Food and Drug Administration
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510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number
K191807

B Applicant
ALPCO

C Proprietary and Established Names
ALPCO Calprotectin Chemiluminescence ELISA, ALPCO Easy Stool Extraction Device

D Regulatory Information:

Product Code(s)	Classification	Regulation Section	Panel
NXO	Class II	21 CFR 866.5180 - Fecal Calprotectin Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:
New Device

B Measurand:
Fecal calprotectin

C Type of Test:
Quantitative, ELISA

III Intended Use/Indications for Use:

A Intended Use(s):
The ALPCO Calprotectin Chemiluminescence ELISA:
The ALPCO Calprotectin Chemiluminescence ELISA is an in vitro diagnostic chemiluminescent assay intended for the quantitative measurement of fecal calprotectin, a neutrophilic protein that is a marker of intestinal mucosal inflammation, in human stool. The ALPCO Calprotectin Chemiluminescence ELISA is intended for in vitro diagnostic use as an aid in the diagnosis of inflammatory bowel disease (IBD), specifically Crohn's disease (CD) and ulcerative colitis

(UC), and as an aid in the differentiation of IBD from irritable bowel syndrome (IBS) in conjunction with other clinical and laboratory findings.
The ALPCO Easy Stool Extraction Device:The ALPCO Easy Stool Extraction Device is intended for use in the preparation of human stool specimens for testing in the ALPCO Calprotectin Chemiluminescence ELISA.

B Indication(s) for Use:
Same as Intended Use

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
Not Applicable

IV Device/System Characteristics:

A Device Description:

Materials provided:
1. Antibody-coated plate: 12x8 wells coated with IgG monoclonal antibody against calprotectin
2. Calibrators: 8 vials containing lyophilized calprotectin at eight concentrations (0, 5, 20, 40, 156, 625, 2500, 10000 µg/g) to be reconstituted using distilled water.
3. Control 1, 2 and 3, 1 vial each, containing lyophilized calprotectin. The range of values is printed on the respective certificate of analysis.
4. Sample Buffer, 2 bottles containing 100 mL sample buffer. Ready-to-use.
5. Detector Antibody (101X), 1 vial containing concentrated detector IgG monoclonal antibody to be diluted in detector antibody buffer.
6. Detector Antibody Buffer, 1 vial containing 15 mL detector antibody buffer. Ready-to-use.
7. Wash Buffer Concentrate (21X), 1 bottle containing 100 mL
8. Streptavidin-HRP (101X), 1 vial containing concentrated SA-HRP to be diluted in SA-HRP buffer.
9. Streptavidin-HRP Buffer, 1 vial containing 15 mL SA-HRP buffer. Ready-to-use.
10. Chemiluminescence Substrate A, 1 vial containing 6 mL substrate A. Ready-to-use.
11. Chemiluminescence Substrate B, 1 vial containing 6 mL substrate B. Ready-to-use.
12. Plate sealers, 3 plate sealers. Ready-to-use.

Materials Required but not Provided:

Feces sample collection:
1. Sample collection tube
2. Transport container

Feces preparation:
1. Disposable, breakable sterile inoculation loops or wooden stick
2. Disposable polypropylene screw cap tubes, 14 ml
3. Eppendorf tubes (1-1.5 ml)
4. Sensitive digital scale (40-150 mg)
5. Extraction buffer (catalog number 900-CALXB)

6. Vortex mixer
7. Shaker
8. Centrifuge (1000 –3000 xg)
9. Freezer (-20°C, -80°C)
10. ALPCO Easy Stool Extraction Device (catalog number 30-EZEX-100)

Equipment for ELISA measurements:
1. Precision pipettes for dispensing up to 100 µL (with disposable tips)
2. Repeating or multi-channel pipette for dispensing up to 100 µL
3. Volumetric containers and pipettes for reagent preparation
4. Distilled or deionized water for reagent preparation
5. Microplate washer or wash bottle
6. Microplate shaker capable of 700-900 rpm
7. Microplate reader capable of reading luminescence

B Principle of Operation:

The ALPCO Calprotectin Chemiluminescence ELISA is performed on stool samples, collected without preservatives. After an extraction procedure of the stool sample, using either the manual weighing or Easy Extraction Device procedure, the test allows the selective measurement of calprotectin-antigen by sandwich ELISA. A monoclonal capture antibody (mAb), specific to the calprotectin heterodimeric and polymeric complexes, is coated onto the microtiter plate. Calibrators, controls and specimen extracts are incubated. After a washing step, a biotinylated secondary monoclonal detection antibody detects the calprotectin molecules bound to the antibody coated onto the plate. After incubation and a further washing step, a Streptavidin-Horseradish Peroxidase Enzyme conjugate binds to the available biotin on the immobilized secondary antibody. A chemiluminescent substrate is added and read when the substrate glows as a result of its oxidation with the enzyme. The signal is then read on a chemiluminescent plate reader.

