510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K160447

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®NG

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®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.

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 (450 nm filter)

I. Device Description:

The Calprest®NG kit contains the following materials:

- Microtiter plate coated with rabbit anti-calprotectin antibodies (12 strips, 8 wells/strip)
- Horse-radish peroxidase (HRP)-labeled mouse anti-calprotectin antibody (1 x 15 mL)
- Substrate (1 x 15 mL)
- Stop solution (1 x 15 mL, 0.5M H_2SO_4)
- 20x Washing solution (1 x 50 mL)
- 10x Diluent solution (1 x 20 mL)
- 2.5x Extraction solution (2 x 50 mL)
- Calibrators (6 x 1.5 mL, 6 vials containing 1.5 mL/vial calprotectin solution at concentration of 0, 2.5, 12.5, 25, 50, 150 ng/mL)
- Control 1 (low) (1 x 1.5 mL)
- Control 2 (high) (1 x 1.5 mL)

J. Substantial Equivalence Information:

1. Predicate device name:

   Calprest®

2. Predicate 510(k) number:

3. Comparison with predicate:

Similarities		
Item	**Device Calprest®NG**	**Predicate Calprest®**
Intended Use/Indication for Use	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	Same
Analyte	Calprotectin	Same
Assay format	Quantitative	Same
Method	Colorimetric ELISA	Same
Sample type	Fecal	Same
Specimen Requirement	1.0–5.0 g stool	Same
Stability (Working solution)	Washing buffer: 20–25°C for 30 days Extraction buffer: 2–8°C for 3 months Dilution buffer: 2–8°C for 30 days	Same
Stability (Open vial reagents)	Conjugate: 2–8°C for 30 days Substrate: 2–8°C for 90 days Calibrators: 2–8°C for 30 days Controls: 2–8°C for 30 days	Same
Sample storage	Stored at 2–8°C for up to 4 days before testing. If not tested immediately, freeze stored samples at -20°C.	Same

Differences		
Item	**Device Calprest®NG**	**Predicate Calprest®**
OD reading	450 nm	405 nm
Detection Antibody	HRP-labeled mouse anti-calprotectin	Alkaline-phosphatase-labeled rabbit anti-calprotectin
Substrate	TMB	pNPP
Stop solution	H_2SO_4 (0.5M)	None
Sample dilution	1:20,000	1:2500
Control	2 controls Control 1: 10–20 ng/mL Control 2: 30–70 ng/mL	2 controls Control 1: 20–40 ng/mL Control 2: 40–85 ng/mL
Calibrators	6 levels:	6 levels:

Differences		
Item	**Device Calprest®NG**	**Predicate Calprest®**
	0, 2.5, 12.5, 25, 50, 150 ng/mL	6.25, 12.5, 25, 50, 100, 200 ng/mL
Conversion factor	20	2.5
Reportable range	27.1–3000 mg/kg	15.6–500 mg/kg
Results interpretation	Normal: 27.1– <50 mg/kg Borderline: 50–120 mg/kg Abnormal: >120 mg/kg	Normal: <15.6–50 mg/kg Borderline: 50–120 mg/kg Abnormal: >120 mg/kg
ELISA Procedure : Sample incubation Conjugate incubation Substrate incubation	 60 min 30 min 15 min	 45 min 45 min 30 min

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

- CLSI EP5-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition
- CLSI EP6-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-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition
- Guidance for Industry and FDA Staff–Class II Special Controls Guidance Document: Fecal Calprotectin Immunological Test Systems

L. Test Principle:

Calprest®NG is a colorimetric enzyme-linked immunosorbent assay (ELISA). The test is performed on stool samples collected without preservatives. An extract is prepared by combining the stool sample with extraction buffer and mixing for 30 minutes followed by centrifugation. The assay uses a polyclonal rabbit antibody against calprotectin as the capture antibody. Calprotectin present in the diluted stool extract sample is bound by the antibody adsorbed onto the surface of the microtiter plate. HRP-conjugated antibodies bind to the captured calprotectin. The enzyme catalyzes the conversion of the substrate (TMB) to a colored product and the optical density (OD) at 450 nm 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 six calibrators and converted to mg of calprotectin per kg of stool using a conversion factor supplied in the package insert.

