# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY

**A. 510(k) Number:**
k050007

**B. Purpose for Submission:**
New device.

**C. Measurand:**
Fecal calprotectin

**D. Type of Test:**
Quantitative, ELISA

**E. Applicant:**
Genova Diagnostics

**F. Proprietary and Established Names:**
PhiCal™ Test

**G. Regulatory Information:**

1. Regulation section:
   None
2. Classification:
   De novo
3. Product Code:
   NXO, Calprotectin, fecal
4. Panel:
   Immunology (82)

**H. Intended Use:**

1. Intended use(s):
   The PhiCal™ test is a quantitative ELISA for measuring, in human stool, concentrations of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The PhiCal™ test 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.
2. Indication(s) for use:
   The PhiCal™ test is a quantitative ELISA for measuring, in human stool, concentrations of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The PhiCal™ test 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; when used in conjunction with other diagnostic testing and the total clinical picture.
3. Special condition for use statement(s):
   The device is for prescription use only.
4. Special instrument requirements:
   ELISA reader (405 nm filter), digital scale (40-150 mg), vortex mixer, shaker, micro-centrifuge

**I. Device Description:**

The PhiCal test assay consists of: microtiter plate coated with polyclonal rabbit antibodies for calprotectin (12 strips, 8 wells per strip); alkaline phosphatase labeled rabbit anti-calprotectin IgG in buffer with Proclin 300; substrate in buffer; 20X washing solution; 10X dilution solution with Proclin 300; 5X extraction solution with Proclin 300; 5 vials of calprotectin solution at concentrations of 6.25, 12.5, 25, 50 and 100 ng/mL; a low and a high control.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):
   Not applicable
2. Predicate K number(s):
   Not applicable
3. Comparison with predicate:

| **Similarities** | | |
|---|---|---|
| Item | Device | Predicate |
| | | |

| **Differences** | | |
|---|---|---|
| Item | Device | Predicate |
| | | |

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

None referenced

**L. Test Principle:**

The test is performed on random stool samples, collected without preservatives. The samples should be tested within 7 days, or frozen at -20° C until tested. An extract is prepared by combining approximately 0.1 gm of stool with 5 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).

The assay uses a polyclonal rabbit antibody against calprotectin as the capture antibody in an enzyme linked immunosorbent assay system. Calprotectin present in the diluted sample is bound by the antibody adsorbed onto the surface of the microtiter plate. The enzyme conjugated antibodies (rabbit IgG antibodies against calprotectin) bind to the captured antigen. A substrate (alkaline phosphatase) is added and subsequently the enzyme catalyses 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. The concentration of calprotectin in the samples is interpreted from a standard curve using 5 calibrators.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:
   *i. Precision/Reproducibility:*
   Intra-Assay precision was determined by extracting one low and one high matrix sample, and assaying each extract twenty times within a single assay run. Precision is calculated as the % CV obtained for each level. Respective CV's were 4.07% and 6.17%. Additionally 12 samples were

assayed in 6 replicates within a singly assay run. Range of %CV was 2.9% to 14.3%. The resultant %CVs demonstrates that the PhiCal Test is precise in the reportable range.

| Calprotectin Intra-Assay Precision ( g/g) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Analysis | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| | Mean | 177.5 | 55.3 | 229.4 | 31.1 | 96.9 | 106.8 | 17.5 | 18.7 | 20.8 | 182.8 | 55.6 | 60.2 |
| | Std Dev | 5.7 | 6.6 | 6.7 | 2.3 | 11.5 | 9.4 | 0.8 | 2.7 | 1.9 | 10.8 | 3.9 | 2.5 |
| | %CV | 3.2 | 12.0 | 2.9 | 7.4 | 11.9 | 8.8 | 4.7 | 14.3 | 9.2 | 5.9 | 7.0 | 4.1 |

Inter-assay precision was assessed by evaluating results for six different pools of stool. Points are from 20 separate extraction and assay runs. The %CV range was 8.9% to 18.1%. Ten different samples (5 positive, 5 negative) were each extracted 5 separate times from individual stool aliquots. Each extract was then assayed in 4 replicates on 5 separate EIA runs performed on 5 different days. The %CV range was 5.8% to 20.1%. These results demonstrate the assay is reproducible within acceptable limits along the reportable range of the assay.