The ALPCO Easy Stool Extraction Device is an alternative to the manual weighing extraction method and it acquires the amount of stool necessary to perform the ALPCO Calprotectin Chemiluminescence ELISA directly from the primary specimen container. The device consists of a tube, containing 1.5 mL of extraction buffer, and a stick shaped with ten 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 yellow screw cap (connected to the plastic stick with grooves) is removed by twisting counter-clockwise. The blue lower part (for retaining the excess material) is removed by twisting clockwise. After completion of the extraction procedure, both the yellow and blue upper parts are removed, allowing access to the prepared stool sample extracts. The device does not collect aqueous stool sample. The ALPCO Easy Stool Extraction Device is sold separately.

V Substantial Equivalence Information: SESE

A Predicate Device Name(s):
Calprest®NG

B Predicate 510(k) Number(s):
K160447

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device: K191807	Predicate Device K160447
General Device Characteristic Similarities		
Assay Intended Use/Indications For Use	The ALPCO Calprotectin Chemiluminescence ELISA is an *in vitro* diagnostic chemiluminescent assay intended for the quantitative measurement of fecal calprotectin, a neutrophilic protein that is a marker of intestinal mucosal inflammation, in human stool. The ALPCO Calprotectin Chemiluminescence ELISA is intended for in vitro diagnostic use as an aid in the diagnosis of inflammatory bowel disease (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC), and as an aid in the differentiation of IBD from irritable bowel syndrome (IBS) in conjunction with other clinical and laboratory findings.	Calprest® NG 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) in conjunction with other clinical and laboratory findings.
Collection Device IU	The ALPCO Easy Stool Extraction Device is intended for use in the preparation of human stool specimens for testing in the ALPCO Calprotectin Chemiluminescence ELISA.	N/A
Analyte	Fecal Calprotectin	Same
Assay Format	Quantitative	Same
Platform	96 well microtiter plate	Same
Assay process	Manual	Same
General Device Characteristic Differences		
Capture Antibody	Monoclonal anti-calprotectin antibody	Polyclonal anti-calprotectin antibody
Detection antibody	Mouse monoclonal anti-calprotectin antibody	Same HRP-labeled mouse anti-calprotectin
Measuring Range	7.9–6000µg/g	27.1–3000 mg/kg
Units	µg/g	mg/kg
Method	Chemiluminescent	Colorimetric
Primary Measurement Units	Relative Light Units (RLU)	Optical Density (OD)

Calibrators	8 levels: 0, 5, 20, 40, 156, 625, 2500, 10000 µg/g	6 levels: 0, 2.5, 12.5, 25, 50, 150 mg/kg
Controls	3 levels Control 1: 20–40 µg/g Control 2: 80–125 µg/g Control 3: 1250–2500 µg/g	2 levels Control 1: 10–20 ng/mL Control 2: 30–70 ng/mL
Calibrator/Control Analyte	Native human calprotectin	Recombinant calprotectin antigen (rAg)
Sample dilution	1:25000	1:20000
Pre-analytical sample processing	Manual weighing extraction method or Easy Extraction Device method	Manual weighing extraction method
Results interpretation	Normal: < 50 µg/g Borderline: 50–100 µg/g Abnormal: >100 µg/g	Normal: <50 mg/kg Borderline: 50–120 mg/kg Abnormal: >120 mg/kg

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition
- CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP07-3rd Edition, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition
- Guidance for Industry and FDA Staff–Class II Special Controls Guidance Document: Fecal Calprotectin Immunological Test Systems

VII Performance Characteristics:

The test results met the company's pre-determined acceptance criteria.

A Analytical Performance:

Samples used to establish the analytical performance characteristics of the assay were of solid and semi-solid consistency.

