510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k130945

B. Purpose for Submission:

New Device

C. Measurand:

Fecal calprotectin

D. Type of Test:

Quantitative, ELISA

E. Applicant:

Eurospital S.p.A.

F. Proprietary and Established Names:

Calprest®

G. Regulatory Information:

1. Regulation section:

   21 CFR§866.5180 – Fecal calprotectin immunological test system

2. Classification:

   Class II

3. Product code:

   NXO, Calprotectin, Fecal

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   Calprest® is a quantitative ELISA for detecting concentration of fecal calprotectin. Calprest® can be used as an in vitro diagnostic to aid in the diagnosis of Inflammatory Bowel Diseases (IBD, Crohn's disease and ulcerative colitis) and to differentiate IBD from Irritable Bowel Syndrome (IBS) in conjunction with other clinical and laboratory findings.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Microtiter plate reader (405 nm filter)

I. Device Description:

The Calprest® kit contains the following materials:

- Microtiter plate coated with rabbit anti-calprotectin antibodies (12 strips, 8 wells/strip)
- Alkaline phosphatase labeled rabbit anti-calprotectin antibody (1x15 mL)
- Substrate (1x15 mL)
- 20x Washing solution (1x50 mL)
- 10x Diluent solution (1x20 mL)
- 2.5x Extraction solution (2x50 mL)
- Calibrators (6x1.0 mL, 6 vial of calprotectin solution at concentration of 6.25, 12.5, 25, 50, 100, and 200 ng/mL)
- Control 1 (low) (1x1.0 mL, Approximate range = 20 – 40 ng/mL or 50 – 100 mg/kg)
- Control 2 (high) (1x1.0 mL, Approximate range = 40 – 80 ng/mL or 100 – 200 mg/kg)

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(K) number(s):

   PhiCal™ Test (k050007)

2. Comparison with predicate:

Similarities		
Item	Device Calprest®	Predicate PhiCal™ Test
Intended Use/Indication for Use	to aid in the diagnosis of Inflammatory Bowel Diseases (IBD, Crohn's disease and ulcerative colitis) and to differentiate IBD from Irritable Bowel Syndrome (IBS) in conjunction with other clinical and laboratory findings.	to aid in the diagnosis of inflammatory bowel diseases (IBD): Crohn's disease and ulcerative colitis, and to differentiate IBD from IBS; when used in conjunction with other diagnostic testing and the total clinical picture.
Analyte	Calprotectin	Same
Assay format	Quantitative	Same
Method	Colorimetric ELISA	Same
Sample type	fecal	Same
Specimen Requirement	1.0 – 5.0 g stool	Same
Instrument	ELISA reader (405 nm)	Same
Capture Antibody	Rabbit anti-human calprotectin	Same
Detection Antibody	Alkaline phosphatase labeled rabbit anti-calprotectin	Same
Substrate	pNPP	Same
Control	2 (high and low)	Same
Stability	Open vial: Conjugate: 2-8℃, 30 days Substrate: 2-8℃, 90 days Calibrators: 2-8℃, 30 days Controls: 2-8℃, 30 days	Same
Sample Transportation	Stool specimen should be received by the laboratory within 10 days of collection. Temperature during shipment should not exceed 37°C. Sample must be extracted within 10 days of collection.	Same

Differences		
Item	Device Calprest®	Predicate PhiCal™ Test
Calibrator	6 levels: (6.25, 12.5, 25, 50, 100, and 200 ng/mL)	5 levels: (6.25, 12.5, 25, 50, and 100 ng/mL)
Measuring range	15.6 – 500 mg/kg	15.6 – 250 mg/kg
Results	Normal: <15.6 – 50 mg/kg	Normal: <15.6 – 50 mg/kg

Differences		
Item	Device Calprest®	Predicate PhiCal™ Test
interpretation	Borderline: 50 – 100 mg/kg Abnormal: >100 mg/kg	Borderline: 50 – 120 mg/kg Abnormal: >120 mg/kg
Stability (Working solution)	Washing buffer: 20-25°C, 30 days Dilution buffer: 2-8°C, 30 days	Washing buffer: 20-25°C, 7 days Dilution buffer: 2-8°C, 7 days
Sample storage	Stored at 2-8°C for up to 4 days before testing. If not tested immediately, freeze stored samples at -20°C.	Samples should be stored at 2-8°C for up to 11 days, or for 1 year at –20°C.

