# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY
ASSAY ONLY

## I Background Information:

### A 510(k) Number

K220763

### B Applicant

ALPCO

### C Proprietary and Established Names

ALPCO Calprotectin Immunoturbidimetric Assay

### 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, immunoturbidimetric assay

## III Intended Use/Indications for Use:

**A Intended Use(s):**

See Indications for Use below.

**B Indication(s) for Use:**

The ALPCO Calprotectin Immunoturbidimetric Assay is an in-vitro diagnostic assay used for the quantitative measurement of human fecal calprotectin in human stool. The ALPCO Calprotectin Immunoturbidimetric Assay is intended for in-vitro diagnostic use as an aid in 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.

**C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only

**D Special Instrument Requirements:**

Beckman AU680 analyzer

## IV Device/System Characteristics:

**A Device Description:**

The ALPCO Calprotectin Immunoturbidimetric Assay consists of the following materials:

Materials provided (100 tests):

- Reagent 1: Tris buffer solution with <0.1% sodium azide, ready to use, 1 x 22.5 mL
- Reagent 2: Suspension of latex particles (< 0.5%) coated with anti-calprotectin antibodies with <0.1% sodium azide, ready to use, 1 x 5.3 mL

Materials Required but not Provided:

*The ALPCO Calprotectin Calibrator Set*

5 levels: 50, 200, 500, 800, 1050 µg/g, 5 x 1 mL, sold separately

*The Calprotectin Immunoturbidimetric Assay Control Set*

2 levels: Control 1: 35 µg/g; Control 2: 135 µg/g), 2 x 1 mL, sold separately

*Feces sample collection*

1) Sample collection tube
2) Transport container

*Feces preparation:*

The ALPCO Calprotectin Immunoturbidimetric Assay can prepare fecal samples by ALPCO Easy Stool Extraction Device (sold separately) or manual weighing method.

*Instrument*:
Beckman AU680 analyzer

**B Principle of Operation:**

The ALPCO Calprotectin Immunoturbidimetric Assay is a particle-enhanced immunoturbidimetric assay. Calprotectin in the sample binds to anti-calprotectin antibodies immobilized on the latex particles, which causes agglutination. This agglutination is detected as an absorbance change and the amount of agglutination is directly proportional to the concentration of calprotectin in the sample. The concentration is determined by interpolation from a calibration curve prepared from calibrators of known concentration.

The fecal samples can be processed and extracted using ALPCO Easy Stool Extraction Device or manual weighing method.

The ALPCO Easy Stool Extraction Device should not be used for the extraction of liquid/watery stool samples. Refer to K191807 for the fecal specimen preparation using the ALPCO Easy Stool Extraction Device.

For manual weighing method, 50 to 100 mg of the stool sample is taken by the inoculation loop and placed it into the pre-weighed tube. The sample is weighed and Extraction Buffer is added (99 times the sample weight volume). Further, the sample is homogenized on a multi-tube vortex by vigorous shaking (at highest speed) for 30 minutes. Next, the sample is centrifuged for 5 minutes at 3000 x g. Finally, decant the supernatant into a fresh labeled tube and continue with the assay procedure or store the extracts at 2–8°C for up to 3 days or at -80°C for up to 14 days.

Results are reported in µg/g of calprotectin in stool. Interpretation of results is shown as follows:

