510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K182698

B. Purpose for Submission:

New device

C. Measurand:

Calprotectin

D. Type of Test:

Quantitative, automated immunoassay

E. Applicant:

DiaSorin, Inc.

F. Proprietary and Established Names:

LIAISON Calprotectin
LIAISON Q.S.E.T. Device

G. Regulatory Information:

1. Regulation section:

   21CFR §866.5180 – Fecal Calprotectin Immunological Test

2. Classification:

   Class II

3. Product code:

   NXO – Calprotectin, Fecal

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   The DiaSorin LIAISON Calprotectin assay is an in vitro diagnostic chemiluminescent immunoassay (CLIA) intended for the quantitative measurement, in human stool, of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The LIAISON Calprotectin assay can be used as an aid in the diagnosis of inflammatory bowel diseases (IBD), specifically Crohn's disease and ulcerative colitis, and as an aid in differentiation of IBD from irritable bowel syndrome (IBS). Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures. The test has to be performed on the LIAISON XL Analyzer.

   The DiaSorin LIAISON Q.S.E.T. Device (Quantitative Stool Extraction and Test) is intended for use in the preparation of human stool specimens for testing in the LIAISON Calprotectin assay.

2. Indications for use:

   Same as Intended Use

3. Special conditions for use statement:

   For prescription use only

4. Special instrument requirements:

   LIAISON XL Analyzer (K181464)

I. Device Description:

The device is an IVD reagent system consisting of:

- paramagnetic capture particles coated with monoclonal mouse antibodies against human calprotectin
- conjugated monoclonal mouse anti-human calprotectin detection antibodies, labeled with an isoluminol derivative
- Q.S.E.T. sample extraction buffer
- sample diluent
- wash buffer
- calibrators and controls
  - controls: two levels of calprotectin, low (50 µg/g) and high (250 µg/g)
  - calibrators: two levels of recombinant calprotectin, low (28.5 µg/g) and high (451 µg/g)
  - calibration verifiers: four levels of recombinant calprotectin, 30–505 µg/g
- (*optional*) Q.S.E.T. device: a probe and container, for sampling and extraction of fecal samples

J. Substantial Equivalence Information:

1. Predicate device name:

   Genova Diagnostics PhiCal Test

2. Predicate 510(k) number:

   DEN060001/K050007

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The DiaSorin LIAISON Calprotectin assay is an in vitro diagnostic chemiluminescent immunoassay (CLIA) intended for the quantitative measurement, in human stool, of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The LIAISON Calprotectin assay can be used as an aid in the diagnosis of inflammatory bowel diseases (IBD), specifically Crohn's disease and ulcerative colitis, and as an aid in differentiation of IBD from irritable bowel syndrome (IBS). Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures.The test has to be performed on the LIAISON XL Analyzer. The DiaSorin LIAISON Q.S.E.T. Device (Quantitative Stool Extraction and Test) is intended for use in the preparation of human stool specimens for testing in the LIAISON Calprotectin assay.	The PhiCal test is a quantitative ELISA for measuring, in human stool, concentrations of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The PhiCal test can be used as an in vitro diagnostic to aid in the diagnosis of inflammatory bowel diseases (IBD):Crohn's disease and ulcerative colitis, and to differentiate IBD from irritable bowel syndrome.
Assay Methodology	Solid-phase (heterogeneous) immunoassay	Same
Assay Output	Quantitative	Same
Measurand	Human calprotectin	Same
Antigen	Recombinant human calprotectin	Same
Interpretation	Normal: <50 µg/g	Same

Similarities		
Item	Device	Predicate
	Borderline: 50 – 120 µg/g Elevated: >120 µg/g	

Differences		
Item	Device	Predicate
Assay processing	Automated	Manual
Instrumentation	LIAISON XL Analyzer	Generic ELISA Reader
Pre-analytical sample processing	Manual weight normalization -or- Q.S.E.T device	Manual weight normalization
Solid-phase	Paramagnetic microparticles	96-well polystyrene plate
Capture antibodies	Mouse monoclonal anti-human calprotectin	Rabbit polyclonal anti-human calprotectin
Detection antibodies	Mouse monoclonal anti-human calprotectin	Rabbit polyclonal anti-human calprotectin
Detection chemistry	Isoluminol chemiluminescence	Chromogenic alkaline phosphatase
Primary measurement units	Relative Light Units (RLU)	Optical Density (OD)
Analytical Measuring Range	5.0 – 800.0 µg/g	27.1 – 3000 µg/g
Extended Analytical Measuring Range	Automated 1:10 dilution: 800.0 – 8000 µg/g	n/a