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

CLSI EP05-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition"

CLSI EP06-A "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline"

CLSI EP7-A2 "Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition"

CLSI EP17-A "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline"

Guidance for Industry and FDA Staff – Class II Special Controls Guidance Document: Fecal Calprotectin Immunological Test Systems

L. Test Principle:

The test is performed on stool samples, collected without preservatives. The samples should be tested within 4 days, or frozen at -20° C if not immediately tested. An extract is prepared by combining the stool sample with extraction buffer at a ratio of 1:50 (e.g., 0.1 gm of stool added with 4.9 mL of extraction buffer) and mixing for 30 minutes. Following centrifugation, 20µL of the supernatant is diluted 1:50 with dilution buffer (final dilution 1:2500).

Calprest® is a colorimetric enzyme-linked immunosorbent assay (ELISA). The assay uses a polyclonal rabbit antibody against calprotectin as the capture antibody. Calprotectin present in the diluted sample is bound by the antibody adsorbed onto the surface of the microtiter plate. Alkaline phosphatase conjugated antibodies (rabbit IgG antibodies) bind to the captured calprotectin. The enzyme catalyzes the conversion of the substrate to a colored product and the optical density (OD) of the sample is read on an ELISA plate reader. The intensity of the color is proportional to the amount of conjugate bound, and thus to the amount of captured calprotectin. The concentration of calprotectin in the extracted sample is

interpreted from a standard curve generated from the 6 calibrators and converted to mg of calprotectin per kg of stool using a conversion of factor supplied by package insert.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Intra-assay precision was conducted by extracting 8 different stool samples. Each extract sample was tested with 10 replicates in a single assay run. The mean, standard deviation (SD) and the coefficient of variation (CV) were calculated. The results met the acceptance criteria with the %CV of each sample <15%.

	#1	#2	#3	#4	#5	#6	#7	#8
Mean (mg/kg)	438.7	355.2	352.9	311.7	199.9	125.7	57.8	27.7
SD (mg/kg)	33.7	43.0	43.7	38.8	6.6	9.8	2.9	1.0
% CV	7.7	12.1	12.4	12.4	3.3	7.8	5.0	3.7

Inter-assay precision was conducted by extracting 8 different stool samples. Each extract was tested using one lot of the test kit with 5 replicates per run, 1 run per day for 6 days with 2 operators. The mean, standard deviation (SD) and the coefficient of variation (CV) were calculated. The results met the acceptance criteria with %CV of each sample <15%.

	#1	#2	#3	#4	#5	#6	#7	#8
Mean (mg/kg)	359.9	209.8	185.5	117.0	70.4	48.8	40.9	20.6
SD (mg/kg)	27.5	17.8	17.8	9.3	6.8	4.5	3.4	2.6
% CV	7.7	8.5	9.6	8.0	9.6	9.3	8.2	12.4

Lot-to-lot reproducibility was done by extracting six different stool samples. Each sample was tested in 5 replicates using 3 different lots of the test kit. The results are shown in the following table.

	#1	#2	#3	#4	#5	#6
Mean (mg/kg)	107.7	63.1	185.1	43.8	36.0	344.9
SD (mg/kg)	5.78	3.68	14.55	1.36	3.00	42.62
% CV	5.4	5.8	7.9	3.1	8.3	12.4

Extraction reproducibility was evaluated by using 3 stool samples with calprotectin concentrations of 28.4, 41.1 and 79.4 mg/kg. Each stool sample was extracted 10 times and each stool extract was tested in duplicate. The %CVs for the 3 stool samples are 13.6%, 8.9% and 7.0%.

b. Linearity/assay reportable range:

Linearity: The reportable range of the Calprest® was evaluated with both aqueous and matrix linearity. For the matrix linearity study, serially diluted samples with calprotectin concentrations ranging from 20.4 to 492.8 mg/kg were prepared by diluting the high positive extracted stool sample pool in the low concentration extracted stool sample pool. Each dilution was tested in triplicate. The percent (%) recovery ranged from 87.0% to 113.2% for tested samples. The linear regression analysis gives the following equation:

$y=1.013x - 3.777$, $R^2= 0.999$

For the aqueous linearity study, samples were prepared by diluting the calprotectin reference materials with sample diluent to generate 7 dilutions with concentrations ranging from 8.6 mg/kg to 550 mg/kg. Each dilution was tested in triplicate. The % recovery for samples ranged from 95.2% to 109.3%. The linear regression analysis gives the following equation:

$y=0.985x + 2.714$, $R^2= 0.999$

The assay was shown to give a linear response over the claimed reportable range (15.6 – 500 mg/kg).

