510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K180971

B. Purpose for Submission:

Addition of optional Fecal Extraction Device (FED) sample collection accessory to cleared assay

C. Measurand:

Fecal Calprotectin

D. Type of Test:

Quantitative chemiluminescent microparticle-enhanced immunoassay

E. Applicant:

Inova Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Flash Calprotectin Reagents

Fecal Extraction Device

G. Regulatory Information:

1. Regulation section:
   866.5180 – Fecal calprotectin immunological test system

2. Classification:

   Class II

3. Product code:

   NXO – Fecal calprotectin immunological test system

4. Panel:

   (82) Immunology

H. Intended Use:

1. Intended use:

   The Fecal Extraction Device is a single use tube containing extraction buffer intended for sampling and extracting human stool specimens and subsequent analysis with the QUANTA Flash Calprotectin assay.

2. Indication for use:

   Same as intended use

3. Special conditions for use statement:

   Prescription use only

4. Special instrument requirements:

   The FED has only been evaluated for use with the QUANTA Flash Calprotectin IVD system. The QUANTA Flash Calprotectin IVD is for use with the BIO-FLASH chemiluminescence detection instrument.

I. Device Description:

The optional Fecal Extraction Device (FED) acquires the amount of stool necessary to perform the QUANTA Flash Calprotectin assay directly from the primary specimen container instead of weighing the sample.

The device consists of a tube containing 2.8 mL of extraction buffer, and a stick shaped with seven grooves for collecting the sample. The upper end of the device is made up of two parts which can be removed with two separate rotations: the white screw cap (connected to the plastic stick with grooves) is removed by twisting counter-clockwise. The red lower part (for retaining the excess material) is removed by twisting clockwise. Once having completed the extraction procedure, remove both the white and red upper parts. The tube containing the extracted sample can be placed directly into the BIO-FLASH instrument sample rack.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   QUANTA Flash Calprotectin Reagents, Controls, Calibrators

2. Predicate 510(k) number(s):
   K170993

3. Comparison with predicate:

Similarities		
Item	Device	Predicate (K170993)
Assay methodology	Solid phase chemiluminescent immunoassay	Same
Sample type	Extracted human stool	Same
Units	milligram of calprotectin per kilogram of stool (mg/kg)	Same
Analytical Measuring Range (AMR)	16.1 – 3500.0 mg/kg	Same
Calibration	Lot-specific Master Curve + three calibrators	Same
Capture antibody	Polyclonal rabbit anti-calprotectin	Same
Detection antibody/Conjugate	Isoluminol-conjugaed monoclonal anti-calprotectin	Same
Assay Kit Shelf life	One year	Same

Differences		
Item	Device	Predicate
Intended use	The Fecal Extraction Device is a single use tube containing extraction buffer intended for sampling and extracting human stool specimens and subsequent analysis with the QUANTA Flash Calprotectin assay.	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).
Specimen sampling	Unitized specimen probe and sample extraction device with extraction buffer	Manual weighing of fecal sample into extraction buffer
Sample normalization	Volumetric	Sample weight
Extraction Shelf Life	Two years	Not applicable

K. Standard/Guidance Document Referenced:

Org	Standard ID	Version	Date	Title
CLSI	EP05	A3	Sep 2014	Evaluation of Precision Performance of Quantitative Measurement Methods

L. Test Principle:

The optional FED collects the amount of stool used to perform the QUANTA Flash Calprotectin assay directly from a primary specimen container instead of manually weighing the sample. The FED also contains buffer for extraction of the sample. Following extraction with the FED, the sample can be used for the QUANTA Flash Calprotectin assay procedure, per K170993.

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, which automatically processes the samples, runs the assay and reports the results.

M. Performance Characteristics:

1. Analytical performance:

   All parameters met the manufacturer's pre-established acceptance criteria.

   a. Precision/Reproducibility:

   FED Validation

   The manufacturer performed a study evaluating the reproducibility of sample collection by the FED probe. Five stool specimens ranging from 2–5 on the Bristol Stool Form Scale (BSFS) were sampled by three operators with five replicates per sample per operator. Samples on the FED were weighed against the empty weight of the FED. Results of the study are summarized below:

Precision of FED sample weight					
Sample	BSFS	Sample Weight (Mean ±SD)	Repeatability %CV	Between-Operator %CV	Within-Laboratory %CV
1	4	54.7 ± 3.6 mg	6.8%	0%	6.8%
2	3	56.9 ± 3.7 mg	6.3%	1.9%	6.6%
3	4	55.6 ± 3.4 mg	6.5%	0%	6.5%
4	5	57.7 ± 4.4 mg	7.4%	2.1%	7.7%
5	2	55.2 ± 3.7 mg	7.1%	0%	7.1%

Repeatability of sample collection weight was assessed across all five samples, replicates and operators. The sample weight collected by the FED is described in the table below:

Sample collection performance of FED	
Mean Sample Weight	56.0 mg
Median Sample Weight	56 mg
Range	50 – 62 mg
95% CI	55 – 57 mg

Std Dev	±3.8 mg
%CV	6.9%

Assay Precision

The sponsor performed a precision study, based on CLSI EP05-A3.