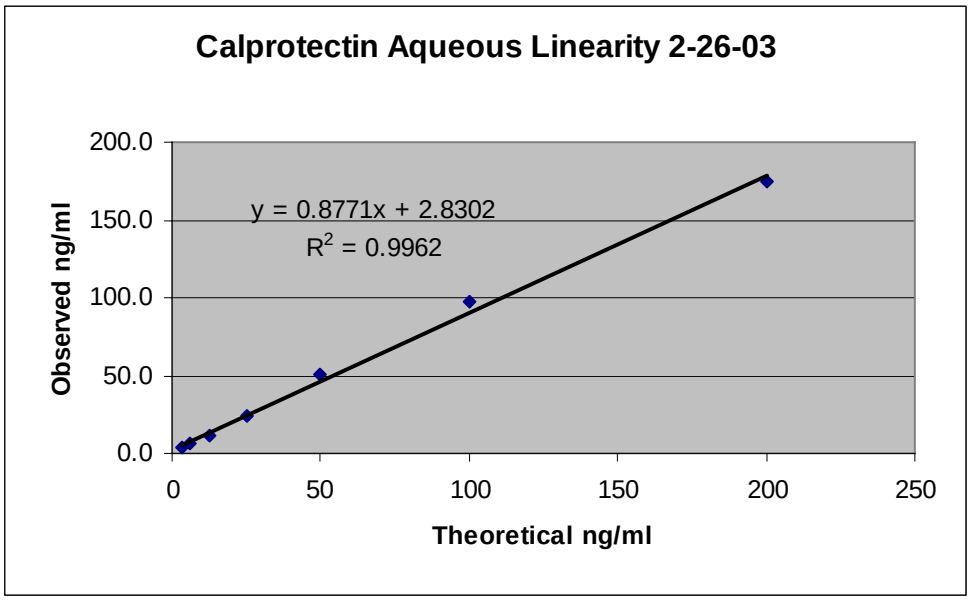
| Calprotectin Inter-Assay Precision ( g/g) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analysis | Replicate | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 |
| | Mean | 36.9 | 12.9 | 16.6 | 20.2 | 26.9 | 131.7 | 188.2 | 89.8 | 101.1 | 63.3 |
| | Std Dev | 2.1 | 2.1 | 2.5 | 0.9 | 2.0 | 10.2 | 36.9 | 10.4 | 13.3 | 12.7 |
| | %CV | 5.8 | 16.7 | 14.9 | 4.4 | 7.6 | 7.7 | 19.6 | 11.6 | 13.2 | 20.1 |

Extraction Repeat Reproducibility: Two samples that represent the low and high ends of the reportable range were tested. Each was extracted 24 times, and each extract was tested. Precision was evaluated according to the %CV obtained for each level. Respective CV's of 12.60% and 12.13% for the low and high levels demonstrate that results are reproducible along the reportable range.

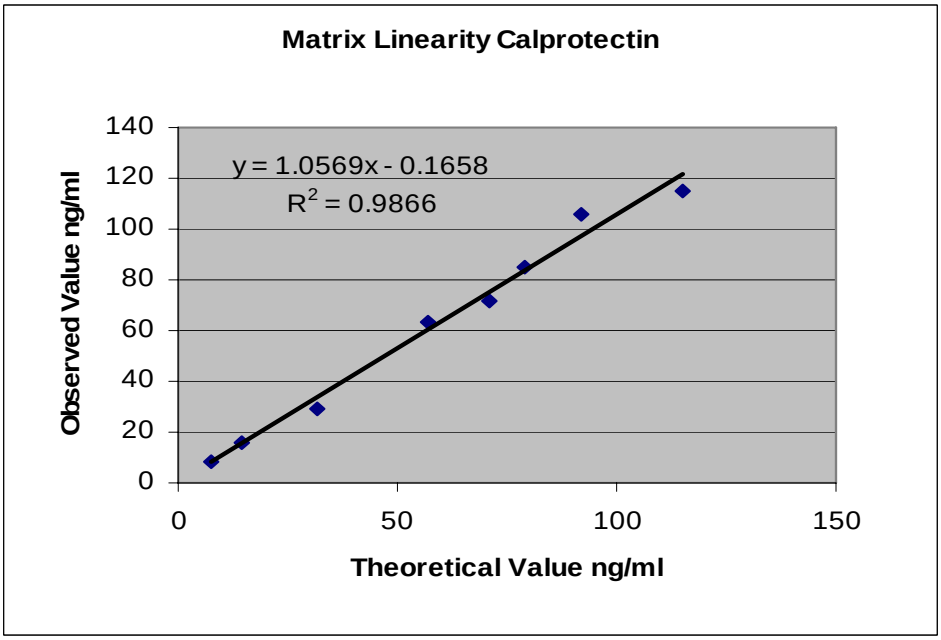
Split Sample Comparison Study:
Forty stool samples that spanned the reportable range of the test were assayed both by GENOVA and by the Fagerhol laboratory in Norway. The forty samples were assayed in small batches over 5 days to introduce day-to-day variability and to minimize any systematic error that might occur in a single run. The comparison plot showed $y = 0.9603x + 9.7691$, $r^2 = 0.9618$.