| 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 |

**V Substantial Equivalence Information:**

**A Predicate Device Name(s):**

ALPCO Calprotectin Chemiluminescence ELISA, ALPCO Easy Stool Extraction Device

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

K191807

**C Comparison with Predicate(s):**

| Device & Predicate Device(s): | K220763 (New Device) | K191807 (Predicate) |
|---|---|---|
| Device Trade Name | ALPCO Calprotectin Immunoturbidimetric Assay | ALPCO Calprotectin Chemiluminescence ELISA |
| **General Device Characteristic Similarities** | | |
| Intended Use / Indications For Use | The ALPCO Calprotectin Immunoturbidimetric Assay is an in-vitro diagnostic assay used for the quantitative measurement of human fecal calprotectin in human stool. The ALPCO Calprotectin Immunoturbidimetric Assay is intended for in-vitro diagnostic use as an aid in 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 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. |
| Analyte | Fecal Calprotectin | Same |
| Type of Test | Quantitative | Same |
| Sample Matrix | Human stool | Same |
| Extraction Procedure | ALPCO Easy Stool Extraction Device or manual weighting method | Same |
| Calibrator / Control Analyte | Native human calprotectin | Same |
| Results Interpretation | Normal: < 50 µg/g<br>Equivocal: 50–100 µg/g<br>Abnormal: >100 µg/g | Same |
| **General Device Characteristic Differences** | | |
| Methodology | Immunoturbidimetric Assay | Chemiluminescence ELISA |
| Analytical Measuring Interval (AMI) | 11.0–1000.0 µg/g | 7.9–6000 µg/g |
| Assay Process | Automated | Manual ELISA |
| Sample Dilution | 1:100 | 1:25000 |
| Instrument | Beckman AU680 analyzer | Not applicable |
| Capture Antibody | Mouse monoclonal IgG antibodies immobilized on latex particles | Mouse monoclonal IgG antibodies immobilized on plate |

| | | |
|---|---|---|
| Detection Antibody | Not applicable | Biotinylated mouse monoclonal anti-calprotectin antibody |
| Calibrators | Ready to use<br>5 levels: 50, 200, 500, 800, 1050 µg/g | Lyophilized<br>8 levels: 0, 5, 20, 40, 156, 625, 2500, 10000 µg/g |
| Controls | Ready to use 2 levels:<br>Control 1: 35 µg/g<br>Control 2: 135 µg/g | Lyophilized, 3 levels:<br>Control 1: 20–40 µg/g<br>Control 2: 80–125 µg/g<br>Control 3: 1250–2500 µg/g |
| Shelf-Life (Kit) | 2–8°C for 24 months | 2–8°C for 18 months |

## VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures, Approved Guideline – Third Edition
- CLSI EP06-Ed2, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07, 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, Approved Guideline – Third Edition
- Class II Special Controls Guidance Document: Fecal Calprotectin Immunological Test Systems - Guidance for Industry and FDA Staff, July (2006)

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

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

1. Precision/Reproducibility:

*Within-Laboratory Precision*

A study was conducted in accordance with CLSI EP05-A3 to evaluate repeatability and within-lab imprecision of the ALPCO Calprotectin Immunoturbidimetric Assay. The study was performed at one site/laboratory, using one reagent lot and calibrators, one Beckman Coulter AU680 analyzer, and one operator. Seven stool extracts were prepared using the ALPCO Easy Stool Extraction Device from seven human stool samples. The extracts were analyzed in duplicate per run, two runs per day, for 20 days to generate a total of 80 replicates per sample. The results (within-run, between-run, between-day, and within-laboratory) are shown in the following table:

| Sample | N | Mean (µg/g) | Within-run | | Between-run | | Between-day | | Within-Lab | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 80 | 14.4 | 1.7 | 12.1 | 1.5 | 10.2 | 1.4 | 9.9 | 2.2 | 15.0 |
| 2 | 80 | 37.6 | 2.2 | 5.9 | 1.9 | 5.1 | 1.5 | 3.9 | 2.5 | 6.7 |
| 3 | 80 | 81.3 | 2.1 | 2.6 | 2.2 | 2.7 | 2.3 | 2.8 | 3.1 | 3.9 |
| 4 | 80 | 173.8 | 3.3 | 1.9 | 4.2 | 2.4 | 6.5 | 3.7 | 7.5 | 4.3 |
| 5 | 80 | 414.5 | 4.2 | 1.0 | 6.7 | 1.6 | 9.2 | 2.2 | 10.8 | 2.6 |
| 6 | 80 | 622.5 | 5.5 | 0.9 | 6.8 | 1.1 | 13.8 | 2.2 | 15.1 | 2.4 |
| 7 | 80 | 854.8 | 9.0 | 1.1 | 9.5 | 1.1 | 12.5 | 1.5 | 15.6 | 1.8 |