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

Org	Std ID	Ver	Date	Title
CLSI	EP05	A3	Sep 2014	Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI	EP06	A	Apr 2003	Evaluation of the Linearity of Quantitative Measurement Procedures
CLSI	EP07	A2	Dec 2002	Interference Testing in Clinical Chemistry
CLSI	EP12	A2	Jan 2014	User Protocol for Evaluation of Qualitative Test Performance
CLSI	EP15	A3	Sep 2014	User Verification of Precision and Estimation of Bias
CLSI	EP17	A2	Jun 2012	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedure
CLSI	EP28	A3c	Oct 2010	Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

L. Test Principle:

The assay is a quantitative sandwich immunoassay, in which capture antibodies, immobilized to the paramagnetic solid phase bind calprotectin from processed fecal samples. Washing eliminates non-specific interactions, allowing for specific detection by conjugate antibodies against calprotectin. Subsequent measurement of light from the bound, conjugated isoluminol is in proportion to the quantity of bound calprotectin. The LIAISON XL Analyzer automates the assay process steps including: incubations, washing, calibration, measurement, and analysis.

Pre-analytical processing of fecal samples is accomplished by either manual weight normalization, followed by homogenization in Q.S.E.T. buffer; or using the optional Q.S.E.T. device for unitized collection and homogenization.

Following measurement, the assay reports calprotectin concentrations in units of microgram-per-gram of starting fecal sample material (µg/g). Interpretation of fecal calprotectin concentration ([Cal]) is as follows:

[Cal] (µg/g)	Interpretation
< 50 µg/g	Negative
50 – 120 µg/g	Borderline
>120 µg/g	Positive

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All analytical studies met the manufacturer's pre-specified acceptance criteria.

a. Precision/Reproducibility:

Within-laboratory Precision:

Six stool samples spanning the analytical measuring range (AMR) were selected for testing and stored frozen. Aliquots were extracted by manual weighing method on each day of testing. Testing was performed by 3 operators × 2 runs/day × 12 days × 2 replicates × 1 lot, for a total of 144 datapoints per sample. The results are summarized in the table below:

Within-Laboratory Precision											
Sample ID	Mean [Cal] (µg/g)	Within Run		Between Run		Between Day		Between Operator		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	21.6	0.7	3.1%	0.4	1.7%	2.9	13.5%	0.6	2.9%	3.1	14.3%
2	24.9	0.7	3.0%	0.7	2.6%	2.9	11.7%	1.0	4.2%	3.3	13.0%
3	39.9	0.9	2.2%	0.8	1.9%	3.4	8.6%	0.0	0.0%	3.6	9.1%
4	155	4.0	2.6%	3.5	2.2%	11.3	7.2%	8.3	5.3%	14.9	9.6%
5	253	7.0	2.8%	8.2	3.2%	20.2	8.0%	2.9	1.1%	23.0	9.1%

Within-Laboratory Precision											
Sample ID	Mean [Cal] (µg/g)	Within Run		Between Run		Between Day		Between Operator		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
6	639	15.9	2.5%	25.3	4.0%	39.2	6.1%	21.0	3.3%	53.6	8.4%

Multi-site Reproducibility:

Six stool samples, spanning the AMR, were selected for testing and stored frozen. Aliquots were extracted by manual weight method on each day of testing. Testing was performed by 3 sites × 2 operators/site × 1 run/day × 5 days × 6 replicates × 1 lot, for a total of 180 datapoints per sample. The results are summarized in the table below:

Multi-site Reproducibility											
Sample ID	Mean [Cal] (µg/g)	Within Run		Between Day		Within Site		Site-to-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	23.4	0.6	2.6%	2.6	11.2%	2.7	11.5%	0.0	0.0%	2.5	10.5%
2	25.9	0.7	2.6%	2.2	8.3%	2.3	8.7%	0.0	0.0%	2.2	8.4%
3	42.1	0.8	1.8%	5.3	12.5%	5.3	12.6%	1.9	4.4%	5.6	13.4%
4	173	4.7	2.7%	20.4	11.9%	20.9	12.1%	20.6	11.9%	29.3	17.0%
5	281	5.6	2.0%	26.9	9.6%	27.4	9.7%	40.1	14.3%	48.6	17.3%
6	695	18.9	2.7%	66.1	9.5%	68.3	9.8%	39.6	5.7%	78.9	11.3%

