample (Negative Control), a sample around the cut-off, a low positive sample (Positive Control), a moderate positive sample and a high positive sample were tested in replicates of 6 (replicates of 9 at time zero) at each time point.
- Acceptance criteria: results should fall within their respective 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 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 Calprotectin:

Negative	<50 mg/kg
Indeterminate	≥50 mg/kg to <120 mg/kg
Positive	≥120 mg/kg

The reference population for establishing the reference interval for the Calprotectin assay consisted of 61 subjects:

Sample Group	N
Apparently healthy donors	53
Squamous Cell Carcinoma	3
Glandular Polyp	2
Hyperplastic Polyp	2
Adenoma	1

All specimens were the same matrix (human stool) 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 95th percentile of the remaining obtained values was calculated as 137.8 mg/kg (90% CI, 125.0 – 148.1 mg/kg).

Additionally, thirty-one diagnosed inflammatory bowel disease (IBD) patient specimens were assayed to aid in the determination of the cutoff. Based on the distribution of result values in these (known) positive samples, the cutoff was established at 120 mg/kg to ensure optimal differentiation between negatives and positives and minimize risk of false negative samples. It was found that none of the IBD samples reported results <120 mg/kg.

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 Calprotectin. A total of 165 characterized samples were included in the Validation Set for the QUANTA Flash Calprotectin. Samples came from studies performed at two different sites. Samples with ID number from 1 to 107 belong to site A, while samples with ID number from 108 to 175 belong to site B.

The remaining samples came from a commercial source.

For site A: Inclusion criteria: samples suspected to suffer from IBD and that a calprotectin test was requested. Samples were consecutive and recruited over a period of 6 months. To allow accurate diagnostic, all patients underwent ileocolonoscopy.

Exclusion criteria: unclear diagnosis (e.g. indeterminate colitis), inability to collect enough fecal samples and age younger of 14 years. Also patients that previously had been diagnosed IBD or had not received ileocolonoscopy were excluded.

IBD diagnostic criteria: diagnostic work-up included physical examination and case history, endoscopic and histologic analysis, radiologic work-up and laboratory tests including ileocolonoscopy. Senior gastroenterologists performed all endoscopies and findings were documented in a computer-based database. The final diagnosis of IBD (i.e. CD and UC) was independently made by a pathologist or gastroenterologist who was blinded for calprotectin results.

For site B: Inclusion criteria: Sixty-eight consecutive samples requested for a calprotectin determination test by a gastroenterologist who had not had a calprotectin level measured before.

Exclusion criteria: samples with excessive mucous, unclear diagnosis, patients who had not received colonoscopy.

IBD diagnostic criteria: Patients were diagnosed after exclusion of organic pathology on the basis of routine blood tests, thyroid function tests, serological screening for coeliac disease, stool examination for bacteria and parasites, ultrasound examination, and eventually colonoscopy using the ROME III criteria. The diagnosis of IBD was made upon clinical, endoscopic and histological findings as described in J Crohn Colitis 2012:6: 965-990 (ulcerative colitis) and J Crohn Colitis 2010:4:7-27 (Crohn's disease).

All samples were run on the QUANTA Flash Calprotectin. The distribution of the cohort and the calprotectin indeterminate and positivity rate is in the Table below:

Patient Diagnosis/Group	N	# Indeterminate	# Positive
Irritable Bowel Disease (IBD)	**57**	**4**	**51**
Crohn's Disease (CD)	31	3	27
Ulcerative Colitis (UC)	26	1	24
Controls	**108**	**15**	**15**
Irritable Bowel Syndrome (IBS)	75	6	5
Chronic Diarrhea *	10	2	5
Recurrent Abdominal Pain *	10	0	2
Celiac Disease	6	2	1
Gastritis	5	3	1
Small Intestine Intestine	1	0	0
Lymfocytic Colitis	1	0	1
Total	**165**	-	-

** These samples have not been included in the clinical performance calculations since they are symptoms that are common in IBD patients and an IBD diagnosis hasn't been ruled out.*

Clinical Performance and predictive value of the QUANTA Flash Calprotectin assay:

Clinical Performance N=145		QUANTA Flash Calprotectin			
		Positive	Indeterminate	Negative	Total
Diagnosis	IBD	51	4	2	57
	Controls	8	11	69	88
	Total	59	15	71	145