*Lot-to-Lot Precision*

The lot-to-lot imprecision of the ALPCO Calprotectin Immunoturbidimetric Assay was evaluated in accordance with CLSI EP05-A3. The study was performed using three reagent lots and one lot of calibrators, one AU680 analyzer, at one site, by one operator. Seven human stool samples containing various fecal calprotectin concentrations were extracted using the ALPCO Easy Stool Extraction Device and analyzed in five replicates, once per day, for 5 days to generate a total of 75 replicates per sample. The results are summarized in the following table:

| Sample | N | Mean (µg/g) | Within-run | | Between-Day | | Between-Lot | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 75 | 14.3 | 1.8 | 12.3 | 1.5 | 10.3 | 1.6 | 11.3 | 2.8 | 19.6 |
| 2 | 75 | 38.4 | 1.9 | 4.9 | 1.4 | 3.6 | 1.8 | 4.6 | 2.9 | 7.6 |
| 3 | 75 | 86.0 | 2.6 | 3.1 | 2.5 | 2.9 | 4.0 | 4.6 | 5.4 | 6.2 |
| 4 | 75 | 173.4 | 3.6 | 2.1 | 3.9 | 2.3 | 5.0 | 2.9 | 7.3 | 4.2 |
| 5 | 75 | 416.5 | 5.0 | 1.2 | 7.8 | 1.9 | 10.1 | 2.4 | 13.8 | 3.3 |
| 6 | 75 | 626.3 | 7.1 | 1.1 | 9.5 | 1.5 | 11.4 | 1.8 | 16.5 | 2.6 |
| 7 | 75 | 869.8 | 7.5 | 0.9 | 8.9 | 1.0 | 11.3 | 1.3 | 16.3 | 1.9 |

*Site-to-Site Reproducibility*

The study was performed using one reagent lot and one lot of calibrators, three AU680 analyzers at three sites, and three operators (one operator per instrument/site). Seven stool samples containing various fecal calprotectin concentrations were extracted using the ALPCO Easy Stool Extraction Device and analyzed in five replicates per run, one run per day, for 5 days to generate a total of 75 replicates per sample. The results are reported in the following tables:

| Sample | N | Mean (µg/g) | Within-run | | Between-Day | | Between-Site | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 75 | 11.2 | 1.8 | 15.7 | 1.7 | 14.9 | 1.8 | 16.2 | 3.0 | 27.1 |
| 2 | 75 | 35.0 | 2.1 | 6.0 | 1.7 | 4.8 | 3.2 | 9.2 | 4.2 | 11.9 |
| 3 | 75 | 77.3 | 3.2 | 4.1 | 2.6 | 3.3 | 3.3 | 4.3 | 5.3 | 6.8 |
| 4 | 75 | 168.5 | 2.9 | 1.7 | 6.1 | 3.6 | 6.7 | 4.0 | 9.5 | 5.6 |
| 5 | 75 | 445.7 | 4.7 | 1.0 | 5.8 | 1.3 | 9.6 | 2.2 | 12.2 | 2.7 |

| Sample | N | Mean (µg/g) | Within-run | | Between-Day | | Between-Site | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 6 | 75 | 643.8 | 6.4 | 1.0 | 10.9 | 1.7 | 8.9 | 1.4 | 15.5 | 2.4 |
| 7 | 75 | 782.2 | 14.3 | 1.8 | 15.2 | 1.9 | 21.2 | 2.7 | 29.7 | 3.8 |

*Extraction Precision*:

Refer to K191807 for extraction device precision using the ALPCO Easy Stool Extraction Device.

2. Linearity:

The linearity study was performed following CLSI EP06-Ed2 using two sample panels. Sample Panel 1 was prepared by dilution of a high native sample pool (576.3 µg/g) with a low native sample pool (9.2 µg/g) to obtain 11 dilutions. Sample Panel 2 was prepared by dilution of a high native sample pool (1285.4 µg/g) with a low native sample pool (10.8 µg/g) to obtain 11 dilutions. The samples were tested in triplicate, using one reagent lot. The expected value, predicted value, and deviation from predicted were calculated. The results are summarized in the following tables:

