**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION MEMORANDUM**

**A. 510(k) Number:**

K170993

**B. Purpose for Submission:**

New Device

**C. Measurand:**

Fecal Calprotectin

**D. Type of Test:**

Quantitative**,** ELISA (chemiluminescent microparticle immunoassay)

**E. Applicant:**

Inova Diagnostics, Inc

**F. Proprietary and Established Names:**

QUANTA Flash® Calprotectin Reagents

QUANTA Flash® Calprotectin Calibrators

QUANTA Flash® Calprotectin Controls

**G. Regulatory Information:**

1. Regulation section:

   866.5180 – Fecal calprotectin immunological test system

   862.1150 – Calibrator

   862.1660– Quality Control Material

2. Classification:

   Class II

3. Product code:

   NXO– Fecal calprotectin immunological test system

   Immunoassay

   JIT– Calibrator

4. Panel:

   Immunology

## H. Intended Use:

1. Intended uses:

   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.

2. Indications for use:

   Same as Intended Use

3. Special conditions for use statement:

   Prescription use only

4. Special instrument requirements:

   BIO-FLASH® instrument

## I. Device Description:

The QUANTA Flash Calprotectin Reagents kit contains the following materials:
One (1) QUANTA Flash Calprotectin Reagent Cartridge

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 antibody in buffer, containing protein stabilizers and preservative.

One (1) bottle of QUANTA Flash Special Wash

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.

## Equivalence Information:

1. Predicate device name:

   Calprest NG, QUANTA Lite® Calprotectin Extended Range ELISA

2. Predicate 510(k) number:

   K160447

3. Comparison with predicate:

| **Similarities** | | |
|---|---|---|
| Item | Device | Predicate |
| Intended use | QUANTA Flash Calprotectin is a chemiluminescent immunoassay for the | Calprest®NG is a quantitative ELISA for detecting concentration of fecal calprotectin, which |

| Similarities | | |
|---|---|---|
| Item | Device | Predicate |
| | 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). | 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) in conjunction with other clinical and laboratory findings. |
| Assay Methodology | Solid phase (heterogeneous) immunoassay | Same |
| Capture antibody | Rabbit polyclonal anti-calprotectin | Same |
| Shelf-life | One year | Same |

| Differences | | |
|---|---|---|
| Item | Device | Predicate |
| 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*

| Similarities | | |
|---|---|---|
| Item | Device | Predicate |
| Analyte | Recombinant calprotectin antigen (rAg) | Same |
| Units | ng/ml | Same |
| Shelf-life | One year | Same |

| Differences | | |
|---|---|---|
| Item | Device | Predicate |
| 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. |
| Method | QUANTA Flash Calprotectin chemiluminescent immunoassay | QUANTA Lite Calprotectin Extended Range ELISA |

*QUANTA Flash Calprotectin Controls*

| Similarities | | |
|---|---|---|
| Item | Device | Predicate |
| | | |
| Analyte | Recombinant calprotectin antigen (rAg) | Same |
| | | |
| Units | ng/ml | Same |
| Shelf-life | One year | Same |
| | | |
| Storage | 2–8°C | Same |

| Differences | | |
|---|---|---|
| Item | Device | Predicate |
| 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. |
| Method | QUANTA Flash Calprotectin chemiluminescent immunoassay | QUANTA Lite Calprotectin Extended Range ELISA |
| Levels | 2 (low and high) | 2 (1 and 2) |

**K. Standard/Guidance Document Referenced (if applicable):**

Class II Special Controls Guidance Document: Fecal Calprotectin Immunological Test Systems

CSLI EP05-A3: *Evaluaiton of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition*

CLSI EP17-A2: *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guidline-Second Edition*

CLSI EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*

CLSI EP28-A3c*: Defining, Establishing, andVerifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition*

**L. Test Principle:**

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 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.

## M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   All results met the manufacturer's pre-determined acceptance criteria.