M. Performance Characteristics:

1. Analytical performance: All results presented below were within the sponsor's pre-determined acceptance criteria for each study.

   a. Precision/Reproducibility:

   Precision: The precision of the Calprest®NG assay was evaluated by extracting seven stool samples containing various concentration of calprotectin. Each extracted sample was tested in duplicate, two runs per day for 10 days by three different operators using one lot of reagent (total of 120 replicates per sample). The results are summarized in the table below

	Mean (mg/kg)	Within-Run		Between-Run		Between-Day		Between-Operator		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	42.60	1.28	3.0	4.30	10.1	0.00	0.0	0.55	1.3	4.52	10.6
2	170.88	3.40	2.0	6.77	4.0	5.51	3.2	0.80	0.5	9.40	5.5
3	248.74	6.44	2.6	11.98	4.8	12.21	4.9	0.00	0.0	18.28	7.3
4	741.60	17.38	2.3	43.12	5.8	55.37	7.5	0.00	0.0	72.30	9.7
5	1006.17	41.69	4.1	39.67	3.9	55.80	5.5	28.24	2.8	84.98	8.4
6	1193.10	52.91	4.4	73.33	6.1	118.78	10.0	60.32	5.1	161.01	13.5
7	2267.29	223.08	9.8	83.22	3.7	155.52	6.9	58.33	2.6	290.31	12.8

   Reproducibility: The lot-to-lot reproducibility was done by extracting four stool samples with calprotectin concentrations of 64.7, 259.2, 778.2, and 2005.2 mg/kg. Each extracted sample was tested in replicates of five using three different lots of the reagents. Mean and %CV for each sample were calculated and %CV values for between-lot reproducibility were 3.1%–7.3% for all samples.

   The site-to-site reproducibility was evaluated by testing a total of eight stool samples at three sites. The samples were run in replicates of five per day for five days at each site, and a total of 75 data points were generated for each sample. Data were analyzed for within-run, between-run, within-site, between site and total reproducibility. The results are summarized in the table below:

	Mean (mg/kg)	Within-Run		Between-Day		Within-Site		Between-Site		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	36.54	3.69	10.1	3.54	9.7	5.11	14.0	0.00	0.0	5.11	14.0
2	62.08	4.19	6.8	3.78	6.1	5.64	9.1	6.60	10.6	8.68	14.0
3	356.84	12.00	3.4	27.57	7.7	30.07	8.4	42.34	11.9	51.93	14.6
4	433.80	13.43	3.1	62.56	14.4	63.99	14.8	8.47	2.0	64.55	14.9
5	682.76	35.88	5.3	34.21	5.0	49.57	7.3	61.45	9.0	78.95	11.6
6	1180.64	71.08	6.0	139.04	11.8	156.16	13.2	0.00	0.0	156.16	13.2
7	1629.44	96.29	5.9	191.96	11.8	214.76	13.2	73.04	4.5	226.84	13.9
8	2152.20	173.58	8.1	254.59	11.8	308.13	14.3	50.20	2.3	312.19	14.5

The extraction reproducibility was evaluated by using four stool samples with calprotectin concentrations of 29.6, 52.7, 227.0, and 1973.3 mg/kg. Each stool sample was extracted 10 times and each stool extract was tested in duplicate. The %CVs for these four stool samples are 14.6%, 11.1%, 6.8%, and 5.9%, respectively.

b. Linearity/assay reportable range:

Linearity: The linearity of Calprest®NG was evaluated according to the CLSI guideline EP6-A. Four sample dilution series each with nine dilution levels were prepared by serially diluting the high concentration stool extract sample pools with the low concentration stool extract sample pools. Each dilution was tested in triplicate. The results are summarized as follows:

Sample	Range (mg/kg)	Slope (95% CI)	Intercept (95% CI)	R^2	% Recovery
1	68.8–519.4	1.01 (0.92–1.11)	3.01 (-27.0–33.1)	0.99	90.9–112.8
2	27.4–198.9	1.03 (0.97–1.08)	-1.05 (-8.08–5.98)	1.00	93.0–108.3
3	365.6–3066.0	0.97 (0.86–1.08)	52.83 (-181.5–287.2)	0.99	86.9–107.1
4	25.2–2832.9	1.05 (0.98–1.05)	6.48 (-11.59–12.89)	0.99	97.9–110.4
All	25.2–3066.0	1.01 (0.99–1.04)	9.28 (-26.54–45.10)	0.99	86.9–112.8

The Calprest®NG is linear for the claimed measuring range of 27.1–3000 mg/kg.

Accuracy/Recovery study: Extracts from seven stool samples were each spiked with the calibrator material. For the recovery of spiked samples expected to be lower than 300 mg/kg, Calprest®NG Calibrator S2 (2.5 ng/ml, 50 mg/kg) was used; for the recovery of spiked samples expected to be higher than 300 mg/kg, sample diluent was used 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)	9.3	51.7	162.7	307.7	458.3	1204.0	1975.2
Spike Value (mg/kg)	32.3	32.3	32.3	115.0	115.0	115.0	115.0
Theoretical (Base+Spike) (mg/kg)	41.7	84.0	195.0	422.7	573.3	1319.0	2090.2
Observed (Base+Spike) (mg/kg)	42.3	79.7	200.7	425.3	592.3	1397.7	2343.8
% Recovery	101.6	94.8	102.9	100.6	103.3	106.0	112.1

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 K130945.

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 0, 2.5, 12.5, 25, 50, and 150 ng/mL. The target values for controls range from 10–20 ng/mL for the Low Control and from 30–70 ng/mL for the High Control.

Stability:

Kit stability: The real time stability was performed using three lots of kits (including calibrators and controls) stored at 2–8°C. Data were collected at time points of 0, 1, 6, 12, 15, 18 months. The accelerated stability study was performed by using three lots of kits stored at 37°C for four weeks. The results support stability of the kits under the recommended storage of 2–8°C for up to 12 months.

Open vial stability: The study was done to evaluate the reagent stability after opening. Three kit lots of Calprest®NG were stored at 2–8°C after first opening. The study 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 three months stability for 1X washing buffer.

Sample stability: Sample stability was established per K130945. Five stool samples which cover the measuring range were stored at 2-8°C up to seven days. Data were collected at time points of 0, 2, 4, 7 days. The results support that stability of stool sample stored at 2–8°C up to four days.

Analyte (Calprotectin) stability: The study was done to determine the stability of extracted stool samples when stored -20°C. The study supports that the stool extracts are stable up to three months when stored at -20°C.

d. Detection limit:

The limit of blank (LoB) was determined by assaying five blank samples with 12 replicates per sample to generate 60 total replicates. LoB was calculated as the 95th percentile using the non-parametric method. The LoB value was determined to be 17.5 mg/kg.

The limit of detection (LoD) was determined by assaying five extracted stool samples with low calprotectin level with 12 replicates per sample to generate 60 total replicates. The LoD value was calculated as the LoB + 1.645 x SD of the replicates for the low level samples and was determined to be 23.4 mg/kg.

The limit of quantitation (LoQ) was determined based on the LoD test results. The low level extracted stool samples were tested on a reference method (Calprest) to obtain bias and calculate Total Error (TE). The LoQ was determined as 27.1 mg/kg with the TE of 10.5 mg/kg.

e. Analytical specificity:

Interference studies were performed according to CLSI guideline EP07-A2 using five pooled stool samples. The pooled samples included two high positives (with calprotectin concentrations of 2068.6 mg/kg and 674.7 mg/kg), one moderate positive (256.0 mg/kg), one sample within borderline range (79.3 mg/kg) and one negative (37.2 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 counts of 1.5×10^7 cfu/ml in stool samples.