1. Precision/Reproducibility:

Precision: precision study was conducted in accordance with CLSI EP05-A3 to evaluate repeatability and within-lab imprecision (between-run, within-day, between-day and within-laboratory). The study was performed at one site/laboratory, using one reagent lot, one ELISA reader and one operator. Eight solid or semi-solid stool samples (Bristol Stool Form Scale [BSFS] Type 1–6) containing various fecal calprotectin concentrations were selected and extracted using the Easy Extraction Device. The extracts were analyzed in duplicate,

twice per day, for 20 days to generate a total of 80 replicates per sample. The results are as follows:

			Within-run		Between-run		Within-day		Between-day		Total	
Sample	N	Mean (µg/g)	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV
1	80	32.9	1.3	3.9	1.2	3.7	1.8	5.3	2.9	8.9	3.4	10.3
2	80	39.1	1.8	4.7	1.6	4.0	2.4	6.1	2.8	7.2	3.7	9.5
3	80	82.2	3.7	4.5	3.3	4.0	4.9	6.0	3.8	4.6	6.2	7.6
4	80	116.6	3.0	2.6	4.3	3.6	5.2	4.5	4.2	3.6	6.7	5.7
5	80	288.7	9.5	3.3	12.3	4.2	15.5	5.4	8.6	3.0	17.7	6.1
6	80	908.3	25.7	2.8	29.2	3.2	38.9	4.3	56.6	6.2	68.7	7.6
7	80	680.3	19.7	2.9	26.2	3.8	32.8	4.8	29.2	4.3	43.9	6.4
8	80	5323.5	220.0	4.1	358.8	6.8	421.7	7.9	410.8	7.7	588.8	11.1

Lot-to-Lot Imprecision: The lot-to-lot imprecision was evaluated in accordance with CLSI EP05-A3. The study was performed using three reagent lots, one ELISA reader, one operator. Seven stool samples ((BSFS 1–6) containing various fecal calprotectin concentrations were extracted using the Easy Extraction method and analyzed in replicates of 5, once per day, for 5 days using each of the kit lots to generate a total of 75 replicates per sample. The results are as follows:

			Within-run		Between-day		Within-lot		Between-lot		Total	
Sample	N	Mean µg/g	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV
1	75	35.4	1.8	5.2	2.1	6.0	2.8	8.0	1.8	5.2	3.4	9.5
2	75	76.7	3.4	4.4	4.5	5.9	5.6	7.3	1.7	2.2	5.9	7.7
3	75	110.8	7.7	6.9	7.3	6.6	10.6	9.5	7.2	6.5	12.8	11.5
4	75	288.6	8.5	2.9	16.5	5.7	18.5	6.4	17.2	5.9	25.3	8.8
5	75	684.2	23.3	3.4	45.1	6.6	50.8	7.4	49.6	7.2	71.0	10.4
6	75	919.2	38.1	4.1	72.3	7.9	81.7	8.9	60.8	6.6	101.9	11.1
7	75	4480.2	321.3	7.2	445.1	9.9	548.9	12.3	298.0	6.7	624.6	13.9

Site-to-Site Reproducibility: The site-to-site reproducibility of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated internally at ALPCO and at two other sites in accordance with CLSI EP05-A3. The study was performed using one reagent lot, by one operator per test site. Seven stool samples (BSFS 1–6) containing various fecal calprotectin concentrations were extracted using the Easy Extraction method at ALPCO, frozen, and then shipped to the two additional sites. Each extract was analyzed in replicates of five, once per day, for five days at each site to generate 25 replicates per site and a total of 75 replicates per sample. The results are as follows:

			Within-run		Between-day		Within-site		Between-site		Total	
Sample	N	Mean µg/g	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV
1	75	20.0	2.4	12.1	1.7	8.6	3.0	14.9	0.3	1.4	3.0	15.0
2	75	21.1	2.2	10.4	1.7	7.9	2.8	13.1	0.0	0.0	2.8	13.1
3	75	46.3	2.6	5.6	3.7	8.1	4.5	9.8	0.7	1.6	4.6	9.9
4	75	138.2	10.1	7.3	15.8	11.4	18.7	13.5	0.0	0.0	18.7	13.5
5	75	195.2	8.9	4.6	21.6	11.0	23.3	12.0	0.0	0.0	23.3	12.0
6	75	446.5	21.5	4.8	27.6	6.2	35.0	7.8	0.0	0.0	35.0	7.8
7	75	2353.7	125.3	5.3	172.9	7.3	213.5	9.1	137.1	5.8	253.7	10.8