*ii. Linearity/assay reportable range:*
To confirm the reportable range of the PhiCal™ assay, standard curves were generated for both aqueous and matrix linearity. The results with serially diluted aqueous calprotectin in triplicate showed acceptable

linearity from 6.25 – 100 ng/ml. The following equation was obtained: Standard curve Aqueous, y = 0.8771x + 2.8302, $R^2$ = 0.9962.




The results with serially diluted fecal calprotectin in triplicate showed acceptable linearity and accuracy from 7.73 – 114.90 ng/ml. The following equation was obtained: Standard curve Matrix, y = 1.0569x – 0.1658, $R^2$ = 0.9866.




Accuracy/ Recovery

Extracts from five different stool samples were each spiked with calprotectin. The calprotectin used for the spike was obtained from established serum pools. The baseline extract for each sample was "spiked" with assay buffer to compensate for volume adjustments made to

the calprotectin-spiked extracts. Each of the spiked extracts was then assayed per kit protocol. Data is shown in table below.

| Calprotectin Recovery Data | | | | | |
|---|---|---|---|---|---|
| | **# 1a** | **# 2a** | **# 3a** | **# 4a** | **# 5a** |
| **Baseline (µg/g)** | 49.1 | 73.4 | 66.4 | 6.4 | 73.6 |
| **Spike Value (µg/g)** | 58.5 | 57.6 | 55.2 | 55.3 | 59.0 |
| **Theoretical (Base + Spike) (µg/g)** | 107.6 | 131.0 | 121.7 | 61.7 | 132.7 |
| **Observed (Base + Spike) (µg/g)** | 106.7 | 129.9 | 144.3 | 66.1 | 140.0 |
| **% Recovery** | **99.2** | **99.1** | **118.6** | **107.2** | **105.5** |

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

Calibrators:

The primary internal reference is calibrated by comparison to the "Gold Standard" preparation provided by Dr. Magne Fagerhol, Norway.

Analyte Stability:

Studies were conducted to determine the stability of calprotectin in neat stool and in stool extracts. To determine stability in neat stool, several temperature conditions were evaluated: stool collected fresh and subsequently stored at 2-8°C; stool collected fresh and then stored at -20°C; and stool collected fresh and subjected to variable temperatures. Stability in stool extract was evaluated by extracting fresh samples and then storing aliquots of the extract at -20°Cm where a different aliquot was thawed for each analysis over 11 days.

For the variable temperature stability study the collected samples were stored at 2-8°C for 24 hours, after which they were stored in an incubator at 37°C for approximately 21 hours. Once removed from the incubator they were left at room temperature for approximately 5½ hours then stored at 2-8°C for the remainder of the study. This study concluded that calprotectin levels stay relatively consistent (no trends observed) for up to 11 days after collection, under conditions that may be experienced during specimen transport. The studies support the specimen collection, transport and storage recommendations in the package insert.

*iv. Detection limit (functional sensitivity):*

Minimum Detection Limit:

The minimum detection limit (MDT) was determined by running 20 replicates of the assay buffer (treated as patient) on one assay plate. Two standard deviations were added to the mean OD reading, and this value was plugged into the calibration curve to arrive at the MDT. The result was below the functional sensitivity of the assay.

Functional sensitivity:

The functional sensitivity was estimated by determining a precision profile at the low concentration range and then selecting the concentration at

which a 20% CV is obtained. To establish the functional sensitivity for the assay, 3 concentrations of kit standards, 12.5 ng/mL, 6.25 ng/mL and 3.125 ng/mL (6.25 ng/mL standard diluted 1:2 with dilution buffer), were assayed in either triplicate or quadruplicate over 6 days. %CV for all three concentrations were <20%. For purposes of result reporting, the low end of the reportable range will be limited to the lowest calibrator, 6.25ng/mL. This corresponds to 15.6 mg calprotectin/kg stool after conversion at the typical sample dilution of 1:2500.

*v. Analytical specificity:*

Microorganisms: The following bacteria, which occur frequently in the stool, or are common to infectious diarrhea were evaluated as potential interfering factors – *Escherichia coli, Citrobacter freundii, and Klebsiella pneumoniae, Salmonella, Shigella, Yersinia.* The presence of these bacteria in stool samples does not interfere with the PhiCal Test.

Oral Pharmaceuticals: The following oral pharmaceuticals and nutritional supplements were tested for potential interference: Prednisone; Sulfamethoxazole, Pentasa; Prevacid; Vancomycin; Asacol; Azathioprine; Ciprofloxacin HCL; Ferrous Sulfate; Multiple vitamin; Vitamin E; Zelnorm. The concentration of the drug in the suspension was sufficient to approximate the concentration to be expected in a patient's stool based on the appropriate dosages and average stool volume per twenty four hours. No interference was noted.

Gastrointestinal Bleeding: Bleeding of as much as 100 mL per day would increase the fecal calprotectin concentration by no more than 6 mg/L (15 µg/g).[23]

*vi. Assay cut-off:*

See clinical cut-off.