QUANTA Flash Calprotectin	Clinical Performance Characteristics (95% Confidence Interval)	
	Indeterminate = Negative	Indeterminate = Positive
Sensitivity	89.5% (78.9 - 95.1%)	96.5% (88.1 - 99.0%)
Specificity	90.9% (83.1 - 95.3%)	78.4% (68.7 - 85.7%)
PPV	86.4% (76.6 - 92.5%)	74.3% (66.0 - 81.2%)
NPV	93.0% (86.2 - 96.6%)	97.2% (89.8 - 99.3%)

Expected values

The expected result for normal population is negative. Calprotectin levels were analyzed using the QUANTA Flash Calprotectin on a panel of 164 apparently healthy stool donors (94 females/70 males, ages from 17 to 89 years, with an average and median age of 44.9 and 42.0 years respectively). With a cut-off of 120 mg/kg, all samples were negative with the QUANTA Flash Calprotectin. The mean concentration was 19.0 mg/kg, and the values ranged from <16.1 to 49.2 mg/kg.

Comparison with predicate device

Samples for method comparison analysis included 137/165 samples from the clinical validation study. These samples were tested on both the QUANTA Flash Calprotectin and on the predicate ELISA. Of the 137 samples tested, 77 samples fell within the AMR of both assays.

Method comparison with the predicate device using all samples tested, treating the samples in the indeterminate range as negative samples.

Method Comparison – All samples (N=137) Indeterminate = Negative		Predicate assay			Percent Agreement (95% Confidence)
		Positive	Negative	Total	
QUANTA Flash Calprotectin	Positive	53	2	55	PPA: 98.1 (90.2 – 99.7)
	Negative	1	81	82	NPA: 97.6 (91.6 – 99.3)
	Total	54	83	137	TPA: 97.8 (93.8 – 99.3)

PPA= Positive Percent Agreement; NPA= Negative Percent Agreement; TPA= Total Percent Agreement

Method comparison with the predicate device using all samples tested, treating the samples in the indeterminate range as positive samples.

Method Comparison – All Samples (N=137) Indeterminate = Positive		Predicate assay			Percent Agreement (95% Confidence)
		Positive	Negative	Total	
QUANTA Flash Calprotectin	Positive	65	4	69	PPA: 98.5 (91.9 – 99.7)
	Negative	1	67	68	NPA: 94.4 (86.4 – 97.8)
	Total	66	71	137	TPA: 96.4 (91.4 – 98.4)

PPA= Positive Percent Agreement; NPA= Negative Percent Agreement; TPA= Total Percent Agreement

Method comparison with the predicate device using samples in the AMR of both assays, treating the samples in the indeterminate range as negative samples.

Method Comparison - Within AMR (N=77) Indeterminate = Negative		Predicate assay			Percent Agreement (95% Confidence)
		Positive	Negative	Total	
QUANTA Flash Calprotectin	Positive	51	2	53	PPA: 98.1 (89.9 – 99.7)
	Negative	1	23	24	NPA: 92.0 (75.0 – 97.8)
	Total	52	25	77	TPA: 96.1 (89.2 – 98.7)

PPA= Positive Percent Agreement; NPA= Negative Percent Agreement; TPA= Total Percent Agreement

Method comparison with the predicate device using samples in the AMR of both assays, treating the samples in the indeterminate range as positive samples.

Method Comparison - Within AMR (N=77) Indeterminate = Positive		Predicate assay			Percent Agreement (95% Confidence)
		Positive	Negative	Total	
QUANTA Flash Calprotectin	Positive	63	4	67	PPA: 98.4 (91.7 – 99.7)
	Negative	1	9	10	NPA: 69.2 (42.4 – 87.3)
	Total	64	13	77	TPA: 93.5 (85.7 – 97.2)

PPA= Positive Percent Agreement; NPA= Negative Percent Agreement; TPA= Total Percent Agreement

Additionally, a quantitative comparison has been performed on the 77 samples in the AMR of both QUANTA Flash Calprotectin and the predicate device assays utilizing a Spearman correlation and a linear regression analysis. The results revealed a Spearman's rs of 0.983 (95% CI, 0.973 – 0.989) and the results for the regression analysis are summarized in the table below:

N	Range (mg/kg)	Slope (95%CI)	Intercept (95%CI)	Correlation Coefficient
77	26.8 - 2681.2	1.10 (1.01 - 1.18)	0.52 (-9.47 - 14.0)	0.956