Operator-to-Operator Imprecision: The operator-to-operator imprecision was evaluated internally at ALPCO in accordance with CLSI EP05-A3. The study was performed using one reagent lot by three operators. Seven stool samples (BSFS 1–6) containing various fecal calprotectin concentrations were extracted using the Easy Extraction method and analyzed by each operator independently in replicates of five, once per day, for five days to generate 25 replicates per sample and per operator and a total of 75 replicates per sample. The results are as follows:

			Within-run		Between-day		Within-operator		Between-operator		Total	
Sample	N	Mean µg/g	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV
1	75	26.7	0.9	3.5	2.1	8.0	2.3	8.7	2.3	8.6	3.3	12.3
2	75	32.5	1.6	5.0	3.2	9.7	3.6	10.9	3.6	11.0	5.0	15.5
3	75	68.1	2.1	3.1	5.8	8.5	6.1	9.0	8.4	12.3	10.4	15.3
4	75	98.8	3.9	3.9	4.8	4.8	6.1	6.2	5.8	5.8	8.4	8.5
5	75	555.8	22.2	4.0	26.2	4.7	34.4	6.2	70.9	12.8	78.8	14.2
6	75	765.0	29.5	3.9	81.3	10.6	86.5	11.3	42.5	5.6	96.4	12.6
7	75	4468.6	299.8	6.7	470.4	10.5	557.9	12.5	225.5	5.0	601.7	13.5

Extraction Method Reproducibility: The extraction reproducibility was evaluated internally at ALPCO. The study was performed using one reagent lot by one operator. Sets of seven stool samples containing various fecal calprotectin concentrations on the Bristol Scale were extracted 10 times using both the Easy Extraction method and manual weighing method. Each stool sample extract was analyzed in duplicate to generate 20 replicates per sample, per extraction method. The results are as follows:

Extraction method reproducibility using the Easy Extraction Device:

			Within extraction		Between extraction		Total imprecision	
Sample	N	Mean µg/g	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV
1	20	33.4	1.7	5.1	0.0	0.0	1.7	5.1
2	20	45.9	4.6	9.9	2.8	6.1	5.3	11.6
3	20	68.3	3.5	5.1	3.2	4.7	4.8	7.0
4	20	123.7	6.9	5.6	3.9	3.1	7.9	6.4
5	20	129.0	1.8	1.4	6.2	4.8	6.5	5.0
6	20	1748.5	186.5	10.7	121.4	6.9	222.6	12.7
7	20	4298.5	317.2	7.4	426.9	9.9	531.8	12.4

Extraction reproducibility using the manual weighing method:

			Within extraction		Between extraction		Total imprecision	
Sample	N	Mean µg/g	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV
1	20	25.7	1.1	4.5	1.6	6.4	2.0	7.8
2	20	69.6	3.2	4.6	0.3	0.5	3.2	4.6
3	20	103.7	2.5	2.4	3.7	3.6	4.5	4.3
4	20	122.0	5.1	4.1	8.9	7.3	10.2	8.4

			Within extraction		Between extraction		Total imprecision	
Sample	N	Mean µg/g	SD µg/g	%CV	SD µg/g	%CV	SD µg/g	%CV
5	20	298.6	13.5	4.5	26.2	8.8	29.5	9.9
6	20	1336.1	42.4	3.2	113.5	8.5	121.2	9.1
7	20	4063.3	389.2	9.6	0.0	0.0	389.2	9.6

2. Linearity:
The matrix linearity and aqueous linearity of the analytical measuring range of the ALPCO Calprotectin Chemiluminescence ELISA were evaluated internally at ALPCO in accordance with CLSI EP06-A.

To evaluate matrix linearity, a stool sample extract containing a high concentration of fecal calprotectin was serially diluted 1:2 with a stool sample extract containing a low concentration of fecal calprotectin to obtain dilution levels with values that cover the AMR. Stool sample extracts were obtained using the Easy Extraction method. Each stool sample extract combination was assayed in duplicate.

To evaluate aqueous linearity, a sample made by diluting native antigen in standard diluent buffer containing stabilizers and preservatives (the same buffer used to make the controls and calibrators) was serially diluted 1:2 (12 dilutions) with standard diluent buffer to obtain dilution levels with values that cover the entire AMR. Each aqueous sample dilution was assayed in duplicate.

