October 25, 2019

ALPCO
Jeffrey Freedman
Regulatory Affairs Specialist
26 Keewaydin Drive, Unit G
Salem, New Hampshire 03079

Re: K191807
Trade/Device Name: ALPCO Calprotectin Chemiluminescence ELISA, ALPCO Easy Stool Extraction Device
Regulation Number: 21 CFR 866.5180
Regulation Name: Fecal Calprotectin Immunological Test System
Regulatory Class: Class II
Product Code: NXO
Dated: July 3, 2019
Received: July 5, 2019

Dear Jeffrey Freedman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Douglas Jeffery, Ph.D.
Chief
Division of Immunology
and Hematology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K191807

Device Name
ALPCO Calprotectin Chemiluminescence ELISA
ALPCO Easy Stool Extraction Device

Indications for Use *(Describe)*

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 is intended for use in the preparation of human stool specimens for testing in the ALPCO Calprotectin Chemiluminescence ELISA.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

Date of Summary: October 22, 2019

Product Name: ALPCO Calprotectin Chemiluminescence ELISA;
ALPCO Easy Stool Extraction Device

Sponsor:
ALPCO
26 Keewaydin Drive, Unit G
Salem, NH 03079

Correspondent:
Jeffrey Freedman, Regulatory Affairs Specialist
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Salem, NH 03079
Phone: (603)893-8914
Fax: (603)898-6854

Common Name:
Fecal calprotectin immunological test system

Regulation Number:
21 CFR 866.5180

Classification:
NXO, Class II

Predicate Device:
Calprest®NG (QUANTA Lite® Calprotectin Extended Range ELISA), 510(k) number: K160447

Intended 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.

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.

Methodology

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) highly specific to the calprotectin heterodimeric and polymeric complexes, respectively, 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.

Substantial Equivalency & Comparison with Predicate

The ALPCO Calprotectin Chemiluminescence ELISA has the same intended use and assay principle as the predicate device. A comparison of the similarities and differences between the devices are provided in the following tables:

Characteristic	**ALPCO** **ALPCO Calprotectin Chemiluminescence ELISA** (New Device)	**Eurospital S.p.A.** **Calprest®NG** K160447 (Predicate Device)
Similarities		
Intended 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.
Analyte	Fecal Calprotectin	Same
Assay Format	Quantitative	Same
Platform	96 well microtiter plate	Same

Characteristic	**ALPCO** **ALPCO Calprotectin Chemiluminescence ELISA** (New Device)	**Eurospital S.p.A.** **Calprest®NG** K160447 (Predicate Device)
Detection antibody	Monoclonal anti-calprotectin antibody	Same
Assay process	Manual	Same
Differences		
Capture Antibody	Monoclonal anti-calprotectin antibody	Polyclonal anti-calprotectin antibody
Analytical Measuring Range	7.9 – 6000 µg/g	27.1-3000.0 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 ng/mL
Controls	3 levels	2 levels
Calibrator/Control Analyte	Native human calprotectin	Recombinant calprotectin antigen (rAg)
Calibrator/Control Physio-chemical Characteristics	Lyophilized	Ready to use
Sample Dilution	1:25000	1:20000
Stop Solution	None	H_2SO_4
Substrate	Luminol	TMB
Incubation Time	30-30-30-5 minutes at room temp	60-30-15 minutes at room temp
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

Standard 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-ED3 Interference Testing in Clinical Chemistry, Third 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 – Third Edition

Performance Data

Precision/Reproducibility Studies

Precision

The precision of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated internally at ALPCO in accordance with CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures. The study was performed using one (1) kit lot of ALPCO Calprotectin Chemiluminescence ELISA using one (1) ELISA reader by one (1) operator. Eight (8) stool samples containing various fecal calprotectin concentrations covering a significant portion of the reportable range of the ALPCO Calprotectin Chemiluminescence ELISA were extracted using the Easy Extraction method and the resultant extracts were analyzed in duplicate, twice per day, for 20 days to generate a total of 80 replicates per sample. Data were analyzed using Analyse-it Method Validation edition and acceptance criteria were met. 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	288.7	9.5	3.3%	12.3	4.2%	15.5	5.4%	8.6	3.0%	17.7	6.1%
2	80	5323.5	220.0	4.1%	359.8	6.8%	421.7	7.9%	410.8	7.7%	588.8	11.1%
3	80	680.3	19.7	2.9%	26.2	3.8%	32.8	4.8%	29.2	4.3%	43.9	6.4%
4	80	908.3	25.7	2.8%	29.2	3.2%	38.9	4.3%	56.6	6.2%	68.7	7.6%
5	80	116.6	3.0	2.6%	4.3	3.6%	5.2	4.5%	4.2	3.6%	6.7	5.7%
6	80	32.9	1.3	3.9%	1.2	3.7%	1.8	5.3%	2.9	8.9%	3.4	10.3%
7	80	82.2	3.7	4.5%	3.3	4.0%	4.9	6.0%	3.8	4.6%	6.2	7.6%
8	80	39.1	1.8	4.7%	1.6	4.0%	2.4	6.1%	2.8	7.2%	3.7	9.5%