Sample Panel 1

| Sample Dilution | Mean Measured Value (µg/g) | Expected Value (µg/g) | Predicted Value (µg/g) | Deviation (µg/g) | % Deviation |
|---|---|---|---|---|---|
| 1 | 9.2 | 9.2 | 9.3 | -0.1 | -0.9% |
| 2 | 60.5 | 65.9 | 66.3 | -5.8 | -8.8% |
| 3 | 113.6 | 122.6 | 123.3 | -9.7 | -7.9% |
| 4 | 166.1 | 179.3 | 180.4 | -14.3 | -7.9% |
| 5 | 221.6 | 236.0 | 237.4 | -15.9 | -6.7% |
| 6 | 275.1 | 292.8 | 294.5 | -19.4 | -6.6% |
| 7 | 341.5 | 349.5 | 351.5 | -10.0 | -2.9% |
| 8 | 417.0 | 406.2 | 408.6 | 8.4 | 2.1% |
| 9 | 471.9 | 462.9 | 465.6 | 6.3 | 1.3% |
| 10 | 543.0 | 519.6 | 522.7 | 20.3 | 3.9% |
| 11 | 576.3 | 576.3 | 579.7 | -3.5 | -0.6% |

Sample Panel 2

| Sample Dilution | Mean Measured Value (µg/g) | Expected Value (µg/g) | Predicted Value (µg/g) | Deviation (µg/g) | % Deviation |
|---|---|---|---|---|---|
| 1 | 10.4 | 10.8 | 10.5 | 0.0 | -0.4% |
| 2 | 95.4 | 100.0 | 97.0 | -1.6 | -1.7% |
| 3 | 185.3 | 199.4 | 193.5 | -8.2 | -4.2% |
| 4 | 290.3 | 304.0 | 294.8 | -4.6 | -1.5% |
| 5 | 398.8 | 399.6 | 387.6 | 11.2 | 2.9% |
| 6 | 487.3 | 501.5 | 486.5 | 0.8 | 0.2% |
| 7 | 604.5 | 603.5 | 585.4 | 19.2 | 3.3% |
| 8 | 716.3 | 702.3 | 681.2 | 35.1 | 5.1% |
| 9 | 798.8 | 801.1 | 777.0 | 21.8 | 2.8% |

| Sample Dilution | Mean Measured Value (µg/g) | Expected Value (µg/g) | Predicted Value (µg/g) | Deviation (µg/g) | % Deviation |
|---|---|---|---|---|---|
| 10 | 887.9 | 899.8 | 872.8 | 15.1 | 1.7% |
| 11 | 995.9 | 998.6 | 968.6 | 27.3 | 2.8% |

Linear Regression Summary

| Dilution Panel | Range (µg/g) | Slope (95%CI) | Intercept (95%CI) | $R^2$ | Recovery Range (%) |
|---|---|---|---|---|---|
| Sample Panel 1 | 9.2–576.3 | 1.03 (0.99–1.07) | -12.74 (-27.18–1.71) | 0.997 | 91.2–103.9 |
| Sample Panel 2 | 10.4–995.9 | 1.01 (0.99–1.03) | -6.95 (-18.40–4.49) | 0.999 | 93.1–102.2 |
| Combined | 9.2–995.9 | 1.04 (1.02–1.06) | -12.19 (-20.28– -4.10) | 0.999 | 91.2–103.9 |

Linearity was supported for the analytical measuring interval from 11.0 µg/g to 1000.0 µg/g.

*Spiking Recovery:*

The recovery of the ALPCO Calprotectin Immunoturbidimetric Assay was evaluated using seven extracted stool samples containing various concentrations of calprotectin across the analytical measuring interval of the assay. Purified native calprotectin diluted in standard diluent was used as the spiking material in a proportion of nine parts of sample to one part spiking material. Recovery was calculated compared to the baseline result. Percent recoveries ranged from 95.5% to 106.5%.

*Hook Effect*

Hook effect was evaluated by testing samples above the analytical measuring interval: from 2,000 to 24,000 µg/g. One lot of reagents was used; five samples were run in duplicate. The results showed no hook effect up to 18,000 µg/g.