Lot-to-Lot Reproducibility:

Six stool samples, spanning the AMR, were selected for testing and stored frozen. Aliquots were extracted by manual weight method on each day of testing. Testing was performed by 1 site × 1 instrument × 3 lots × 5 days × 6 replicates, for a total of 90 datapoints per sample. The results are summarized in the table below:

Lot-to-Lot Reproducibility					
Sample ID	Mean [Cal] (µg/g)	Between Lot		Total	
		SD	%CV	SD	%CV
1	27.6	2.5	9.0%	4.5	16.4%
2	31.7	2.5	7.8%	4.1	12.9%
3	53.0	3.0	5.7%	13.0	24.5%
4	179	11.3	6.3%	18.3	10.2%
5	288	16.7	5.8%	33.8	11.7%
6	743	62.2	8.4%	88.3	11.9%

Q.S.E.T Extraction Device Validation:

Seven stool specimens, representing a range of qualitative consistency (Bristol stool form scale 2–6), were used to evaluate the reproducibility of sample weight collected by the Q.S.E.T. extraction device. Specimens were sampled with the Q.S.E.T.

extraction device by three operators × five replicates per sample per operator. The Q.S.E.T. device was weighed before and after sampling; the sample weight (final weight – tare weight) was calculated for precision parameters, summarized in the table below:

Precision of Q.S.E.T. Device sample weight								
Sample ID	BSFS	Mean Weight (g)	Repeatability		Between-Operator		Total	
			SD	%CV	SD	%CV	SD	%CV
1	2	10.7	0.39	3.6%	0.28	2.6%	0.52	4.9%
2	3	10.4	0.39	3.7%	0.18	1.7%	0.48	4.7%
3	4	10.3	0.25	2.5%	0.12	1.1%	0.32	3.1%
4	4	10.3	0.56	5.4%	0.25	2.4%	0.70	6.8%
5	4	10.7	0.42	3.9%	0.37	3.4%	0.59	5.5%
6	5	10.6	0.03	2.6%	0.21	2.0%	0.37	3.5%
7	6	10.6	0.15	1.4%	0.42	4.0%	0.40	3.8%

Repeatability of sample collection weight was calculated across all seven samples, replicates, and operators. The sample weight collected by the Q.S.E.T. Extraction Device is described in the table below:

Sample collection performance of Q.S.E.T. Device	
Mean Sample Weight	10.5 mg
Median Sample Weight	10.5 mg
Range	9.0–12.0 mg
95% CI	10.4–10.6 mg
Std Dev	0.51 mg
%CV	4.9%

Q.S.E.T. Extraction Device Reproducibility:

Five stool samples spanning the AMR were each extracted a total of 10 times using the Q.S.E.T device over three days and tested in triplicate using one reagent lot. The results are summarized in the table below:

Q.S.E.T. Extraction Device Reproducibility											
Sample ID	Mean [Cal] (µg/g)	Repeatability		Between-Day		Within-Operator		Between-Operator		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	21.9	0.64	2.9%	3.18	14.5%	3.23	14.8%	0.95	4.3%	3.05	13.9%
2	26.2	0.81	3.1%	1.95	7.4%	2.09	7.9%	1.84	7.0%	2.64	10.1%
3	38.6	1.43	3.7%	4.73	12.2%	4.90	12.7%	1.34	3.5%	4.62	12.0%
4	166	6.14	3.7%	9.11	5.5%	10.7	6.5%	8.14	4.9%	12.8	7.7%
5	297	11.3	3.8%	28.6	9.6%	30.4	10.2%	6.59	2.2%	28.4	9.6%
6	505	18.6	3.7%	59.4	11.8%	61.8	12.2%	34.4	6.8%	65.5	13.0%