Lot-to-Lot Reproducibility

The lot-to-lot reproducibility of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated internally at ALPCO in accordance with CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures. The study was performed using three (3) kit lots of ALPCO Calprotectin Chemiluminescence ELISA using one (1) ELISA reader by one (1) operator. Seven (7) stool samples containing various fecal calprotectin concentrations covering a fraction of the

reportable range of the ALPCO Calprotectin Chemiluminescence ELISA 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. Data were analyzed using Analyse-it Method Validation edition and acceptance criteria were met. 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	288.6	8.5	2.9%	16.5	5.7%	18.5	6.4%	17.2	5.9%	25.3	8.8%
2	75	4480.2	321.3	7.2%	445.1	9.9%	548.9	12.3%	298.0	6.7%	624.6	13.9%
3	75	684.2	23.3	3.4%	45.1	6.6%	50.8	7.4%	49.6	7.2%	71.0	10.4%
4	75	919.2	38.1	4.1%	72.3	7.9%	81.7	8.9%	60.8	6.6%	101.9	11.1%
5	75	110.8	7.7	6.9%	7.3	6.6%	10.6	9.5%	7.2	6.5%	12.8	11.5%
6	75	35.4	1.8	5.2%	2.1	6.0%	2.8	8.0%	1.8	5.2%	3.4	9.5%
7	75	76.7	3.4	4.4%	4.5	5.9%	5.6	7.3%	1.7	2.2%	5.9	7.7%

Site-to-Site Reproducibility

The site-to-site reproducibility of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated internally at ALPCO and at two other analytical test sites in accordance with CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures. The study was performed using one (1) kit lot of ALPCO Calprotectin Chemiluminescence ELISA by one (1) operator per test site. Seven (7) stool samples containing various fecal calprotectin concentrations of the reportable range of the ALPCO Calprotectin Chemiluminescence ELISA 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 5, once per day, for 5 days at each site to generate 25 replicates per site and a total of 75 replicates per sample. Data were analyzed using Analyse-it Method Validation edition and acceptance criteria were met. 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	46.3	2.6	5.6%	3.7	8.1%	4.5	9.8%	0.7	1.6%	4.6	9.9%
2	75	195.2	8.9	4.6%	21.6	11.0%	23.3	12.0%	0.0	0.0%	23.3	12.0%
3	75	20.0	2.4	12.1%	1.7	8.6%	3.0	14.9%	0.3	1.4%	3.0	15.0%
4	75	446.5	21.5	4.8%	27.6	6.2%	35.0	7.8%	0.0	0.0%	35.0	7.8%
5	75	138.2	10.1	7.3%	15.8	11.4%	18.7	13.5%	0.0	0.0%	18.7	13.5%
6	75	21.1	2.2	10.4%	1.7	7.9%	2.8	13.1%	0.0	0.0%	2.8	13.1%
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 Reproducibility

The operator-to-operator reproducibility of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated internally at ALPCO in accordance with CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures. The study was performed using one (1) kit lot of ALPCO Calprotectin Chemiluminescence ELISA by three (3) operators. Seven (7) stool samples containing various fecal calprotectin concentrations covering a significant portion of the reportable range of the ALPCO Calprotectin Chemiluminescence ELISA were extracted using the Easy Extraction method and analyzed by each operator independently in replicates of 5, once per day, for 5 days to generate 25 replicates per sample, per operator and a total of 75 replicates per sample. Data were analyzed using Analyse-it Method Validation edition and acceptance criteria were met. The results are as follows:

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

Extraction Method Reproducibility

The extraction reproducibility of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated internally at ALPCO. The study was performed using one (1) kit lot of ALPCO Calprotectin Chemiluminescence ELISA by one (1) operator. Sets of seven (7) stool samples containing various fecal calprotectin concentrations, including stool samples that are near the clinical cut-offs of the ALPCO Calprotectin Chemiluminescence ELISA 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. Data were analyzed using Analyse-it Method Validation edition and acceptance criteria were met. The results are as follows:

Easy 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	129.0	1.8	1.4%	6.2	4.8%	6.5	5.0%
2	20	45.9	4.6	9.9%	2.8	6.1%	5.3	11.6%
3	20	4298.5	317.2	7.4%	426.9	9.9%	531.8	12.4%
4	20	123.7	6.9	5.6%	3.9	3.1%	7.9	6.4%
5	20	1748.5	186.5	10.7%	121.4	6.9%	222.6	12.7%
6	20	68.3	3.5	5.1%	3.2	4.7%	4.8	7.0%
7	20	33.4	1.7	5.1%	0.0	0.0%	1.7	5.1%

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	4063.3	389.2	9.6%	0.0	0.0%	389.2	9.6%
2	20	1336.1	42.4	3.2%	113.5	8.5%	121.2	9.1%
3	20	122.0	5.1	4.1%	8.9	7.3%	10.2	8.4%
4	20	25.7	1.1	4.5%	1.6	6.4%	2.0	7.8%
5	20	103.7	2.5	2.4%	3.7	3.6%	4.5	4.3%
6	20	69.6	3.2	4.6%	0.3	0.5%	3.2	4.6%
7	20	298.6	13.5	4.5%	26.2	8.8%	29.5	9.9%

Analytical Sensitivity Studies

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 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 by the parametric method.

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 mean and

within laboratory precision were calculated for each sample for each reagent lot. For each reagent lot, the observed precision (%CV) (Y-axis) was plotted vs. the sample calprotectin concentration (X-axis) to give a precision profile, and fit by a constant variance function using Analyse-it Method Validation edition. The LoQ estimate for each reagent lot was determined as the measurand concentration at the intersection of the precision profile curve with the accuracy goal of 20 %CV. The LoQ was determined to be 7.9 µg/g.

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, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. 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 entire AMR. Stool sample extracts were obtained using the Easy Extraction method. Each stool sample extract combination was assayed in duplicate in a single analysis using one reagent lot of the ALPCO Calprotectin Chemiluminescence ELISA. 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 with standard diluent buffer to obtain dilution levels with values that cover the entire AMR. Each aqueous sample dilution was assayed in duplicate in a single analysis using one reagent lot of the ALPCO Calprotectin Chemiluminescence ELISA. Results were analyzed using Analyse-it Method Validation edition to determine the best fitting polynomial. For linearity to be claimed, the best fitting polynomial had to be linear (first order) or the difference between the best fitting nonlinear polynomial (second or third order) and the linear polynomial could not exceed ±15%. The plots were further analyzed by linear regression. The slope and y-intercept, and the 95% confidence intervals thereof, and R^2 of the regression analysis were calculated. In addition, the recovery of each replicate value included in the regression analysis for the linearity dilutions was calculated and the average recovery was calculated. Acceptance criteria were met, the ALPCO Calprotectin Chemiluminescence ELISA is acceptably linear over the analytical measuring range. The results are as follows:

Sample Type	Test Range (µg/g)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average Recovery (%)
Matrix	2.5 – 5780.1	1.003 (0.994 to 1.013)	1.77 (-15.87 to 19.42)	1.00	99.2
Aqueous	3.0 – 6221.7	0.99 (0.98 to 1.00)	0.03 (-22.42 to 22.47)	0.99	100.6

Accuracy/Recovery

The accuracy/recovery of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated using seven (7) extracted stool samples containing various concentrations of calprotectin across the analytical measuring range of the assay, samples were extracted using the Easy Extraction

method. Native calprotectin diluted in standard diluent was used as the spiking material. The stool sample extracts were mixed with the spiking material in a proportion of 9:1 (9 parts sample:1 part spiking material) to calculate recovery: Samples with calprotectin concentrations lower than 200 µg/g were spiked with 42.8 µg/g. Samples with calprotectin concentrations higher than 200 µg/g were spiked with 129.3 µg/g. Each baseline sample extract, control spike and spiked sample were tested in duplicate in the same assay run. Recovery was calculated compared to the baseline result and acceptance criteria were met. 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 (%)
1	248.5	129.3	353.0	391.0	110.8
2	5392.3	129.3	4982.4	4469.3	89.7
3	615.5	129.3	683.2	754.1	110.4
4	94.8	42.8	128.1	123.5	96.4
5	28.1	42.8	68.1	72.1	105.9
6	77.7	42.8	112.7	123.6	109.7
7	36.2	42.8	75.3	79.0	104.9