Drugs and Nutrients: The data demonstrated that Calprest®NG 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 (Vitamin D: 1.1 U/50 mg stool, Vitamin A: 8.0 U/50 mg stool, Vitamin C: 0.05 mg/50 mg stool), and Vitamin E (0.10 mg/50 mg stool). The concentration level of tested drugs and nutrients was sufficient to reflect the average concentration to be expected in a patient's 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®NG.

f. Assay cut-off:

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

Calprotectin (mg/kg)	Interpretation	Suggested follow-up
< 27.1–50	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 182 stool samples including 123 samples from patients diagnosed with IBD (Crohn's disease, ulcerative colitis (UC), intermediate colitis (IC), suspected IBD, and diversion disease) and 59 samples from patients with IBS, chronic diarrhea, recurrent abdominal pain (RAP), celiac disease, and other conditions were assayed using Calprest®NG and the predicate device Calprest® in accordance with the instructions for use in the package inserts. From the total sample size of 182, results for 157 samples were within the measuring ranges of both assays. The regression analysis of the results comparing the Calprest®NG and the predicate Calprest® is shown below:

N	Range (mg/kg)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
157	15.60–494.57	0.92 (0.84–1.01)	0.13 (-4.64–4.90)	0.84

The observed low correlation coefficient of the regression analysis is likely due to the different detection antibody and detection system used for the Calprest NG and the predicate.

Additional method comparison analysis was performed using the cut-offs of both assays. The results are shown below:

		Predicate (Calprest®)			
		Positive	Borderline	Negative	Total
Calprest® NG	Positive	73	3	0	76
	Borderline	2	35	0	37
	Negative	1	3	40	44
	Total	76	41	40	157

Borderline as positive:
Positive agreement (95% CI): 96.6% (91.5–98.7%)
Negative agreement (95% CI): 100.0% (91.2–100.0%)
Total agreement (95% CI): 97.5% (93.6–99.0%)

Borderline as negative:
Positive agreement (95% CI): 96.1% (89.0–98.6%)
Negative agreement (95% CI): 96.3% (89.7–98.7%)
Total agreement (95% CI): 96.2% (91.9–98.2%)

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and specificity

The 273 stool samples used in the clinical validation for the Calprest®NG assay include 130 samples from patients diagnosed with IBD and 143 samples from patients with IBS and other diseases/conditions with gastrointestinal symptoms. Among the 130 IBS samples, 71 were from patients diagnosed with Crohn's disease including six samples from patients in remission, 41 were from patients diagnosed with UC including seven samples from patients in remission, 12 were from patients with IC, and six were from patients with diversion disease. The patients with IBD were diagnosed by clinical findings and/or confirmed with colonoscopy. Among 143 non-IBD samples, 51 samples were from patients diagnosed with IBS and 92 samples from patients with chronic diarrhea, RAP, celiac disease and other non-IBD conditions. The patients with IBS were defined based on the Rome III diagnostic criteria. The clinical performance of Calprest®NG was evaluated for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The results are summarized in the following tables:

		Clinical Diagnosis of IBD		
		Positive	Negative	Total
Calprest® NG	Abnormal (>120 mg/kg)	108	3	111
	Borderline (50–120 mg/kg)	15	11	26
	Normal (<50 mg/kg)	7	129	136
	Total	130	143	273

Borderline considered as abnormal:
Sensitivity (95% CI): 94.6% (89.2–97.8%)
Specificity (95% CI): 90.2% (8 4.1–94.5%)
PPV (95% CI): 89.8% (83.4–94.3%)
NPV (95% CI): 94.9% (89.7–97.9%)

Borderline considered as normal:
Sensitivity (95% CI): 83.1% (75.5–89.1%)
Specificity (95% CI): 97.9% (94.0–99.6%)
PPV (95% CI): 97.3% (92.3–99.4%)
NPV (95% CI): 86.4% (80.2–91.3%)

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

Røseth *et al*. (1992)*.

Ninety-two 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 89 out of 92 samples (96.7 %) from normal healthy people tested negative and the remaining three samples fell in the range of 56.3–64.4 mg/kg. Of the 42 samples from patients with CD, UC, IBS and IC, 39 (92.9%) were 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.