   *a. Precision/Reproducibility:*

   *Precision*
   Precision was evaluated according to *CLSI EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Procedures - Approved Guideline*. Calprotectin was extracted from stool samples from ten samples with concentrations across the analytical measuring range (AMR) of the device. Samples were evaluated in duplicate, twice per day, for twenty days. Each sample had a total of 80 replicates. The results are presented in the table below:

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

*Reproducibility*

*Between-Site*:
Extracts were prepared from eight stool samples at a single site, frozen, then shipped to two additional sites. At the three different sites, the eight extracts with calprotectin concentrations across the AMR of the device were run in replicates of five, once per day, for five days, to generate a total of 25 replicates per site, and a total of 75 replicates per sample. The results are presented in the table below:

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

*Between-Lot*:
Ten samples with concentration across the AMR were tested using three independent lots of reagent. Samples were run in replicates of five, once per day, for five days, to

generate a total of 75 replicates at a single site. The results are presented in the table below:

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

*Extraction reproducibility (Between-operator)*:
Five frozen stool samples were extracted three times per day, and by three different operators. Samples were run in replicates of five, once a day, for five days, to generate 15 data points per operator. A total of 75 replicates for each sample were measured. The results are presented in the table below:

| Sample | Number of Replicates | Mean (mg/kg) | Between Operator Precision | |
|---|---|---|---|---|
| | | | SD (mg/kg) | CV (%) |
| 1 | 75 | 59.01 | 4.3 | 7.2% |
| 2 | 75 | 108.10 | 9.0 | 8.3% |
| 3 | 75 | 131.41 | 6.0 | 4.5% |
| 4 | 75 | 220.93 | 21.6 | 9.8% |
| 5 | 75 | 1465.60 | 95.7 | 6.5% |

b. *Linearity/assay reportable range:*

The linearity of the AMR of the QUANTA Flash Calprotectin was evaluated according to *CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. The linearity was evaluated using extracted stool samples. Three extracted stool samples with various calprotectin antigen concentrations were combined with another extracted stool sample containing low levels of calprotectin antigen to obtain values that cover the entire AMR. The dilutions were assayed in duplicates. The results are presented in the table below, including the regression statistics using standard linear regression.

| 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 (0.97–1.06) | 16.1 (-90.1–122.3) | 1.00 | 102.6% |
| 2 | 890.3 to 89.0 | 0.95 (0.90–1.00) | 11.9 (-14.2–38.0) | 1.00 | 100.0% |
| 3 | 155.6 to 15.6 | 1.12 (0.98–126) | -5.5 (-19.0–8.0) | 0.98 | 100.8% |
| Combined | 4102.8 to 15.6 | 1.02 (1.01–1.03) | -5.8 (-25.6–14.1) | 1.00 | 101.1% |

Accuracy/Recovery

Assay recovery was evaluated using seven extracted stool samples containing various concentrations of calprotectin antigen covering the AMR of the assay. Extracts 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 of 9:1. Each sample, calibrator and spiked sample was tested in duplicate and recovery values were calculated. The results are presented in the table below:

| Sample | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---|---|---|---|---|---|---|---|
| Baseline (mg/kg) | 49.6 | 83.1 | 126.9 | 155.3 | 252.3 | 833.2 | 2501.6 |
| Spike Value (mg/kg) | 740.6 | 740.6 | 740.6 | 740.6 | 2624.4 | 2624.4 | 2624.4 |
| Theoretical (9:1) (Base+Spike) (mg/kg) | 118.7 | 148.8 | 188.2 | 213.8 | 489.5 | 1012.3 | 2513.9 |
| Observed (9:1) (Base+Spike) (mg/kg) | 128.3 | 141.9 | 202.3 | 212.7 | 518.4 | 1112 | 2380 |
| % Recovery | 108.10% | 95.40% | 107.50% | 99.50% | 105.90% | 109.80% | 94.70% |

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

*Traceability:* There is no international reference material for calprotectin. Calibrators and Controls are traceable to internal standards made from recombinant antigen.