3. Analytical Specificity/Interference:

The interference testing of the ALPCO Calprotectin Immunoturbidimetric Assay was evaluated following CLSI guideline EP07-Ed3. The study was performed using three samples: one high positive stool sample (477–488 µg/g, one moderately high positive stool sample (261–287 µg/g), and one low positive stool sample (41–46 µg/g). Interfering substances were spiked into each stool sample at 10% of the total specimen volume; a series of endogenous and exogenous interfering substances of different concentrations were tested. 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 tested in duplicate. Percent recovery was calculated to evaluate the differences between blank and control samples. No interference (% recovery range within 90–110%) of the ALPCO Calprotectin Immunoturbidimetric Assay was found to the concentration level tested for the following substances:

| Interference Substance | Spiking Concentration |
|---|---|
| Oral Pharmaceuticals | |
| Iron (II) sulfate | 0.11 mg/50 mg stool |
| Prednisone | 0.31 mg/50 mg stool |
| Azathioprine | 0.19 mg/50 mg stool |
| Mesalamine; 5-ASA | 5.21 mg/50 mg stool |
| Lansoprazol | 0.18 mg/50 mg stool |
| Vancomycin | 2.0 mg/50 mg stool |
| Sulfamethoxazole | 1.6 mg/50 mg stool |
| Trimethoprim | 0.35 mg/50 mg stool |
| Ciprofloxacin | 1.25 mg/50 mg stool |
| Nutritional Supplements | |
| Vitamin E (DL-α Tocopherol Acetate) | 0.3 mg/50 mg stool |
| Multi-Vitamins (A, B1, B2, B3, B5, B6, B8, B9, B12, C, D, and E) and minerals | 1.06 mg/50 mg stool |
| Vitamin B7 (biotin) | 1750 ng/50 mg stool |
| Microorganisms | |
| *Escherichia coli* | $1.5 \times 10^7$ cfu/mL |
| *Salmonella enterica subsp. enterica* | $1.5 \times 10^7$ cfu/mL |
| *Klebsiella pneumoniae subsp. pneumonia* | $1.5 \times 10^7$ cfu/mL |
| *Citrobacter freundii* | $1.5 \times 10^7$ cfu/mL |
| *Shigella flexneri* | $1.5 \times 10^7$ cfu/mL |
| *Yersinia enterocolitica subsp. enterocolitica* | $1.5 \times 10^7$ cfu/mL |
| Other | |
| Human anti-mouse antibody (HAMA) | 0.241 µg/50 mg stool |
| Hemoglobin | 7.5 mg/50 mg stool |
| Rheumatoid Factor (RF) | 500 IU/50 mg stool |

4. Assay Reportable Range:

   The analytical measuring interval (AMI) of the ALPCO Calprotectin Immunoturbidimetric Assay is 11.0–1000.0 µg/g.

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

   *Traceability:*

   No international reference materials or reference measurement procedures are currently available for calprotectin. The ALPCO Calprotectin Immunoturbidimetric Assay is traceable to an internal standard that was value assigned using the ALPCO Calprotectin Chemiluminescent ELISA (K191807) predicate device. Calprotectin calibrators are prepared by spiking human recombinant calprotectin in a buffered matrix with stabilizers and preservatives to the target calprotectin concentrations.

*Stability:*

a. Shelf-Life Stability
The real-time and accelerated reagent stability studies were performed using two native stool sample pools and two controls. For real-time stability, three reagent lots of the ALPCO Calprotectin Immunoturbidimetric Assay (reagents, calibrators, and controls) were stored at 2–8°C, and the samples were tested every 4 months. For accelerated stability, three lots of the reagents were stored at 25°C, and 37°C, and the samples were tested every 7 days up to 35 days. Percent (%) recovery was calculated for each sample at each testing timepoint compared to the value at Day 0 stored under the same condition. The results support that the ALPCO Calprotectin Immunoturbidimetric Assay is stable at 2–8°C for 24 months.