Accuracy/Recovery study: Extracts from 7 stool samples were each spiked with the kit calibrator material (S3) that has a calprotectin concentration target of 25 ng/mL. The baseline extract for each sample was "spiked" with sample diluent to compensate for volume adjustments made to the calprotectin-spiked extracts. Each of the spiked extracts was then assayed in triplicate and results are shown in table below.

Sample	#1	#2	#3	#4	#5	#6	#7
Baseline (mg/kg)	18.3	47.5	59.4	66.5	115.2	232.5	421.9
Spike Value (mg/kg)	31.2	31.2	31.2	31.2	31.2	31.2	31.2
Theoretical (Base+Spike) (mg/kg)	49.6	78.8	90.6	97.8	146.4	263.7	452.1
Observed (Base+Spike) (mg/kg)	49.8	81.1	100.1	108.7	166.0	271.3	475.0
% Recovery	100.5	103.0	110.5	111.1	113.4	102.8	112.6

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

Traceability: There is no international reference material for calprotectin. The calibrators and controls are manufactured using an approved reference lot which is traceable to the internal reference material cleared in k050007.

The calibrator S6, prepared from the master stock solution, was used to make the calibrators and controls. New lots of the calibrator set and controls were value assigned and validated by a comparison against an approved lot of calibrators and controls in three different assay runs and further testing with a panel of 12 internal quality control samples. The target values for calibrators are 6.25, 12.5, 25, 50, 100 and 200 ng/mL, and the target values for controls range from 20 to 40 ng/mL for the

Low Control and 40 to 80 ng/mL for the High Control.

Stability:

Kit stability: The real time stability was performed using three lots of Calprest® kits (including calibrators and controls) stored under the recommended temperature at 2 – 8°C. Data were collected at point 0, 6, 12, 18 and 19 months. The results support stability of the kits under the recommended storage of 2 – 8°C for up to 18 months.

Open vial stability: The study was done to evaluate the reagent stability after opening. Three kit lots of Calprest® were stored at 2 – 8°C after first opening. Data were collected at 0, 30 and 60 days. The results support that the reagents are stable once opened for up to 30 days when stored at 2 – 8°C.

Working solutions stability: The study was done to evaluate the stability of working solutions including dilution buffer, washing buffer and extraction solution. The 1X working solution for each buffer was prepared and stored at 2 – 8°C. Data were collected at different time points. The results support up to 30 days stability for 1X dilution buffer and extraction buffer and 3 months stability for 1X washing buffer.

Sample stability: Five (5) stool samples which cover the measuring range of Calprest® were stored at 2-8°C for 2, 4, and 7 days. The acceptance criteria for % recovery of calprotectin in the samples tested at each time point should be within ±5% of the initial reading taken at day 0. The results support that stability of stool sample stored at 2-8°C up to 4 days.

Analyte (Calprotectin) stability: The study was done to determine the stability of extracted stool samples when stored -20°C. A total of 10 stool samples (1 with concentration above 100 mg/kg, 2 around 100 mg/kg, 5 around 50 mg/kg, and 2 around 20 mg/kg) were tested. Data were collected at time 0 and 3 months. The acceptance criteria for %recovery of calprotectin at 3 months should be within ±15% of the initial reading. All samples met the acceptance criteria.

d. Detection limit:

The limit of blank (LoB) was determined by assaying 5 blank samples 12 times. The LoB value was estimated to be 3.04 mg/kg. The limit of detection (LoD) was determined by assaying 5 extracted stool samples with low calprotectin level 12 times. The LoD value was calculated as the LoB + 1.645 x SD of the replicates for the low level samples and was found to be 3.98 mg/kg.

The lower limit of the reportable assay range for Calprest® is 15.6 mg/kg which corresponds to the lowest calibrator with a concentration of 6.25 ng/mL after conversion to mg of calprotectin per kg stool sample according to the assay's instructions for use.

e. Analytical specificity:

Interference studies were performed according to EP7-A2 using 5 pooled stool samples, each containing a mixture of 3 different stool samples. The pooled samples included 2 high positives (with calprotectin concentrations around 460 mg/kg and 270 mg/kg), 1 low positive (145 mg/kg), 1 sample within borderline range (68 mg/kg) and 1 negative (30 mg/kg). For non-interference to be claimed, the % recovery of the spiked sample should be within 95-105% of the neat sample. Stool samples were tested for potential interference by:

Microorganisms: The data demonstrated that Calprest® was not affected by *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella spp*., *Shigella spp*., and *Yersinia spp*. (at cell count of $1.5x10^7$ cfu/ml) in stool samples.