Interfering Substances

The interference testing of the ALPCO Calprotectin Chemiluminescence ELISA was evaluated internally at ALPCO using CLSI EP07-ED3, Interference Testing in Clinical Chemistry as a guideline. The study was performed using seven stool samples containing various concentrations of fecal calprotectin. 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. The stool sample containing the interferents and the control stool sample were extracted using the Easy Extraction method. Recovery of the test samples ranged from 91.0 – 108.1%, no interference was observed at the concentrations tested. The list of interferents and the concentrations tested are as follows:

Oral Pharmaceuticals		
Trade Name	**Active Component**	**Spiking Concentration**
Ferro-Gradumet	Iron (II) sulfate	0.02 mg/50 mg stool
Prednisone	Prednisone	0.065 mg/50 mg stool
Imurek	Azathioprine	0.035 mg/50 mg stool
Pentasa/Asacol	Mesalamine; 5-ASA	1.0 mg/50 mg stool
Prevacid	Lansoprazol	0.035 mg/50 mg stool
Vancocin	Vancomycin	0.40 mg/50 mg stool
Sulfamethoxazole	Sulfamethoxazole	0.32 mg/50 mg stool
Trimethoprim	Trimethoprim lactate	0.065 mg/50 mg stool
Cipro	Ciprofloxacin	0.04 mg/50 mg stool
Nutritional Supplements		
Trade Name	**Active Component**	**Spiking Concentration**
Vitamin E	DL-α Tocopherol Acetate	0.06 mg/50 mg stool
Multiple Vitamin	A, B1, B2, B3, B5, B6, B8, B9, B12, C, D, E, and minerals	0.215 mg/50 mg stool
Biotin	B7	1750 ng/50 mg stool
Other		
Human anti-mouse antibody (HAMA)		0.5 µg/50 mg stool
Hemoglobin		0.25 mg/50 mg stool
Microorganisms		
Escherichia coli		1.5×10^7 cfu/mL
Salmonella enterica subsp. enterica		1.5×10^7 cfu/mL
Klebsiella pneumoniae subsp. pneumonia		1.5×10^7 cfu/mL
Citrobacter freundii		1.5×10^7 cfu/mL
Shigella flexneri		1.5×10^7 cfu/mL
Yersinia enterocolitica subsp. enterocolitica		1.5×10^7 cfu/mL

Product Stability

Real-Time Reagent Stability Summary – The ALPCO Calprotectin Chemiluminescence ELISA reagents are stable for 18 months at 2-8°C.

Open Vial Stability Summary – The ALPCO Calprotectin Chemiluminescence ELISA reagents are stable for 7 days at 2-8°C once opened.

Transport Stability Summary - The ALPCO Calprotectin Chemiluminescence ELISA reagents are stable at 28°C for 14 days.

Raw Stool Sample Stability

Storage Conditions	Stability Duration
2-8°C	14 days
-20°C	14 days
Freeze/Thaw Cycles	3 cycles

Stool Sample Extract Stability

Storage Conditions	Stability Duration
2-8°C	3 days
-80°C	14 days
Freeze/Thaw Cycles	3 cycles

Stool Sample Extraction Method Comparison

The extraction methods of the ALPCO Calprotectin Chemiluminescence ELISA were evaluated internally at ALPCO to determine if the extraction methods provide similar results. The study was performed using one (1) kit lot of ALPCO Calprotectin Chemiluminescence ELISA by one (1) operator. Sixty-eight (68) stool samples varying in consistency and containing various fecal calprotectin concentrations over a significant fraction of 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. Each resultant stool sample extract was analyzed in singlicate. 61 of the samples were within the AMR of the ALPCO Calprotectin Chemiluminescence ELISA 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 using Analyse-it Method Validation Edition. The Y-Intercept, slope, and bootstrap 95% confidence intervals thereof, and correlation r were calculated. The results are as follows:

Stool Sample Extraction Analytical Method Comparison	
N	61
Slope (95% CI)	1.014 (1.004 to 1.031)
Y-Intercept (95% CI)	-1.296 (-1.986 to -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 as follows:

Stool Sample Extraction Qualitative Method Comparison

		Manual weighing method			Totals
		Positive	Equivocal	Negative	
Easy Extraction method	Positive	25	0	0	25
	Equivocal	1	13	0	14
	Negative	0	0	22	22
	Totals	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%)	

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 according to the manufacturer-supplied labeling.