The results are summarized in the table below:

Sample	Range (µg/g)	Slope (95%CI)	Intercept (95%CI)	R^2	Percent Recovery Range
Matrix (Solid sample)	2.5–5780.1	1.003 (0.99 to 1.01)	1.77 (-15.87 to 19.42)	1.00	Avg. 99.2%
Aqueous sample	3.0–6221.7	0.99 (0.98 to 1.00)	0.03 (-22.42 to 22.47)	0.99	Avg. 100.6%

Accuracy/Recovery:
The accuracy/recovery of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated using seven extracted stool samples containing various concentrations of calprotectin across the analytical measuring range of the assay, samples were extracted using the Easy Extraction method. The stool sample extracts were mixed with the spiking material in a proportion of 9:1 (9 parts sample: 1-part spiking material). Native calprotectin diluted in standard diluent was used as the spiking material. Samples were run in duplicate. Percent recovery ranges from 89.7% to 110.8%. The results are as follows:

Sample	Mean Baseline Result (µg/g)	Spike Value (µg/g)	*Theoretical Post-Spike Result (µg/g)	Observed Post-Spike Result (µg/g)	**Recovery (%) (O/E)
1	28.1	42.8	68.1	72.1	105.9
2	36.2	42.8	75.3	79.0	104.9

Sample	Mean Baseline Result (µg/g)	Spike Value (µg/g)	*Theoretical Post-Spike Result (µg/g)	Observed Post-Spike Result (µg/g)	**Recovery (%) (O/E)
3	77.7	42.8	112.7	123.6	109.7
4	94.8	42.8	128.1	123.5	96.4
5	248.5	129.3	353.0	391.0	110.8
6	615.5	129.3	683.2	754.1	110.4
7	5392.3	129.3	4982.4	4469.3	89.7

*Theoretical concentration of spiked sample = 0.9x (mean baseline sample concentration) + spike value

** % Recovery = (Observed concentration of spiked sample mean / Theoretical concentration of spiked sample) x 100

3. Analytical Specificity/Interference: The interference testing of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated internally at ALPCO following CLSI guideline EP07-3rd Edition. The study was performed using seven samples, one very high positive stool sample, one high positive stool sample, one moderately high positive stool sample, one low positive stool sample, one stool sample in the indeterminate range, and two negative stool samples. Interfering substances were spiked into each stool sample at 10% of the total specimen volume. The solvent used to create the interferent stock was spiked into each stool sample at 10% of the total specimen volume to generate a control sample. Samples were run in singlicate. The results are summarized in the table below:

The interfering substances and concentrations tested were as follows:

Trade Name	**Active Component**	**Spiking Concentration**	**Recovery range**
Oral Pharmaceuticals			
Ferro-Gradumet	Iron (II) sulfate	0.02 mg/50 mg stool	97.9-102.6
Prednisone	Prednisone	0.065 mg/50 mg stool	98.0 -101.5
Imurek	Azathioprine	0.035 mg/50 mg stool	98.6-105.0
Pentasa/Asacol	Mesalamine; 5-ASA	1.0 mg/50 mg stool	91.2-103.0
Prevacid	Lansoprazol	0.035 mg/50 mg stool	98.1-104.5
Vancocin	Vancomycin	0.40 mg/50 mg stool	99.1-103.4
Sulfamethoxazole	Sulfamethoxazole	0.32 mg/50 mg stool	98.2-108.1
Trimethoprim	Trimethoprim lactate	0.065 mg/50 mg stool	98.7-105.7
Cipro	Ciprofloxacin	0.04 mg/50 mg stool	96.8-107.1
Nutritional Supplements			**Recovery range**
Vitamin E	DL-α Tocopherol Acetate	0.06 mg/50 mg stool	94.7-105.8
Multiple Vitamin	A, B1, B2, B3, B5, B6, B8, B9, B12, C, D, E, and minerals	0.215 mg/50 mg stool	98.2-105.1
Biotin	B7	1750 ng/50 mg stool	97.5–104.1
Microorganisms			**Recovery range**
Escherichia coli		1.5×10^7 cfu/mL	93.1-103.1
Salmonella enterica subsp. enterica		1.5×10^7 cfu/mL	97.5-105.5
Klebsiella pneumoniae subsp. pneumonia		1.5×10^7 cfu/mL	98.5-103.5
Citrobacter freundii		1.5×10^7 cfu/mL	97.9-103.0
Shigella flexneri		1.5×10^7 cfu/mL	91.0-105.8
Yersinia enterocolitica subsp. enterocolitica		1.5×10^7 cfu/mL	95.2-104.9
Other			**Recovery range**
Human anti-mouse antibody (HAMA)		0.5 µg/50 mg stool	94.9–106.6
Hemoglobin		0.25 mg/50 mg stool	95.1-105.4

4. Assay Reportable Range:
   The assay range is 7.9–6000µg/g.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Traceability: No international reference material or reference measurement procedures are available for calprotectin. Standard and control values are directly traceable to in-house reference standards made of recombinant calprotectin in a buffer containing stabilizers and preservatives.