Q.S.E.T. Extraction Device Reproducibility											
Sample ID	Mean [Cal] (µg/g)	Repeatability		Between-Day		Within-Operator		Between-Operator		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
7	2381	62.6	2.6%	410.4	17.2%	414.4	17.4%	254.7	10.7%	450.5	18.9%
8	4140	126.8	3.1%	309.2	7.5%	330.1	8.0%	228.9	5.5%	377.1	9.1%

b. Linearity/assay reportable range:

Linearity:

A pool of three stool extracts with endogenous calprotectin levels above the AMR was diluted into 13 intervals over the range of the AMR. Diluted samples were tested in triplicate, in random sequence, using one reagent lot. The results are summarized in the table below:

Linearity	
[Cal] Range (µg/g)	5.0 – 800 µg/g
Slope (95% CI)	0.96 (0.93–0.99)
Y-Intercept (95% CI)	4.14 (−13.82–22.1)
R^2	1.00

Extended Analytical Measuring Range (AMR):

The AMR for the assay was set as the linear range of 5.0 – 800 µg/g. An extended AMR for the LIAISON Calprotectin assay was evaluated using auto-dilution features incorporated into the LIAISON XL Analyzer. To evaluate the extended AMR (800-8000 µg/g), automated dilutions were compared to manual dilutions for three stool samples with calprotectin concentrations above 800 µg/g, extracted using the Q.S.E.T. device, and tested in triplicate. The results are summarized in the table below:

Extended Analytical Measuring Range Dilution				
Sample ID	BSFS	1:10 Dilution [Cal] (µg/g ± SD)		% Difference
		Manual	Automated	
1	7	3646 ± 132	3827 ± 146	4.93%
2	5	3807 ± 11.5	4127 ± 167	8.14%
3	7	7557 ± 405	7990 ± 567	5.69%

Hook Effect:

Antigen excess was evaluated by testing three stool extracts spiked with exogenous recombinant calprotectin to a final concentration of 100,000 µg/g. The spiked samples were serially diluted 1:10 to a final concentration of 10 µg/g. Dilutions, the undiluted

spiked samples, and the unspiked native samples were tested in triplicate, using one reagent lot. No high-dose hook effect was observed for calprotectin concentrations up to 100,000 µg/g.

Assay Recovery:

To evaluate recovery, five high concentration stool extracts and five low concentration stool extracts were tested neat. Recovery samples were prepared by mixing defined ratios of high and low samples (i.e. 2:1, 1:1, 1:2), and tested in five replicates using one reagent lot. Recovery was calculated as the observed calprotectin concentration, as a percent ratio of the expected calprotectin concentration. Mean recovery across all five sample pairs, replicates, and dilution ratios was 103% (± 3%).

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

Traceability:

Recognized international standards for calprotectin measurement are not available. The LIAISON calprotectin calibrators are referenced to an in-house standard preparation of recombinant calprotectin. Reagent integral lots include two calibrators to establish working curves based on 10-point master curves stored on the analyzer.

Stability:

Independent evaluations of the stability of the LIAISON Calprotectin kit, the Q.S.E.T extraction buffer, calibrators, controls, calibration verifiers, and Q.S.E.T. device are summarized below.

Kit Stability: LIAISON Calprotectin Test Components		
Component	Storage Conditions	Shelf Life
Kit	2–8°C	18 months
Q.S.E.T. Extraction Buffer	2–8°C	18 months
Calibration Curve	onboard instrument	28 days
Open Kit	onboard instrument	56 days
Open Q.S.E.T. Extraction Buffer	2–8°C	56 days
Reconstituted Calibrators	2–8°C	28 days
Reconstituted Calibrators	RT	6 hours
Kit Control Materials	2–8°C	18 months
Reconstituted Control Materials	2–8°C	28 days
Reconstituted Control Materials	RT	6 hours
Reconstituted Control Materials	−20°C	8 weeks
Reconstituted Control Materials	freeze/thaw cycles	4 cycles
Calibration Verifiers	2–8°C	18 months
Reconstituted Calibration Verifiers	2–8°C	28 days
Reconstituted Calibration Verifiers	RT	6 hours

Kit Stability: LIAISON Calprotectin Test Components		
Component	Storage Conditions	Shelf Life
Reconstituted Calibration Verifiers	−20°C	8 weeks
Reconstituted Calibration Verifiers	freeze/thaw cycles	4 cycles