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:

	ALPCO Calprotectin Chemiluminescence ELISA			
Predicate	Normal (< 50 µg/g)	Equivocal (50 – 100 µg/g)	Abnormal (> 100 µg/g)	Total
Normal (< 50 mg/kg)	229	5	12	246
Equivocal (50 – 120 mg/kg)	60	11	9	80
Abnormal (> 120 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 using Analyse-it Method Validation Edition. 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 as follows:

Analytical Method Comparison with Predicate Device	
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

Clinical Performance

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 or IBS (Rome IV).

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

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

Estimates of sensitivity, specificity, PPV, and NPV, along with 95% confidence intervals (Wilson score method for sensitivity/specificity and Mercaldo-Wald logit method for predictive value) were calculated for the ALPCO Calprotectin Chemiluminescence ELISA as an aid in the diagnosis of IBD (n = 424). The estimates of sensitivity, specificity, PPV, and NPV were calculated considering equivocal results as both positive and negative:

Equivocal results = positive	**ALPCO Test Result**		
Clinical Diagnosis	> 50	<= 50	Total
IBD	70	6	76
Non-IBD	26	322	348
Total	96	328	424
	Fraction	**%**	**95% CI**
Sensitivity	70/76	92.1	83.8 – 96.3
Specificity	322/348	92.5	89.3 – 94.9
PPV	70/96	72.9	64.9 – 79.7
NPV	322/328	98.2	96.1 – 99.1

Equivocal results = negative	**ALPCO Test Result**		
Clinical Diagnosis	> 100	<= 100	Total
IBD	50	26	76
Non-IBD	13	335	348
Total	63	361	424
	Fraction	**%**	**95% CI**
Sensitivity	50/76	65.8	54.6 – 75.5
Specificity	335/348	96.3	93.7 – 97.8
PPV	50/63	79.4	68.8 – 87.0
NPV	335/361	92.8	90.4 – 94.6

Estimates of sensitivity, specificity, PPV, and NPV, along with 95% confidence intervals (Wilson score method for sensitivity/specificity and Mercaldo-Wald logit method for predictive value) were calculated for the ALPCO Calprotectin Chemiluminescence ELISA as an aid in the differentiation of IBD vs IBS (n = 198). The estimates of sensitivity, specificity, PPV, and NPV were calculated considering equivocal results as both positive and negative:

Equivocal results = positive	**ALPCO Test Result**		
Clinical Diagnosis	> 50	<= 50	Total
IBD	70	6	76
IBS	6	116	122
Total	76	122	198
	Fraction	**%**	**95% CI**
Sensitivity	70/76	92.1	83.8 – 96.3
Specificity	116/122	95.1	89.7 – 97.7
PPV	70/76	92.1	84.2 – 96.2
NPV	116/122	95.1	90.0 – 97.7

Equivocal results = negative	**ALPCO Test Result**		
Clinical Diagnosis	> 100	<= 100	Total
IBD	50	26	76
IBS	2	120	122
Total	52	146	198
	Fraction	**%**	**95% CI**
Sensitivity	50/76	65.8	54.6 – 75.5
Specificity	120/122	98.4	94.2 – 99.5
PPV	50/52	96.2	86.2 – 99.0
NPV	120/146	82.2	77.1 – 86.3

Expected Values

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. The expected result for the "normal"/asymptomatic population is < 50 µg/g (normal). Calprotectin levels were analyzed using the ALPCO Calprotectin Chemiluminescence ELISA on 131 samples obtained from apparently healthy individuals with the following demographics:

- Gender: 65 females / 66 males
- Age: 22 to 82 years old, mean age 42.4 years

With a cut-off of 50 µg/g, 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, (N+1)p in accordance with CLSI EP28-A3c, *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, 3rd Edition* using Analyse-it Method Validation Edition. 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)