To study precision, eight stool samples representing the span of the analytical measuring range (AMR) were extracted using the FED and tested once per day × five replicates × five days × three operators, for a total of 75 measurements per sample. Each FED extraction was performed daily by each operator independently. The results of this study are summarized below:

Fecal calprotectin reproducibility, using the fecal extraction device											
Mean fCal (mg/kg)	***n***	**Repeatability**		**Between-Day**		**Within-Operator**		**Between-Operator**		**Within-Laboratory**	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
29.5	75	2.2	7.3%	7.2	24.4%	7.5	25.4%	0.0	0.0%	7.5	25.4%
40.0	75	2.3	5.7%	6.0	15.0%	6.4	16.1%	2.2	5.5%	6.8	17.0%
53.2	75	2.1	3.9%	5.4	10.2%	5.8	10.9%	4.8	9.0%	7.5	14.1%
108.2	75	4.3	4.0%	8.0	7.4%	9.1	8.4%	7.3	6.8%	11.7	10.8%
222.9	75	7.3	3.3%	20.9	9.4%	22.1	9.9%	5.4	2.4%	22.8	10.2%
535.4	75	15.7	2.9%	41.2	7.7%	44.1	8.2%	21.9	4.1%	49.2	9.2%
1278.3	75	45.0	3.5%	114.6	9.0%	123.2	9.6%	8.9	0.7%	123.5	9.7%
1560.0	75	63.9	4.1%	197.1	12.6%	207.2	13.3%	0.0	0.0%	207.2	13.3%

The manufacturer has included a statement in the labeling alerting users that the assay may have greater imprecision when used with the FED, as compared to the predicate manual weighing method.

b. Linearity/assay reportable range:

Assay linearity was evaluated in K170993.

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

Sample Stability

Sample stability was evaluated using seven stool samples selected to span the AMR, with particular attention to the equivocal range (Eq): 50–120 mg/kg fCal. Samples were extracted using the FED and tested in duplicate with the QUANTA Flash fCal assay for up to 73 hours stored at room temperature, up to 21 days stored at 2–8° C, and after up to five cycles of freeze-thaw. All results met the manufacturers pre-specified acceptance criteria.These results support the manufacturer's recommendation for sample storage up to 72 hours at room temperature, up to 14 days at 2–8° C, and up to four freeze-thaw cycles.

FED Shelf-Life Stability

Real-time stability was evaluated to determine shelf-life for the FED. pH values for the extraction buffer in the device were assessed on three lots every 3–6 months for a total of 24–26 months. pH values varied less than 5% within the timespan of the study.

The stability of the FED was evaluated using fCal assay values as a performance output. Fifteen stool samples selected to span the AMR were extracted using the FED from two lots at expiration date, and one newly produced FED lot for comparison. From these extracted samples, fCal values were tested with the QUANTA Flash fCal assay. These results support the manufacturer's recommendation for FED expiration date up to two years.

FED Stability: Room Temperature Storage

Three independent lots of FED were evaluated for room temperature stability. Six stool samples, selected to span the AMR, were extracted using FED devices stored at room temperature for up to 73 hours, as compared to FED devices stored at 2–8° C during this interval. From these extracted samples, fCal values were tested with the QUANTA Flash fCal assay. These results support the manufacturer's recommendation for FED storage at room temperature up to 72 hours.

d. Detection limit:

Assay detection capabilities was evaluated in K170993.

e. Analytical specificity:

Assay analytical specificity was evaluated in K170993.

f. Assay cut-off:

Assay cutoff was evaluated in K170993.

2. Comparison studies:

a. Method comparison with predicate device:

Ninety-seven (97) human stool samples selected to represent the span of the AMR were extracted by the FED as well as by the manual weighing method of the predicate device. Samples from both processing methods were tested in singlicate by the QUANTA Flash fCal assay. Linear regression by Passing-Bablok is summarized below:

Method Comparison performance of FED versus manual extraction	
Slope (95% CI)	0.93 (0.82–1.06)
Intercept (95% CI)	-1.2 (-9.7–6.4)
Bias at 120 mg/kg fCal	-7.8% (-16.0%–0.6%)
R^2	0.975

Qualitative agreement measures derived from the FED were compared to the manual weighing method to evaluate the comparative performance of the FED. Per FDA Guidance, "Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests", the positive percent agreement (PPA) and negative percent agreement (NPA) were calculated with equivocal results considered positive as well as negative.

Qualitative performance of FED extracted samples compared to manually-weighed samples					
		Predicate (Manual)			
		fCal(+)	**fCal(Eq)**	**fCal(–)**	
FED	**fCal(+)**	43	3	0	46
	fCal(Eq)	3	20	3	26
	fCal(–)	0	5	20	25
		46	28	23	97

fCal(Eq) considered positive **(+)**	**PPA** (95% CI)	93.2% (85.1–97.1%)
	NPA (95% CI)	87.0% (67.9–95.5%)
fCal(Eq) considered negative **(–)**	**PPA** (95% CI)	93.5% (82.5–97.8%)
	NPA (95% CI)	94.1% (84.1–98.0%)

b. Matrix comparison:

Human stool is the only claimed matrix. Variations in human stool consistency according to the BSFS index are represented as an independent variable in Precision and Method Comparison studies.

3. Clinical studies:

Clinical performance of the fecal calprotectin assay was evaluated in K170993.

4. Clinical cut-off**:**

Not Applicable

5. Expected values/Reference range:

Reference range and expected values of the fecal calprotectin assay were evaluated in K170993

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable

O. Conclusion:

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