In addition, critical storage parameters for stool samples were evaluated for stability, using six samples. In addition to storing native, unextracted sample, extracts derived from the manual extraction method or the Q.S.ET. device were stored in different conditions, and evaluated. Data from these sample stability studies are summarized below:

Sample Stability: Human Stool Samples tested by LIAISON Calprotectin		
Sample Extraction for Storage	Condition	Stability
none, native	Refrigerated: 2–8°C	72 hours
none, native	Frozen: −20°C	16 weeks
none, native	freeze/thaw cycles	3 cycles
Manual	Refrigerated: 2–8°C	7 days
Q.S.E.T. Device, without centrifugation	Refrigerated: 2–8°C	6 hours
Q.S.E.T. Device, with centrifugation	Refrigerated: 2–8°C	8 days
Manual	Room Temperature	8 hours
Q.S.E.T. Device, without centrifugation	Room Temperature	4 hours

d. Detection limit:

Limit of Blank (LoB): Five blank extraction buffer samples were tested in duplicate in six runs × two operators/instruments × two lots. The LoB was calculated by the parametric method as the 95th percentile of a corrected, normal distribution on the mean and standard deviation. The greater value from two lots, calculated independently, was selected.

Limit of Detection (LoD): Four low stool samples were tested in duplicate in six runs over three days × two operators/instruments × two lots. The LoD was calculated by the parametric method, as the 95th percentile of a corrected, normal distribution on the mean and standard deviation, added to the LoB. The greater value from two lots, calculated independently, was selected.

Limit of Quantitation (LoQ): Nine low stool samples were tested in duplicate in six runs over three days × two operators/instruments × two lots. The LoQ was calculated by precision profile, calculated from the inverse power regression function intercepting a precision target of 20%. The greater value from two lots, calculated independently, was selected; a third lot was used for verification.

Values for detection capabilities are summarized in the table below:

Detection Limits for LIAISON Calprotectin	
LoB	0.107 µg/g
LoD	0.395 µg/g
LoQ	0.400 µg/g

e. *Analytical specificity:*

One stool sample extract, containing a calprotectin concentration of ~50 µg/g was tested in triplicate in the presence of each interferent or vehicle buffer, using one assay reagent lot. A total of four endogenous, six microbial, and 23 exogenous interferents were evaluated, each at a single concentration. Interference was calculated as a percent ratio of the observed calprotectin concentration to the expected calprotectin concentration.

Interference studies are summarized in the table below:

Interference testing for LIAISON Calprotectin		
Interferent	Concentration	Interference
Endogenous interferents		
Hemoglobin	6.7 mg/mL	1.6%
Mucin	3.33 mg/mL	−4.0%
Palmitic acid	1.3 mg/mL	−4.0%
S100A12	21 µg/mL	0.3%
Stearic acid	2.65 mg/mL	−4.5%
Microbial interferents		
Escherichia coli	1.2×10^8 cfu	3.0%
Klebsiella pneumonia	1.2×10^8 cfu	4.0%
Salmonella enterica	1.2×10^8 cfu	−1.8%
Shigella boydii	1.2×10^8 cfu	−1.4%
Yersinia enterocolitica	1.2×10^8 cfu	−1.9%
Citrobacter freundii	1.2×10^8 cfu	1.5%
Exogenous interferents		
Barium sulfate	5.0 mg/mL	−1.0%
Imodium AD	6.67 mg/mL	2.5%
Kaopectate	0.87 mg/mL	2.4%
Mylanta	4.2 mg/mL	−1.9%
Pepto-Bismol	0.87 mg/mL	0.4%
Tums ($CaCO_3$)	0.5 mg/mL	−0.8%
Polyethylene glycol 3350	79.05 mg/mL	−2.1%
Simethicone	0.625 mg/mL	−0.4%
Lansoprazole (Prevacid)	0.2 mg/mL	2.2%
Omeprazole (Prilosec)	0.5 mg/mL	−2.6%
Cimetidine (Tagamet)	0.5 mg/mL	1.9%
Ciprofloxacin	1.25 mg/mL	−1.8%

