July 10, 2019

Eurospital S.p.A.
Chiara Pelillo
Regulatory Affairs Assistant
Via Flavia 122
Trieste, 34147
Italy

Re: K191592
Trade/Device Name: CalprestNG, EasyCal
Regulation Number: 21 CFR 866.5180
Regulation Name: Fecal Calprotectin Immunological Test System
Regulatory Class: Class II
Product Code: NXO
Dated: June 11, 2019
Received: June 14, 2019

Dear Chiara Pelillo:

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,

Doug Jeffery, Ph.D.
Branch Chief
Immunology and Flow Cytometry Branch
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)*
K191592

Device Name
CalprestNG

Indications for Use *(Describe)*
CalprestNG is a quantitative ELISA for detecting concentration of fecal calprotectin. CalprestNG 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.

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.**

This section applies only to requirements of the Paperwork Reduction Act of 1995.

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This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

## 1. Administrative data

| | |
|---|---|
| Submitter: | Eurospital S.p.A. |
| Submitter's Address: | Via Flavia, 122<br>34147 Trieste<br>Italy |
| Submitter's contact: | Chiara Pelillo<br>Regulatory Affairs Specialist<br>Phone: 0039 0408997266<br>Fax: 0039 040280944<br>Email: cpelillo@eurospital.it |
| Device name: | Proprietary name: Calprest®NG<br>Common name: Fecal Calprotectin enzyme-linked immunosorbent assay with EasyCal |
| Regulation Description: | Fecal Calprotectin Immunological Test System |
| Regulation Medical Specialty | Immunology |
| Review Panel | Immunology |
| Product Code | NXO |
| Regulation Number | 21 CFR 866.5180 |
| Device Class | 2 |
| Date of 510(k) Preparation: | June 11th, 2019 |

## 2. Predicate device

Calprest®NG (using manual extraction), 510 (k) number K160447. Date of clearance: November 10th, 2016.

## 3. Device description

Calprest®NG is an enzyme-linked immunosorbent assay (ELISA) system with colorimetric detection based on the use of antibodies against calprotectin. Calprotectin present in the diluted sample is bound by the antibody adsorbed to the surface of the plastic well. The enzyme conjugated antibody binds to the captured antigen and subsequently the enzyme catalyzes the conversion of the substrate to a colored product. The intensity of the color is proportional to the amount of conjugate bound, and thus to the amount of captured calprotectin. Concentration of calprotectin in the samples is calculated using the provided standards.

EasyCal is a single-use device for stool sample pre-analytical processing that allows the extraction of calprotectin from the specific amount of collected fecal sample required to perform Eurospital's Calprest® and Calprest®NG assays.

The device consists of a tube, containing 2.8 ml of extraction solution, a stick shaped with seven grooves for collecting the sample. The upper end is made up by two components which can be removed by opposite rotations. The screw cap (white) connected to the shaped stick traps the sample excess and can be then removed by counter-clockwise rotation. Once the extraction procedure has been completed, the sample can be transferred to an automated ELISA instrumentation, placing it directly into the sample rack. EasyCal allows an easy, reliable and reproducible way to sample from primary containers and analyze the extract directly from the device, without the need to weight the stool sample.

The EasyCal kit provides the materials listed below:

- One hundred (100) EasyCal kits.

Each tube of EasyCal contains:

- 2,8 mL of extraction buffer, colorless, ready to use.

## 4. Intended use and Indications for use

Calprest®NG is a quantitative ELISA for detecting concentration of fecal calprotectin. Calprest®NG 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.

## 5. Pre-Analytical Processing

The device object of the present submission is the same in terms of chemical composition, formulation, principle of operations to the device cleared in 2016 (K160447). The change is due to the introduction of the EasyCal, which is an optional single-use device for stool sample pre-analytical processing sold separately from Calprest®NG. It allows the extraction of calprotectin from the specific amount of collected fecal sample required to perform Eurospital's Calprest® and Calprest®NG calprotectin determination assays.

## 6. Substantial equivalence

The new device and the predicate Device (K160447) have the same Intended Use/Indications for Use, same fundamental technological characteristics, principles of operation and comparable performances characteristics. The modifications consist in the addition of an optional accessory, the EasyCal device, which is a single-use device for stool sample pre-analytical processing as alternative to the manual extraction procedure. It allows the extraction of calprotectin from the specific amount of collected fecal sample required to perform Eurospital's Calprest® and Calprest®NG calprotectin determination assays.

As evidenced by Risk Assessment and Validation Studies, no new questions were raised regarding the Safety, Effectiveness, Performance, Indication for Use, Technology and the Principles of Operation. Therefore, Calprest®NG in combination with EasyCal as stool extraction device performs equivalently as the predicate device, Calprest®NG in combination with manual extraction procedure Device.