   Antigen for calibrators and controls: The native calprotectin antigen is isolated from human neutrophils and obtained from commercial sources.

   Calibrators and controls: Calibrators and controls are manufactured by diluting native calprotectin antigen. The target concentrations of the standards and controls are as follows:

Material	Manufacturing Target Value
Standard A	10,000 µg/g
Standard B	2,500 µg/g
Standard C	625 µg/g
Standard D	156 µg/g
Standard E	40 µg/g
Standard F	20 µg/g
Standard G	5 µg/g
Standard H	0 µg/g
Control 1	20 - 40 µg/g
Control 2	80 - 125 µg/g
Control 3	1250 - 2500 µg/g

   Stability:

   Stability claims summary:

Stability Item	Storage Condition	Stability claim
Reagent Shelf Life Stability	2 – 8°C	18 months
Reagent Open Vial Stability	2 – 8°C	7 days
Reagent Transport Stability	Room temperature (28°C)	14 days
Unextracted Stool Sample Stability	2-8°C	14 days
	-20°C	14 days
	Freeze/Thaw Cycles	3 F/T cycles
Extracted Stool Sample Stability	2 – 8°C	3 days
	-80°C	14 days
	Freeze/Thaw Cycles	3 F/T cycles

6. Detection Limit:

Limit of Blank (LoB)
The limit of blank of the ALPCO Calprotectin Chemiluminescence ELISA was determined in accordance with CLSI EP17-A2. Four blank stool samples were extracted and tested in replicates of six on two reagent lots across three days. The LoB was estimated as the 95th percentile for the distribution of blank sample results. The LoB was determined to be 3.6 µg/g by the nonparametric method.

Limit of Detection (LoD)
The limit of detection of the ALPCO Calprotectin Chemiluminescence ELISA was determined in accordance with CLSI EP17-A2. Four stool samples containing a low level of calprotectin were extracted and tested in replicates of five on two reagent lots across three days. The LoD was determined to be 7.7 µg/g.

Limit of Quantitation (LoQ)
The limit of quantitation of the ALPCO Calprotectin Chemiluminescence ELISA was determined in accordance with CLSI EP17-A2. Six stool samples containing a low level of calprotectin were extracted and tested in replicates of five on two reagent lots across three days. The LoQ estimate for each reagent lot was determined as the measurand concentration at the intersection of the precision profile curve with the accuracy total within laboratory imprecision goal of 20 %CV. The maximum value obtained for each reagent lot was reported as the LoQ. The LoQ was determined to be 7.9 µg/g.

7. Assay Cut-Off:
To verify the low clinical cut-off value (50 µg/g), OPKO tested normal stool samples obtained from asymptomatic individuals with no abdominal complaints and no history of IBS, IBD or other chronic intestinal disorders. The ALPCO Calprotectin Chemiluminescence ELISA cut-offs are summarized in the table below:

Calprotectin Concentration	Interpretation	Follow-Up
< 50 µg/g	Normal	None
50 - 100 µg/g	Equivocal	Retest in 4-6 weeks
> 100 µg/g	Elevated	Repeat as clinically indicated

B Comparison Studies:

1. Method Comparison with Predicate Device:
A method comparison study was performed comparing the ALPCO Calprotectin Chemiluminescence ELISA and the predicate device. 400 samples that were used to determine the clinical performance characteristics of the ALPCO Calprotectin Chemiluminescence ELISA were tested on the predicate device and the subject device according to the manufacturer-supplied labeling. Testing using the predicate device was conducted at two U.S. sites. Results are calculated considering equivocal results as both positive and negative.