Drug and Nutrients: The data demonstrated that Calprest® was not affected by the following oral pharmaceuticals and nutritional supplements: Vancomycin (0.67 mg/50 mg stool); Ciprofloxacin HCL (0.50 mg/50 mg stool); Prevacid (0.02 mg/50 mg stool); Azathioprine (0.07 mg/50 mg stool); Prednisone (0.01 mg/50 mg stool); Pentasa (1.33 mg/50 mg stool); Asacol (1.33 mg/50 mg stool); multiple vitamin (Vit D: 1.1 UI/50 mg stool, Vit A: 8.0 UI/50 mg stool), Vit C: 0.05 mg/50 mg stool) and Vitamin E (0.10 mg/50 mg stool).

Hemoglobin: The results showed that the addition of hemoglobin into the stool samples at level of 5.83 mg/50 mg stool did not interfere with Calprest®.

f. Assay cut-off:

The assay cut-off for Calprest® is as follows:

Calprotectin (mg/kg)	Interpretation	Suggested follow-up
<15.6 – 50	Normal	None
50 – 120	Borderline	Re-evaluate at 4-6 weeks
>120	Abnormal	Repeat as clinically indicated.

2. Comparison studies:

a. Method comparison with predicate device:

A total of 131 clinical samples including 70 samples from patients diagnosed with Crohn's disease, Crohn's disease in remission, ulcerative colitis (UC), UC in remission, Diverticulitis, or intermediate colitis (IC), and 61samples from patients with irritable bowel syndrome (IBS), anemia, chronic diarrhea, recurrent abdominal pain (RAP), celiac disease, and other conditions were assayed using Calprest® and the predicate device PhiCal in accordance with the instructions for use in the package inserts. The values of the samples tested covered the measuring range of both devices. Deming regression analyses are based on the balance of the paired results,

and the data are as follows:

N=	Sample range (Calprest®) (mg/kg)	Regression analysis
131	15.6 – 266.50	y = 0.98x – 1.85 Slope (95% CI): 0.96 – 1.01 Intercept (95% CI): -3.96 – 0.96

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and specificity

A total of 138 samples were tested with Calprest® assay: 98 were from patients diagnosed with Crohn's disease, UC and intermediate colitis (IC), and 40 were from patients with IBS, chronic diarrhea, RAP and celiac disease. The IBD patients were diagnosed by clinical findings and/or confirmed with colonoscopy.

The results are summarized in the following tables:

		Clinical Diagnosis of IBD		
		Positive	Negative	Total
Calprest®	Abnormal (>120 mg/kg)	85	3	88
	Borderline (50 – 120 mg/kg)	10	3	13
	Normal (<50 mg/kg)	3	34	37
	Total	98	40	138

Borderline considered as abnormal:
Sensitivity: 96.9% (95% CI: 91.3 – 99.4%)
Specificity: 85.0% (95% CI: 70.2 – 94.3%)
PPV: 94.1% (95% CI: 87.5 – 97.8%)
NPV: 91.9% (95% CI: 78.1 – 98.3%)

Borderline considered as normal:
Sensitivity: 79.6% (95%CI: 70.3 – 87.1%)
Specificity: 92.5% (95% CI: 79.6 – 98.4%)
PPV: 96.3% (95% CI: 89.6 – 99.2%)
NPV: 64.9% (95% CI: 51.1 – 77.1%)

b. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   The expected value in the normal healthy population is generally <50 mg/kg according to study done by Røseth *et al*. (1992)*.

   Ninety (90) samples from normal healthy people and 42 samples from patients with CD, UC, IBS, and IC were tested with Calprest® to validate the reference range value. The results showed that 85 out of 90 samples (92.2%) from normal healthy people tested negative and the remaining 5 samples fell in the range of 51.8 – 60.4 mg/kg. Of the 42 samples from patients with CD, UC, IBS and IC, 36 (85.7%) tested positive by the assay.

   * Røseth AG, Fagerhol MK, Aadland E, Schonsby H. (1992). Assessment of the neutrophil-dominating protein calprotectin in feces. A methodology study. *Scand J. Gastroenterol.* 27(9): 793-798.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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