## 7. Comparison to predicate device

| **Similarities** | | |
|---|---|---|
| **Item** | **New device** | **Predicate (K160447)** |
| | **Calprest®NG with EasyCal** | **Calprest®NG** |
| Intended use | Calprest®NG is a quantitative ELISA for detecting concentration of fecal calprotectin. Calprest®NG 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. | Calprest®NG is a quantitative ELISA for detecting concentration of fecal calprotectin. Calprest®NG 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. |
| Assay methodology | ELISA | Same |
| Antigen | Calprotectin | Same |
| Conjugate | Horseradish peroxidase | Same |
| Shelf life | 18 months | Same |
| Sample type | Extracted human stool | Same |
| Sample dilution | 1:20000 | Same |
| Sample units | mg/kg (mg of calprotectinper kg of stool) | Same |
| Detection/ operating principle | Colorimetric assay | Same |
| Analytical Measuring Range | 27.1 - 3000 mg/kg | Same |
| Calibration | 6 calibrators: 0, 2.5, 12.5, 25, 50, 150 ng/ml | Same |

| **Differences** | | |
|---|---|---|
| **Item** | **New device** | **Predicate (K160447)** |
| | **Calprest®NG with EasyCal** | **Calprest®NG** |
| Pre-analytical processing procedure | Manual extraction procedure<br>or<br>Stool extraction procedure using EasyCal, a tube containing 2.8 ml of extraction solution and a stick shaped with seven grooves for collecting the sample. | Manual extraction procedure. |

## 8. Analytical performance characteristics

Same as approved in 510(k) submission #K160447 plus the following additional data.

### 8.1. Stool extraction method comparison: EasyCal vs manual extraction procedure

One hundred (100) stool samples containing different levels of calprotectin evenly distributed and covering the quantification range of Calprest®NG, were extracted in parallel with the EasyCal and using the manual extraction procedure. All the extracted stool samples were tested in duplicate for calprotectin concentration using the Calprest®NG assay according to the package insert. The following parameters were analyzed with Passing-Bablok regression and Analyse-it software: intercept, slope, predicted bias at cut-off, 95% CI, correlation index (r). The study was conducted according to the CLSI EP09c "Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 3rd Edition" (June 2018).

The results are summarized in the table below:

Table 1. **Method comparison Calprest®NG with EasyCal (y) versus Calprest®NG with manual extraction procedure (x)**

| **Passing-Bablok regression** | Calprest®NG with EasyCal = 0.8495 + 0.9778 Calprest®NG manual weight |
|---|---|
| **Slope (95% CI)** | 0.9778 (0.901 to 1.003) |
| **Y-intercept (95% CI)** | 0.8495 (-0.8360 to 3.978) |
| **Correlation - r** | 0.954 |
| **Bias at 120 mg/kg (95% CI)** | -1.5% (-9.0% to 1.5%) |

| **Qualitative agreement Calprest NG with EasyCal versus Calprest NG with manual weight** | | | |
|---|---|---|---|
| | **Calprest NG with EasyCal** | | |
| **Borderline** | **Negative** | **Positive** | **Total** |
| 9 | 0 | 0 | 9 |
| 1 | 25 | 0 | 26 |
| 3 | 0 | 62 | 65 |
| 13 | 25 | 62 | 100 |

| | |
|---|---|
| **Borderline as positive** | |
| Negative Agreement (95% CI) | 96.2% (81.1 to 99.3%) |
| Positive Agreement (95% CI) | 100.0% (95.1 to 100%) |
| Total Agreement (95% CI) | 99.0% (94.6 to 99.8%) |
| **Borderline as negative** | |
| Negative Agreement (95% CI) | 100.0% (90.1 to 100%) |
| Positive Agreement (95% CI) | 95.4% 87.3 to 98.4%) |
| Total Agreement (95% CI) | 97.0% (91.5 to 99.0%) |

The results demonstrate that the performances of Calprest®NG with EasyCal is comparable to Calprest®NG with manual extraction procedure.

### 8.2. Stool sample collection performance of EasyCal

To validate the amount of fecal material that is collected by the EasyCal, five different human stool samples, encompassing different stool consistencies, were collected using EasyCal in replicates of five by three independent operators. Data were analyzed with Analyse-it software to calculate weight distribution.

The results obtained confirm that the stool sample mean weight collected by EasyCal is 56 mg.

### 8.3. Reproducibility study: Extraction Reproducibility

Eight (8) stool samples covering the quantification range of Calprest®NG assay were extracted and tested in replicate of five, once a day, for five days, by three different operators (scheme 5x5x3).

CV(%) and standard deviation were calculated for each set of analysis performed with Analyse-it software.