b. Reagent On-Board Stability
Reagent on-board stability was tested using one lot of the ALPCO Calprotectin Immunoturbidimetric Assay reagents on one instrument (AU680 chemistry analyzer) using one calibration event. Two native sample pools and two controls were tested every 7 days using this lot of reagents stored on-board. The percent (%) recovery was evaluated for each sample compared to its value at Day 0. The results support the ALPCO Calprotectin Immunoturbidimetric Assay is stable when stored on-board for 30 days.

c. Transport Stability
The Calprotectin Immunoturbidimetric Assay (reagents, calibrators, and controls) transport stability was performed using one lot of reagents, one lot of calibrators, one instrument, and one lot of controls. Two sample pools and two controls were tested in two replicates. The reagents, calibrators, and controls were shipped using standards shipping packaging with two ice packs via commercial shipping for 2 days during peak summer conditions, and upon arrival the assay was stored at 30°C for an additional 14 days. Percent (%) recovery was calculated to evaluate the difference between transported and not transported reagents. Reagent percent (%) recovery ranged 91.0%–103.4%; for the calibrators: 94.9%–104.8%; and for the controls: 98.3%–101.7%.

d. Sample Stability
Refer to K191807

6. Detection Limit:

*Limit of Blank (LoB*):

The LoB of the ALPCO Calprotectin Immunoturbidimetric Assay y was determined in accordance with CLSI EP17-A2. Four blank stool samples were extracted and tested in replicates of five on two reagent lots across 3 days. The LoB was estimated as the 95th percentile for the distribution of blank sample results using nonparametric method. The LoB was determined to be 2.2 µg/g.

*Limit of Detection (LoD):*

The LoD of the ALPCO Calprotectin Immunoturbidimetric Assay 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 with two reagent lots across 4 days. The reported LoD measurement is the maximum value of the individual LoDs obtained for each reagent lot. The LoD was determined to be 3.9 µg/g.

*Limit of Quantitation (LoQ):*

The LoQ of the ALPCO Calprotectin Immunoturbidimetric Assay was determined in accordance with CLSI EP17-A2. Five stool samples containing a low level of calprotectin were tested in replicates of five on two reagent lots across 4 days. The LoQ estimate for each reagent lot was determined as the measurand concentration interpolated from the intersection of the precision profile curve with the 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 11.0 µg/g.

7. Assay Cut-Off:

The cut-offs of the ALPCO Calprotectin Immunoturbidimetric Assay are presented in the following table:

| 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 by testing 306 stool sample extracts in singlicate using the ALPCO Calprotectin Immunoturbidimetric Assay and the predicate device. Of the 306 samples, 168 tested within the analytical measuring intervals of both assays and further evaluated by qualitative agreement and regression analysis. Among the 168 samples, 12 samples were native samples spiked with purified native human calprotectin to supplement high values. The comparison results were summarized in the following tables:

| ALPCO Calprotectin Immunoturbidimetric Assay | Predicate Device | | | Total |
|---|---|---|---|---|
| | Elevated (>100 µg/g) | Equivocal (50–100 µg/g) | Normal (<50 µg/g) | |
| **Elevated (>100 µg/g)** | 80 | 6 | 3 | 89 |
| **Equivocal (50–100 µg/g)** | 6 | 18 | 7 | 31 |
| **Normal (<50 µg/g)** | 0 | 8 | 40 | 48 |
| **Total** | 86 | 32 | 50 | 168 |

*Equivocal results considered as elevated:*

| | |
|---|---|
| Negative Percent Agreement (NPA) | 80.00% (95%CI: 68.28–89.97%) |
| Positive Percent Agreement (PPA) | 93.22% (95% CI: 87.08–97.03%) |
| Total Percent Agreement (TPA) | 89.29% (95%CI: 83.60–93.53%) |

*Equivocal results considered as normal:*

| | |
|---|---|
| Negative Percent Agreement (NPA) | 89.02% (95% CI: 80.18–94.86%) |
| Positive Percent Agreement (PPA) | 93.02% (95% CI: 84.43–97.40%) |
| Total Percent Agreement (TPA) | 91.07% (95% CI: 85.70–94.92%) |