A qualitative agreement analysis between the ALPCO Calprotectin Chemiluminescence ELISA and the predicate device was conducted including all samples that were tested on both devices (N=400) using the cut-offs of both products to calculate the positive percent

agreement (PPA), negative percent agreement (NPA), and total percent agreement (TPA), and 95% CIs (Wilson score method) thereof considering equivocal results as both positive and negative. The results are as follows:

Qualitative Method Comparison with Predicate Device Data Summary:

	ALPCO Calprotectin Chemiluminescence ELISA			
Predicate	Normal	Equivocal	Abnormal	Total
Normal (<50 mg/kg)	229	5	12	246
Equivocal (50-120 mg/kg)	60	11	9	80
Abnormal (>20 mg/kg)	22	14	38	74
Total	311	30	59	400

Equivocal results considered positive (95% CI):

PPA	72/154	46.8% (39.0–54.6%)
NPA	229/246	93.1% (89.2–95.6%)
TPA	301/400	75.3% (70.8–79.2%)

Equivocal results considered negative (95% CI):

PPA	38/74	51.4% (40.2–62.4%)
NPA	305/326	93.6% (90.4–95.7%)
TPA	343/400	85.8% (82.0–88.8%)

An analytical method comparison was conducted including the samples that were within the analytical measuring range of both assays (N=169); results had to measure within 27.1–3000 mg/kg on the predicate device and 7.9–6000 µg/g on the ALPCO Calprotectin Chemiluminescence ELISA. A scatter plot was created by plotting the values obtained with the ALPCO Calprotectin Chemiluminescence ELISA (Y-axis) against the predicate device (X-axis) and analyzed by Passing-Bablok regression analysis. The slope, y-intercept, and correlation-r were determined. The 95% CI of the slope and intercept was determined using the bootstrap technique. The results are summarized in the table below:

N	169
Slope (95% CI)	0.6919 (0.5760, 0.8777)
Y-Intercept (95% CI)	-18.35 (-29.54, -11.13)
Correlation-r	0.784

2. Extraction Method Comparison:
The Easy Extraction method and manual weighing method of the ALPCO Calprotectin Chemiluminescence ELISA were evaluated to determine if the extraction methods provide similar results. The study was performed using one kit lot of ALPCO Calprotectin Chemiluminescence ELISA by one operator. Sixty-eight stool samples with varying consistency on the Bristol Scale and various fecal calprotectin concentrations across the reportable range and near the clinical cut-offs of the ALPCO Calprotectin Chemiluminescence ELISA were extracted in parallel using both the Easy Extraction method and manual weighing method. Results were analyzed in singlicate. 61 of the samples were within the AMR and included in the analysis. A scatter plot was created by plotting the values obtained from the Easy Extraction method extracts (Y-axis) against the manual weighing method extracts (X-axis) and analyzed by Passing-Bablok regression analysis. The

Y-Intercept, slope, and bootstrap 95% confidence intervals thereof, and correlation r were calculated. The results are as follows:

Easy Extraction method and manual weighing method Comparison:

N	61
Slope (95% CI)	1.014 (1.004, 1.031)
Y-Intercept (95% CI)	-1.296 (-1.986, -0.3179)
Correlation-r	0.997

Qualitative agreement analysis between the Easy Extraction method and manual weighing method was also conducted to calculate the positive percent agreement, negative percent agreement, and total percent agreement and Wilson 95% CI considering equivocal results as both positive and negative. The results are summarized below:

		Manual weighing method			
		Positive	Equivocal	Negative	Total
Easy Extraction method	Positive	25	0	0	25
	Equivocal	1	13	0	14
	Negative	0	0	22	22
	Total	26	13	22	61

Equivocal results considered positive (95% CI):
PPA 39/39 100% (91.0–100%)
NPA 22/22 100% (85.1–100%)
TPA 61/61 100% (94.1–100%)
Equivocal results considered negative (95% CI):
PPA 25/26 96.2% (81.1–99.3%)
NPA 35/35 100% (90.1–100%)
TPA 60/61 98.4% (91.3–99.7%)

C Clinical Studies

To evaluate the clinical performance of the ALPCO Calprotectin Chemiluminescence ELISA 424 samples were obtained from the intended use population. The following clinical samples were collected:

Clinical Diagnosis	Number of Subjects	Total
Inflammatory Bowel Disease (IBD)		76
Ulcerative Colitis (UC)	34	
Crohn's Disease (CD)	30	
Indeterminant/Undefined	12	
Irritable Bowel Syndrome (IBS)	122	122
Other GI conditions	226	226
Total		424

Clinical Sensitivity/Clinical Specificity:
The estimates of clinical sensitivity, clinical specificity, positive predictive value (PPV), and negative predictive value (NPV) of the ALPCO Calprotectin Chemiluminescence ELISA were

determined by comparing analytical test results of the prospectively collected stool specimens against the clinical diagnosis made by the clinical investigator/gastroenterologist (reference standard):

- IBD diagnosis was based on endoscopy results and/or histology of biopsies taken during the endoscopy.
- IBS diagnosis was based on the Rome IV criteria and confirmed by negative endoscopy including the colon and terminal ileum.
- Subjects were diagnosed with "Other GI conditions" when they did not meet the diagnostic criteria for IBD and IBS (Rome IV).