2. Comparison studies:

*i. Method comparison with predicate device:*

Not applicable.

*ii. Matrix comparison:*

Not applicable.

3. Clinical studies:

*a. Clinical sensitivity and specificity:*

Fecal calprotectin results from the complete cohort of 908 subjects were used to calculate the overall clinical sensitivity and specificity of the PhiCal test. The 908 subjects included IBD n=255, IBS n=410, other bowel diseases n=82, IBD, and normal subjects n=161. The results and calculations are shown below:

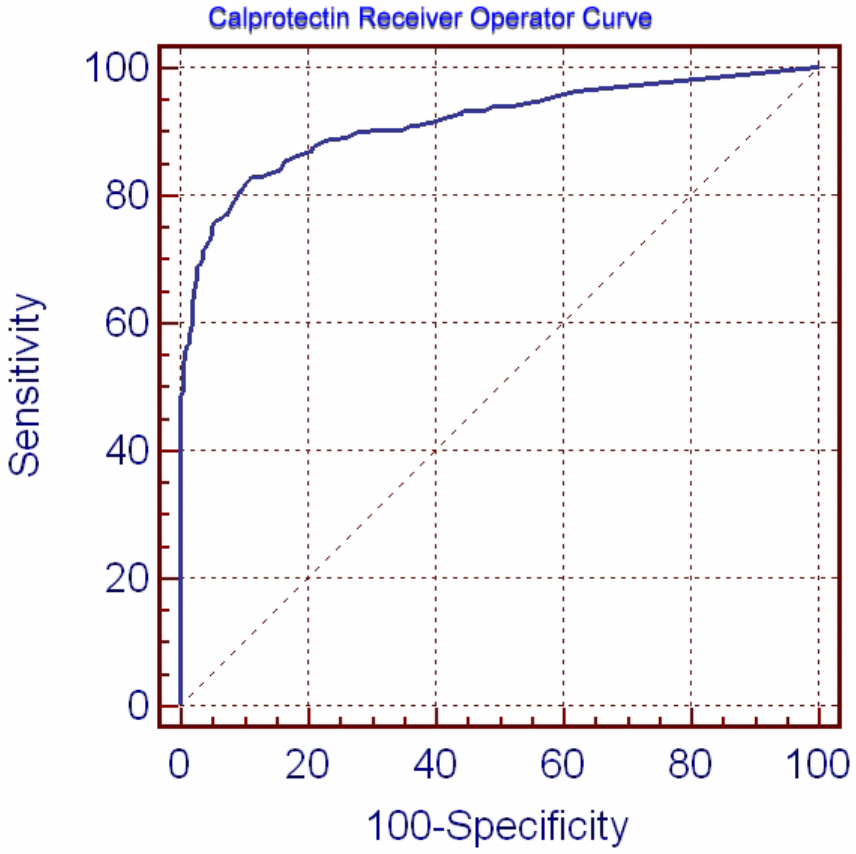
| **N = 908** | **IBD/ Inflamed "Organic"** | **IBS/ Normal "Non-Organic"** | **Totals** |
|---|---|---|---|
| >120 mcg/gm | 254 | 29 | 283 |
| 50-120 mcg/gm | 43 | 89 | 132 |

| | | | |
|---|---|---|---|
| <50 mcg/gm | 42 | 451 | 483 |
| **Totals** | 296+43 | 480+89 | 776+132 = 908 |

When borderline cases (n=132) are excluded from the calculations:
Clinical sensitivity: 86% (254/296)
Clinical specificity: 94% (451/480)

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

4. Clinical cut-off:
Establishing the cut-off for the assay was done by testing 124 normal sera. The mean value was 40µg/g, and with a standard deviation of 40µg/g, the upper cut point was 120µg/g. In addition, ROC analysis using results from the total 908 patients showed that 120 µg/g yielded 95% specificity and 70% sensitivity.




| Calprotectin Concentration | Interpretation | Follow-Up |
|---|---|---|
| <15.625 – 50 µg/g | Normal | None |
| 50 – 120 µg/g | Borderline | Re-evaluate @ 4-6 weeks |
| >120 µg/g | Abnormal | Repeat as clinically indicated |

5. Expected values/Reference range:
   The expected value in the normal population is <50 μg/g. The highest levels are found in Crohn's disease and ulcerative colitis with levels as high as five to several thousand times the upper limit for healthy individuals. Calprotectin is generally not elevated in healthy subjects or in irritable bowel syndrome but levels can overlap with those of organic disease.

**N. Proposed Labeling:**

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

**O. Conclusion:**

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.5180 with special controls. The special control guidance document, "Class II Special Controls Guidance Document: Fecal Calprotectin Immunological Test Systems" will be available shortly.