*Stability*

*Reagent Stability:* The study was done to support the shelf-life stability of the the kit reagents (Beads, tracer, calibrators, low and high controls, extraction buffer, and special wash). Accelerated stability was performed using three lots of reagents at 37°C for three weeks, where one week is equal to six months a 5 ± 3 °C. The results support a stability claim up to 12 months at 5 ± 3 °C.

*Real-time reagent Stability*: Real time stability testing was completed up to six months at the time of the submission. Additional real-time stability testing is on-going.

*In-use (onboard) Stability:* The study was performed to determine the stability of reagents used over a period of time or for a number of assay runs. The results support calibrator stability for up to four calibrations over an eight hour period. The results support the claim that controls can be used up to 15 times. For reagent cartridges, the result support an in-use stability claim of 90 days. For Calprotectin Extraction Buffer diluted to 1X, the results support a stability claim of 90 days when stored at 2–8°C. For the special wash, 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.

*Sample Stability*: The stool stability claim for four days at 2–8°C was established by K160447 and acceptable because both devices use the same capture and detection antibodies. Further, the stability claim was up and supported by *Walsham et.al., Clin. Exp. Gastroenteriology, 2016; 9: 21–29*.

*Analyte Stability*: The study was performed to determine the stability of extracted stool samples when stored at various temperatures. The study results support stability of extracts for 72 hours at room temperature; 24 days at 2–8°C; and up to three months at -20°C. The study results also supports extract stability up to five freeze-thaw cycles.

d. *Detection limit:*

The LoB was determined using four blank samples, using two different lots of reagents. Samples were run in replicates of five, for three days, for a total of 60 replicates per reagent lot. The LoB was determined at the 95$^{th}$ percentile using the parametric method for one lot and the non-parametric methodod for the second lot. The LoB was determined to be 0.0 mg/kg.

The LoD was determined using four samples, two reagent lots, five replicates per run, for three days, consistent with CLSI EP17-A2 guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%. The determination was based on 240 measurements, with 60 measurements on blank samples and 60 measurements of low level samples per reagent lot. LoD was

determined to be 2.4 mg/kg.

For determination of the LoQ, 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 defined as the lowest concentration at which total imprecision was <20%. The LoQ for the QUANTA Flash Calprotectin assay was determined to be 16.1 mg/kg, which has been set as the lower limit of the AMR.

*e. Analytical specificity:*

Interference was performed according to CLSI EP07-A2: *Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition*. Six human stool specimens were tested, 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). Interfering compounds 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. Stool samples were tested for potential interference by:

Microorganisms: The data demonstrate that the results of the QUANTA Flash® Calprotectin was not affected by *Yersinia ruckeri*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Salmonella enterica* and *Escherichia coli* at 1.5x107 cfu/mL for each individual bacterial culture.

Drugs, Antibiotics, and Nutrients: The data demonstrate that the results of the QUANTA Flash® Calprotectin was not affected by the following oral pharmaceuticals: mesalamine (1.33 mg/50 mg stool), prednisone (0.01 mg/50 mg stool), vancomycin (0.67 mg/50 mg stool), gamma-tocopherol (0.0010 mg/50 mg stool), tacrolimus (0.07 mg/50 mg stool), beta-carotene (0.0048 mg/50 mg stool), ciprofloxacin (0.50 mg/50 mg stool), cholecalciferol (0.275 ng/50 mg stool), lansoprazole (lansoprazole 0.02 mg/50 mg stool), and ascorbic acid (0.05 mg/50 mg stool).

Others: The results demonstrate that the results of the QUANTA Flash® Calprotectin was not affected by the addition of Hemoglobin into the stool samples at 5.56mg/50mg stool.