The 168 samples within the analytical measuring intervals of both assays were evaluated by Passing-Bablok regression. The results are summarized in the table below:

| N | Range (µg/g) | Slope (95%CI) | Intercept (95%CI) | $R^2$ |
|---|---|---|---|---|
| 168 | 11.0−975.3 | 0.98 (0.95–1.02) | -0.52 (-4.74–3.23) | 0.99 |

2. Sample Extraction Method Comparison:

Extraction method comparison was performed to demonstrate equivalency of the ALPCO Easy Stool Extraction Device and manual weighing method. A total of 60 native stool samples were extracted using the ALPCO Easy Stool Extraction Device and manual weighing method, respectively. The paired samples were run using one lot of the ALPCO Calprotectin Immunoturbidimetric Assay reagent, calibrators, and controls. Test results of 53 paired samples across the analytical measuring interval were evaluated by Passing-Bablok regression analysis. The results are summarized in the following table:

| N | Range (µg/g) | Slope (95%CI) | Intercept (95%CI) | $R^2$ |
|---|---|---|---|---|
| 53 | 11.2−780.8 | 1.01 (0.99–1.03) | 0.71 (-0.41–2.30) | 0.99 |

## C Clinical Studies:

*a. Clinical Sensitivity and specificity*

The clinical performance of the Calprotectin Immunoturbidimetric Assay was evaluated. A total of 349 stool specimens from the intended use population were prospectively collected at 15 sites. All enrolled patients were scheduled to undergo colonoscopy and were further screened to determine eligibility based upon the below inclusion/exclusion criteria:

- *Inclusion criteria:*
  ≥ 22 years of age; possible inflammatory bowel disease or irritable bowel syndrome with symptoms such as abdominal pain, diarrhea, altered appetite, weight loss, or anemia; able to provide a stool sample within 24–48 hours prior to colonoscopy preparation or 3–14 days following colonoscopy; able to understand the study and the tasks required and sign the Informed Consent Form.

- *Exclusion criteria:*
  Unable or unwilling to provide a stool specimen; known intestinal cancer; receiving chemotherapy or systemic immunosuppressive drugs; previously diagnosed IBD managed with immunomodulators, 5-ASA or biologic therapies or who have undergone a surgical resection or diversion procedure; known intestinal infection; known upper Gl disease (i.e., esophagitis or gastritis); have taken proton pump inhibitors (PPIs) or H2-receptor antagonists for the previous 2 weeks; have taken NSAIDS (including aspirin) on seven or more days during the 2 weeks before providing the sample.

Clinical diagnoses were established by the clinical investigator/gastroenterologist:
- 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.

The clinical diagnosis of the samples in the study were summarized in the following table:

| Clinical Diagnosis | Number of Subjects |
|---|---|
| Inflammatory Bowel Disease (IBD) | 63 |
| Ulcerative Colitis (UC) | 29 |
| Crohn's Disease (CD) | 24 |
| Indeterminant/Undefined | 10 |
| Irritable Bowel Syndrome (IBS) | 105 |
| Other GI conditions | 181 |
| Total | 349 |

The stool samples were de-identified and were tested using the ALPCO Calprotectin Immunoturbidimetric Assay at ALPCO site and at two external sites. The results are summarized in the following table:

| ALPCO Calprotectin Immunoturbidimetric Assay | Clinical Diagnosis | | | Total |
|---|---|---|---|---|
| | IBD | IBS | Other GI | |
| **Elevated (>100 µg/g)** | 48 | 4 | 3 | 55 |
| **Equivocal (50–100 µg/g)** | 9 | 6 | 6 | 21 |
| **Normal (<50 µg/g)** | 6 | 95 | 172 | 273 |
| **Total** | 63 | 105 | 181 | 349 |

*IBD vs. non-IBD*

The performance of the Calprotectin Immunoturbidimetric Assay as an aid in the diagnosis of IBD was evaluated by calculate the estimates of the clinical sensitivity, clinical specificity, positive predictive value (PPV), and negative predictive value (NPV). The results are summarized in the following tables:

*Equivocal results as Elevated*

| ALPCO Calprotectin Immunoturbidimetric Assay | Clinical Diagnosis | | Total |
|---|---|---|---|
| | IBD | Non-IBD | |
| **Elevated (≥50 µg/g)** | 57 | 19 | 76 |
| **Normal (<50 µg/g)** | 6 | 267 | 273 |
| **Total** | 63 | 286 | 349 |
| Sensitivity: 90.5% (95% CI: 80.7–95.6%)<br>Specificity: 93.4% (95% CI: 89.9–95.7%)<br>PPV: 75.0% (95% CI: 65.8–82.4%)<br>NPV: 97.8% (95% CI: 95.4–99.0%) | | | |

*Equivocal results as Normal:*

| ALPCO Calprotectin Immunoturbidimetric Assay | Clinical Diagnosis | | Total |
|---|---|---|---|
| | IBD | Non-IBD | |
| **Elevated (>100 µg/g)** | 48 | 7 | 55 |
| **Normal (≤100 µg/g)** | 15 | 279 | 294 |
| **Total** | 63 | 286 | 349 |
| Sensitivity: 76.2% (95% CI: 64.4–85.0%)<br>Specificity: 97.6% (95%CI: 94.8–98.9%)<br>PPV: 87.3% (95% CI: 76.5–93.5%)<br>NPV: 94.9% (95% CI: 91.8–96.9%) | | | |

*IBD vs. IBS*

The performance of the Calprotectin Immunoturbidimetric Assay as an aid to differentiate the IBD and IBS was evaluated by calculate the estimates of the clinical sensitivity, clinical specificity, positive predictive value (PPV), and negative predictive value (NPV). The results are summarized in the following tables:

*Equivocal results as Elevated:*

| ALPCO Calprotectin Immunoturbidimetric Assay | Clinical Diagnosis | | Total |
|---|---|---|---|
| | IBD | IBS | |
| **Elevated (≥50 µg/g)** | 57 | 10 | 67 |
| **Normal (<50 µg/g)** | 6 | 95 | 101 |
| **Total** | 63 | 105 | 168 |
| Sensitivity: 90.5% (95% CI: 80.7–95.6%)<br>Specificity: 90.5% (95% CI: 83.4–94.7%)<br>PPV: 85.1% (95% CI: 75.9–91.2%)<br>NPV: 94.1% (95% CI: 88.1–97.1%) | | | |

*Equivocal results as Normal*:

| ALPCO Calprotectin Immunoturbidimetric Assay | Clinical Diagnosis | | Total |
|---|---|---|---|
| | IBD | IBS | |
| **Elevated (>100 µg/g)** | 48 | 4 | 52 |
| **Normal (≤100 µg/g)** | 15 | 101 | 116 |
| **Total** | 63 | 105 | 168 |

| |
|---|
| Sensitivity: 76.2% (95% CI: 64.4–85.0%)<br>Specificity: 96.2% (95% CI: 90.6–98.5%)<br>PPV: 92.3% (95% CI: 82.0–96.9%)<br>NPV: 87.1% (95% CI: 81.2–91.3%) |

2. Other Clinical Supportive Data (When 1 Is Not Applicable):

Not applicable

**D Clinical Cut-Off:**

Same as assay cut-off

**E Expected Values/Reference Range:**

A study was performed using 120 stool samples to establish the reference range for the ALPCO Calprotectin Immunoturbidimetric Assay following CLSI EP28-A3c. The stool samples were obtained from 120 asymptomatic individuals with no abdominal complaints and no history of IBS, IBD or other chronic intestinal disorders. The cohort contained 65 females and 55 males, 22 to 82 years old with the mean age of 43.8 years.

Among 120 samples, the value of stool calprotectin level for 104 samples were below 11 µg/g, the lower end of the AMI. Two samples were tested with elevated value of stool calprotectin, i.e., 111.2 and 130.3 µg/g, and one with equivocal, value 68.5 µg/g. The results are summarized in the following table:

| | **Calprotectin Concentration (µg/g)** |
|---|---|
| Range | <3.9–130.3 |
| Mean | 8.6 |
| Median | 3.9 |
| Lower Limit (90% CI) | 3.9 (3.9–3.9) |
| Upper Limit (90% CI) | 67.5 (20.0–130.3) |

**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.