Interference testing for LIAISON Calprotectin		
Interferent	Concentration	Interference
Metronidazole	12.5 mg/mL	−0.8%
Sulfamethoxazole	1.6 mg/mL	0.1%
Vancomycin HCl	2.5 mg/mL	−1.3%
Azathioprine	0.2 mg/mL	1.2%
Mesalamine (5-ASA)	5.0 mg/mL	7.3%
Prednisone	0.3 mg/mL	0.3%
Provitamin A	5.0 mg/mL	−0.6%
Vitamin C	0.1 mg/mL	−0.2%
Vitamin D_3	0.1 µg/mL	−0.3%
Vitamin E	0.3 mg/mL	−0.4%

f. Assay cut-off:

Assay cut-off	
Normal:	<50 µg/g
Borderline:	50–120 µg/g
Elevated:	>120 µg/g

2. Comparison studies:

a. Method comparison with predicate device:

Clinical stool samples (*n*=182) were collected prospectively and tested in in singlicate using the LIAISON calprotectin assay and the predicate device, tested in duplicate according to the predicate instructions for use. Samples above (*n*=16) or below (*n*=2) the AMR for either respective assay were excluded. Results of the Passing-Bablok regression of the comparison are summarized in the table below:

Method Comparison: Passing-Bablok regression	
[Cal] Range (µg/g)	27.1 – 800 µg/g
Slope (95% CI)	0.97 (0.89 – 1.00)
Y-Intercept (95% CI)	1.50 (−1.55 – 5.40)
R^2	0.96

In addition, qualitative interpretative agreement between the two assays was compared.

		Method Comparison: Qualitative agreement			
		Predicate			
		Abnormal	Borderline	Normal	Totals
LIAISON Calprotectin	Elevated	90	4	0	94
	Borderline	2	25	4	34
	Normal	0	4	32	36
	Totals	92	36	36	164

Borderline results considered elevated/abnormal (95% CI)			
PPA:	124/128	96.9%	(92.2–99.1%)
NPA	32/36	88.9%	(73.9–96.9%)
Borderline results considered normal (95% CI)			
PPA:	90/92	97.8%	(92.4–99.7%)
NPA:	68/72	94.4%	(86.3–98.5%)

The sponsor evaluated the accuracy of the Q.S.E.T. extraction device, comparing calprotectin values for the LIAISON calprotectin assay obtained by the manual weight extraction method, to LIAISON calprotectin values for the same 128 samples, selected to represent the AMR, obtained using the Q.S.E.T. device. Extracts were tested in singlicate using one reagent lot. Results were evaluated by linear regression by the method of Passing and Bablok, summarized in the table below:

Q.S.E.T. Device Accuracy: Passing-Bablok regression	
[Cal] Range (µg/g)	5.31 – 744 µg/g
Slope (95% CI)	0.96 (0.92 – 1.02)
Y-Intercept (95% CI)	−1.12 (−2.81 – 0.60)
R^2	0.97
Bias (at 50 µg/g, w/95%CI))	−2.91 (−5.15 – −0.53)
Bias (at 120 µg/g, w/95%CI)	−5.43 (−9.58 – −0.45)

The comparison of outputs between calprotectin the Q.S.E.T. device was also evaluated for qualitative outcomes, presented in the table below:

		Method Comparison: Qualitative agreement of Q.S.E.T. values with manual extraction			
		Manual Extraction			
		Elevated	Borderline	Normal	Totals
Q.S.E.T. Extraction	Elevated	36	0	0	36
	Borderline	6	23	3	32
	Normal	0	7	53	60
	Totals	42	30	56	128

Borderline results considered elevated (95% CI)			
PPA:	65/72	90.3%	(81.3–95.2%)
NPA	53/56	94.6%	(85.4–98.2%)
Intermediate results considered normal (95% CI)			
PPA:	36/42	85.7%	(72.2–93.3%)
NPA:	86/86	100%	(95.7–100%)

b. Matrix comparison:

Stool is the only matrix for this assay.

3. Clinical studies:

To study the clinical performance of the LIAISON calprotectin assay, prospective specimens were collected at 14 sites within the U.S. . The population consisted of subjects undergoing endoscopy for evaluation of signs and symptoms of Inflammatory Bowel Disease (IBD) or Irritable Bowel Syndrome (IBS). A total of 240 evaluable subjects meeting inclusion and exclusion criteria remained (see table below) after an initial 411 subjects were recruited. These subjects included 160 female, 80 male, and 19 pediatric (<22 years of age) subjects.