The study was conducted according to the CLSI EP05A3 "Evaluation of Precision of Quantitative Measurement Procedures, 3rd Edition" (October 2014).

| Faecal extracts tested with Calprest® NG | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Repeatability | | Between-Day | | Within Operator | | Between Operator | | Within Laboratory | |
| Sample ID | N | Mean (mg/kg) | SD (mg/kg) | CV (%) | SD (mg/kg) | CV (%) | SD (mg/kg) | CV (%) | SD (mg/kg) | CV (%) | SD (mg/kg) | CV (%) |
| Sample 1 | 75 | 32.61 | 2.03 | 6.2% | 0.76 | 2.3% | 2.16 | 6.6% | 0.69 | 2.1% | 2.27 | 7.0% |
| Sample 2 | 75 | 57.77 | 2.22 | 3.8% | 5.10 | 8.8% | 5.56 | 9.6% | 0.51 | 0.9% | 5.59 | 9.7% |
| Sample 3 | 75 | 232.86 | 6.40 | 2.7% | 26.02 | 11.2% | 26.79 | 11.5% | 9.61 | 4.1% | 28.47 | 12.2% |
| Sample 4 | 75 | 335.40 | 10.48 | 3.1% | 47.95 | 14.3% | 49.08 | 14.6% | 27.56 | 8.2% | 56.29 | 16.8% |
| Sample 5 | 75 | 529.88 | 18.46 | 3.5% | 95.44 | 18.0% | 97.21 | 18.3% | 0.00 | 0.0% | 97.21 | 18.3% |
| Sample 6 | 75 | 871.67 | 28.92 | 3.3% | 44.80 | 5.1% | 53.32 | 6.1% | 50.52 | 5.8% | 73.46 | 8.4% |
| Sample 7 | 75 | 1284.61 | 70.34 | 5.5% | 87.01 | 6.8% | 111.89 | 8.7% | 0.00 | 0.0% | 111.89 | 8.7% |
| Sample 8 | 75 | 2539.14 | 174.85 | 6.9% | 75.74 | 3.0% | 190.55 | 7.5% | 17.25 | 0.7% | 191.33 | 7.5% |

Reproducibility of results obtained with Calprest® with EasyCal is confirmed.

### 8.4. Samples stability and handling

Eight (8) stool samples covering the quantification range of Calprest®NG assay were extracted with EasyCal and tested, using Calprest®NG assay, for stability of the extracted stool sample at 2-8 °C up to 21 days (day 0, day 1, day 3, day 7, day 14 and day 21), at room temperature up to 73h (0h, 16h, 24h, 48h, 72h and 73h) and after up to 5 freeze/thaw cycles (0 cycle, 1 cycle, 2 cycles, 3 cycles, 4 cycles and 5 cycles). Data related to the extracted stool samples were analyzed compared to those obtained at time zero/cycle zero and the percentage of recovery was calculated.

All samples met the acceptance criteria at 21 days, 73 hours and after five cycle of freeze-thaw, therefore, the extracted stool samples in the EasyCal can be stored: up to 14 days at 2-8 °C and up to 72h at RT. Even though after five freeze-thaw cycles all samples met the acceptance criteria, we recommend not to exceed 4 freeze-thaw cycles to remain in a safety stability condition.

### 8.5. EasyCal device stability

#### 8.5.1. Shelf life (Real time stability study)

To evaluate the real time stability of EasyCal, pH values for the extraction buffer were assessed and variation of the volume (evaporation) was verified by weighting the devices at time zero and up to 25 months (0; 7; 13; 19; 24 and 25 months) on three lots. Data were compared to those obtained at time zero.

The pH measures were between 7.77 and 7.84 (mean 7.80) and volume variation expressed in percentage was between 99.4% and 100.6% (mean 100.0%); all measures met the acceptance criteria up to 25 months.

Additionally, to confirm that EasyCal stability guarantees no changes in Calprest®NG's performances, twelve (12) samples were extracted with three lots of EasyCal stored at 2-8 °C up to 25 months (24 and 25 months) and with a freshly produced one for comparison. The extracted stool samples were tested using Calprest®NG assay.

All acceptance criteria were met at 25 months, therefore the EasyCal device is stable for 24 months when kept at 4 °C and does not affect Calprest®NG's performances.

#### 8.5.2. Calprest®NG performances with EasyCal stored at room temperature

Eight (8) stool samples, covering the quantification range of Calprest®NG assay, were extracted with three different lots of EasyCal stored at 2-8 °C or at room temperature up to 73h and tested using Calprest®NG assay. The results obtained with the EasyCal stored at room temperature were compared to those obtained with the EasyCal stored at 2-8 °C and the percentage of recovery calculated.

All acceptance criteria were met, therefore, the device is stable for 72h at room temperature and doesn't affect Calprest®NG performances.

## 9. Clinical performance characteristics

Same as approved in 510(k) submission # K160447

## 10. Conclusions

The Calprest®NG, with EasyCal is a modification of existing and legally marketed IVD device Calprest®NG. The modification consist in the introduction of a stand-alone optional accessory, the EasyCal, which is a single-use device for stool sample pre-analytical processing. It allows the extraction of calprotectin from the specific amount of collected fecal sample required to perform Eurospital's Calprest®NG calprotectin determination assays. This modification does not affect their intended use either the technological characteristics of the cleared Calprest®NG assay (K160447).

The design validation of Calprest®NG plus EasyCal has been performed following same standards and verification-validation testing already approved for Calprest®NG.

The IVD medical device design validation revealed compliant and without affecting the requirements of safety and effectiveness of Calprest®NG.

Therefore, the Calprest®NG plus EasyCal is substantially equivalent to the cleared Calprest®NG (K160447).