*f. Assay cut-off:*

| Concentration (mg/kg) | Interpretation |
|---|---|
| <50 | Negative |
| ≥50 – <120 | Intermediate |
| ≥120 | Positive |

2. Comparison studies:

*a. Method comparison with predicate device:*

A total of 137 samples including 48 samples from patients diagnosed with IBD (Crohn's disease, ulcerative colitis, Lymphocytic colitis), 89 patients diagnosed with IBS, chronic diarrhea, recurrent abdominal pain, Celiac disease, and gastritis were assayed with QUANTA Flash® Calprotectin and the predicate device QUANTA Lite Calprotectin Extended Range in accordance with the instruction fror use in the package inserts. From these samples 77 samples were within the AMR of both devices, and were used to calculate agreement between the two devices. The regression analysis of the results comparing the QUANTA Flash® Calprotectin and QUANTA Lite Calprotectin Extended Range are shown below:

| N | Range (mg/kg) | Slope (95% CI) | Intercept (95% CI) | Correlation Coefficient |
|---|---|---|---|---|
| 77 | 27.42–2865.12 | 1.10 (1.009-1.179) | 0.5165 (-9.469-14.03) | 0.956 |

| | | Predicate (QUANTA Lite Calprotectin Extended Range) | | | |
|---|---|---|---|---|---|
| | | **Positive** | **Indeterminate** | **Negative** | **Total** |
| **QUANTA Flash Calprotectin** | **Positive** | 53 | 2 | 0 | 55 |
| | **Borderline** | 1 | 9 | 4 | 14 |
| | **Negative** | 0 | 1 | 67 | 68 |
| | **Total** | 54 | 12 | 71 | 137 |

| | | |
|---|---|---|
| **Borderline as positive:** | | |
| Positive agreement (95%CI): | 98.5% | (91.9–99.7) |
| Negative agreement (95% CI): | 94.4% | (86.4–97.8) |
| Total Agreement (95% CI): | 96.4% | (91.4–98.4) |
| | | |
| **Indeterminate as negative:** | | |
| Positive agreement (95%CI): | 98.1% | (90.2–99.7) |
| Negative agreement (95% CI): | 97.6% | (91.6–99.3) |
| Total Agreement (95% CI): | 97.8% | (93.8–99.3) |

*b. Matrix comparison:*

Not applicable

3. Clinical studies:

***a.*** *Clinical sensitivity and specificity**:***

Stool Samples from 165 individuals were collected for clinical validation of the QUANTA Flash Calprotectin device. Of these samples, 58 samples were from individuals diagnosed with IBD, 107 samples were from patients diagnosed with IBS and other diseases/conditions with gastrointestinal symptoms. Of the 58 IBD samples, 31 samples were from individuals with Crohn's disease, and 26 were from patients with Ulcerative Colitis, and one patient with Lymphocytic colitis. Among the non-IBD samples, 75 were from individuals diagnosed with IBS, 6 samples from patients diagnosed with Celiac Disease, 10 patients with chronic diarrhea, 5 patients with gastritis, 10 patients with Recurrant Abdominal Pain (RAP), and 1 patient with small intestine obstruction. The clinical performance was evaluated for sensitivity, specificity, positive predictive value, and negative predictive value.

| **Clinical Performance N=165** | | **Clinical Diagnosis** | | |
|---|---|---|---|---|
| | | **IBD** | **Controls** | **Total** |
| **QUANTA FLASH Calprotectin** | **Positive** | 51 | 15 | 66 |
| | **Indeterminate** | 4 | 13 | 17 |
| | **Negative** | 2 | 80 | 82 |
| | **Total** | 57 | 108 | 165 |

| **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 | 86.1% (78.3–91.4) | 74.1% (65.1–81.4) |
| PPV | 77.3% (67.8–84.6) | 66.3% (58.7–73.1) |
| NPV | 93.9% (87.9–97.1) | 97.6% (91.1–99.4) |

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   Samples were collected from 164 apparently healthy individuals (95 females and 70 males, ages 17 to 89, with an average and median age of 44.9 and 42.0 years, respectively). With a positive 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.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.