Inclusion Criteria	Exclusion Criteria	Sample Ineligibility
· ≥4 years of age · Signs and symptoms of IBD or IBS · Diagnosis of IBD, IBS, or other GI disorder by colonoscopy · Informed consent · Able to follow sample collection procedure	· Surgical resection or diversion procedure · Currently taking NSAIDs within seven days of colonoscopy and sample collection · Immunomodulator or biologic therapy within six months of colonoscopy and sample collection · Currently pregnant or lactating · Unable or unwilling to provide informed consent · Unable or unwilling to perform required study procedures	· Insufficient quantity (<5g) · Collected with urine and/or toilet water · Not stored frozen · Not received frozen by clinical site

Of the 240 evaluable subjects, additional metadata collected included demographic information (age, sex, race/ethnicity), current medications, signs and symptoms, clinical lab test results, and colonoscopy and biopsy results.

For 102 IBD patients, disease status was based on the Simple Endoscopic Score for Crohn's disease and the Mayo Endoscopic Score for Ulcerative Colitis and Indeterminate Colitis. For 67 IBS patients, diagnosis was based on negative colonoscopy within the previous year and the Rome III criteria. Of the 71 patients labeled with other GI conditions, seven diagnoses other than IBD or IBS were represented, also based in part on negative colonoscopic findings. In order of representation, these Other GI conditions included (with total number): diverticular disease (35), chronic diarrhea (16), recurrent abdominal pain (11), *Clostridium difficile* infection (3), other GI infection (3), celiac disease (2), and small bowel obstruction (1). Qualitative interpretation for the LIAISON Calprotectin test for these disease categories is summarized in the table below:

		Clinical Study: Qualitative comparison			
		Diagnostic Category			
		IBD	IBS	Other GI	Totals
LIAISON Calprotectin	Elevated	90	8	5	103
	Borderline	10	15	15	40
	Normal	2	44	51	97
	Totals	102	67	71	240

Clinical Study: Qualitative evaluation of IBD versus non-IBD			
Borderline results considered elevated (95% CI)			
Sensitivity:	100/102	98.0%	(93.1–99.8%)
Specificity:	95/138	66.8%	(60.4–76.7%)
Borderline results considered normal (95% CI)			
Sensitivity:	90/102	88.2%	(80.4–93.8%)
Specificity:	125/138	90.6%	(84.4–94.9%)

In addition to the above determination, where the specificity calculation is based on including both IBS and Other GI as a "Non-IBD" category (n=138), sensitivity and specificity values were also calculated for differential comparison of IBD and IBS:

Clinical Study: Qualitative evaluation of IBD versus IBS			
Borderline results considered elevated (95% CI)			
Sensitivity:	100/102	98.0%	(93.1–99.8%)
Specificity:	44/67	65.7%	(53.1–76.9%)
Borderline results considered normal (95% CI)			
Sensitivity:	90/102	88.2%	(80.4–93.8%)
Specificity:	59/67	88.1%	(77.8–94.7%)

4. Clinical cut-off**:**

See assay cut-off

5. Expected values/Reference range:

Stool samples from apparently healthy individuals were obtained from multiple sources. The study population fitting the inclusion and exclusion criteria comprised 112 adults and 15 children (3–21 years of age).

Inclusion Criteria	Exclusion Criteria	Sample Eligibility
· Informed consent	· History of prolonged abdominal complaints · History of IBD, IBS, or other chronic intestinal disorders · Currently taking NSAIDs	· Received frozen within 16 weeks of collection date

The values obtained for the study are summarized in the table below:

Reference Range	
Mean:	23.5 µg/g
Median:	15.0 µg/g
Range:	5.0 to 103 µg/g
90% Central Interval (95% CI)	5.0 µg/g (5.0–5.4 µg/g) to 79.8 µg/g (55.6–96.8 µg/g)

Qualitative interpretation for the LIAISON Calprotectin test for the reference population is summarized in the table below:

		Reference Range: Qualitative evaluation		
		Reference Population		
		Adult (≥22yo)	Pediatric (<22yo)	Totals
LIAISON Calprotectin	Elevated	0 (0%)	0 (0%)	0 (0%)
	Intermediate	12 (10.7%)	3 (20%)	15 (11.8%)
	Normal	100 (89.3%)	12 (80%)	112 (88.2%)
	Totals	112	15	127

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type under 21 CFR §866.5180.

O. Conclusion:

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