The results are summarized below:

Clinical Diagnosis	Number of Results in ALPCO Calprotectin Chemiluminescence ELISA Range			Total
	<50 µg/g	50–100 µg/g	>100 µg/g	
IBD	6	20	50	76
IBS	116	4	2	122
GI Other	206	9	11	226
Total	328	33	63	424

IBD vs. Clinical Diagnosis:
The estimates of sensitivity, specificity, PPV, and NPV were calculated considering equivocal results as both positive and negative:

Equivocal results as positive

Equivocal results as positive	ALPCO Test Result		Total (N)
Clinical Diagnosis Results	>50 µg/g)	≤50 µg/g)	
IBD	70	6	76
Non-IBD	26	322	348
Total	96	328	424
Sensitivity: 70/76, 92.1%, 95%CI (83.8–96.3) Specificity: 322/348, 92.5%, 95%CI (89.3–94.9) PPV: 70/96, 72.9%, 95%CI (64.9–79.7) NPV: 322/328, 98.2, 95%CI (96.1–99.1)			

Equivocal results as negative

Equivocal results negative	ALPCO Test Result		Total (N)
Clinical Diagnosis results	100 µg/g	≤100 µg/g	
IBD	50	26	76
Non-IBD	13	335	348
Total	63	361	424
Sensitivity: 50/76, 65.8%, 95%CI (54.6–75.5) Specificity: 335/348, 96.3%, 95%CI (93.7–97.8) PPV: 50/63 79.4%, 95%CI (68.8–87.0) NPV: 335/361, 92.8%, 95%CI (90.4–94.6)			

IBD versus IBS

The estimates of sensitivity, specificity, PPV, and NPV were calculated considering equivocal results as both positive and negative:

Equivocal results as positive:

Equivocal results As positive	ALPCO Test Result		Total (N)
Clinical Diagnosis Results	>50	≤50	
IBD	70	6	76
IBS	6	116	122
Total	76	328	198
Sensitivity: 70/76, 92.1%, 95%CI (83.8–96.3) Specificity: 116/122, 95.1%, 95%CI (89.7–97.7) PPV: 70/76, 92.1%, 95%CI (84.2–96.2) NPV: 116/122, 95.1%, 95%CI (90.0–97.7)			

Equivocal results as Negative:

Equivocal results as negative	ALPCO Test Result		Total (N)
Clinical Diagnosis Results	>100	≤100	
IBD	50	26	76
IBS	2	120	122
Total	52	146	198
Sensitivity: 50/76, 65.8%, 95%CI (54.6–75.5) Specificity: 120/122, 98.4%, 95%CI (94.2–99.5) PPV: 50/52, 96.2%, 95%CI (86.2–99.0) NPV: 120/146, 82.2%, 95%CI (77.1–86.3)			

Other Clinical Supportive Data

Not applicable

D Clinical Cut-Off:

See assay cut-off (Section A.7)

E Expected Values/Reference Range:

To verify the low clinical cut-off value (50 µg/g), normal stool samples were obtained from asymptomatic individuals with no abdominal complaints and no history of IBS, IBD or other chronic intestinal disorders. This study cohort was separate from those used to establish estimates of the clinical performance of the test device. 130/131 of the samples were normal/negative with the ALPCO Calprotectin Chemiluminescence ELISA. Values ranged from <7.9 µg/g to 75.1 µg/g, with 86/131 samples measuring below the lower end of the AMR.

The 90% confidence intervals for the lower and upper 95% reference limits of the 131 healthy individuals were determined by the non-parametric quantile method, in accordance with CLSI guidline EP28-A3c. The results are as follows:

Lower Limit (90% CI): 0.7 µg/g (0.5–0.9 µg/g)

Upper Limit (90% CI): 37.7 µg/g (30.9–75.1 µg/g)

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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