September 24, 2019

BÜHLMANN Laboratories AG
Roshana Ahmed
President
Quaras, LLC
2101 Camino Rey
Fullerton, California 92833

Re: K191718
Trade/Device Name: BÜHLMANN fCAL turbo and CALEX Cap
Regulation Number: 21 CFR 866.5180
Regulation Name: Fecal calprotectin immunological test system
Regulatory Class: Class II
Product Code: NXO
Dated: June 26, 2019
Received: June 26, 2019

Dear Roshana Ahmed:

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,

for

Douglas Jeffery, Ph.D.
Branch Chief
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)*

K191718

Device Name
BÜHLMANN fCAL turbo, CALEX Cap

Indications for Use *(Describe)*

The BÜHLMANN fCAL turbo is an in vitro diagnostic assay intended for the quantitative measurement of fecal calprotectin, a neutrophilic protein that is a marker of intestinal mucosal inflammation, in human stool. The BÜHLMANN fCAL turbo aids in the diagnosis of inflammatory bowel disease (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC) and aids in the differentiation of IBD from irritable bowel syndrome (IBS) in conjunction with other laboratory and clinical findings.

The BÜHLMANN CALEX Cap is a single use tube intended for the preparation of human stool samples to be used with the BÜHLMANN fCAL turbo.

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.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) Summary

I. Submitter

BÜHLMANN Laboratories AG
Baselstrasse 55
Schönenbuch CH-4124
Switzerland
Phone: +41 61 487 12 50

Contact Person: Laura Zurbrügg
Date Prepared: September 11, 2019

II. Device

Device Proprietary Names:	BÜHLMANN fCAL® turbo, CALEX® Cap
Common or Usual Name:	Fecal calprotectin immunological test system
Classification Name:	Calprotectin, Fecal
Regulation Number:	21 CFR 866.5180
Product Code:	NXO
Device Classification:	II

III. Predicate Device

Substantial equivalence is claimed to the following device:

- BÜHLMANN fCAL® turbo, K190784, BÜHLMANN Laboratories AG

IV. Device Description

The BÜHLMANN fCAL® turbo, a particle-enhanced turbidimetric immunoassay (PETIA), is performed using patient stool extracts collected without preservatives. Calprotectin within the sample extract mediates immunoparticle agglutination; sample turbidity is proportional to calprotectin concentration. The detected light absorbance allows quantification of calprotectin concentration via interpolation of an established calibration curve. The assay is validated for use on clinical chemistry analyzers such as the Roche cobas® c501/c502 platforms.

The BÜHLMANN fCAL® turbo Reagent Kit is to be used in conjunction with the BÜHLMANN fCAL® turbo Calibrator Kit and BÜHLMANN fCAL® turbo Control Kit, which are available separately.

Sample extracts may be prepared using manual weighing extraction methods or the CALEX® Cap.

The CALEX® Cap is a single use tube filled with extraction buffer. The sampling pin houses a dosing tip which is used to obtain sufficient stool sample for the extraction process. The extraction method leads to stool specimen extracts which can be measured directly using the BÜHLMANN fCAL® turbo assay.

V. Indications for Use

The BÜHLMANN fCAL® turbo is an *in vitro* diagnostic assay intended for the quantitative measurement of fecal calprotectin, a neutrophilic protein that is a marker of intestinal mucosal inflammation, in human stool. The BÜHLMANN fCAL® turbo aids in the diagnosis of inflammatory bowel disease (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC) and aids in the differentiation of IBD from irritable bowel syndrome (IBS) in conjunction with other laboratory and clinical findings.

The BÜHLMANN CALEX® Cap is a single use tube intended for the preparation of human stool samples to be used with the BÜHLMANN fCAL® turbo.

VI. Comparison of Technological Characteristics

The subject and predicate device utilize the same exact assay, controls, and calibrators. The only difference between the two products is the provision of the CALEX® Cap as an alternative sample extraction method to manual weighing.

The tables below compare key technological features between the subject and predicate devices.

Technological comparison

Comparison of Assay

	BÜHLMANN fCAL® turbo and CALEX® Cap	**BÜHLMANN fCAL® turbo (K190784)**
Analyte	Human fecal calprotectin (MRP8/14)	Human fecal calprotectin (MRP8/14)
Assay format	Quantitative	Quantitative
Specimen type	Human stool	Human stool
Clinical Decision Thresholds	Normal: < 80 µg/g Gray-zone/borderline: 80 - 160 µg/g Elevated: > 160 µg/g	Normal: < 80 µg/g Gray-zone/borderline: 80 - 160 µg/g Elevated: > 160 µg/g
Method	PETIA	PETIA
Automation	Automated	Automated
Solid phase	Polystyrene nanoparticles (beads)	Polystyrene nanoparticles (beads)
Detection method	Automated clinical chemistry analyzer read at 546 nm	Automated clinical chemistry analyzer read at 546 nm
Analyte-specific antibody components	Polyclonal antibodies against human calprotectin coated on polystyrene beads	Polyclonal antibodies against human calprotectin coated on polystyrene beads

	BÜHLMANN fCAL® turbo and CALEX® Cap	**BÜHLMANN fCAL® turbo (K190784)**
Measuring range	Direct measuring range: 30 - 2000 µg/g Measuring range with automatic dilution: 30 – 10,000 µg/g	Direct measuring range: 30 - 2000 µg/g Measuring range with automatic dilution: 30 – 10,000 µg/g
Extraction Method	Manual Weighing (1:50 dilution in Extraction Buffer)	Manual Weighing (1:50 dilution in Extraction Buffer)
	CALEX® Cap (1:500 dilution in Extraction Buffer)	N/A

Comparison of Calibrators

	BÜHLMANN fCAL® turbo	**BÜHLMANN fCAL® turbo (K190784)**
Indications for Use	The BÜHLMANN fCAL® turbo Calibrator Kit is intended for use with the BÜHLMANN fCAL® turbo Reagent Kit for the determination of fecal calprotectin levels in extracted stool samples. Comprised of six (6) calibrators, each calibrator establishes a point of reference for the working curve that is used to calculate test results from patient samples.	The BÜHLMANN fCAL® turbo Calibrator Kit is intended for use with the BÜHLMANN fCAL® turbo Reagent Kit for the determination of fecal calprotectin levels in extracted stool samples. Comprised of six (6) calibrators, each calibrator establishes a point of reference for the working curve that is used to calculate test results from patient samples.
Method	BÜHLMANN fCAL® turbo	BÜHLMANN fCAL® turbo
Analyte	Native human calprotectin Source: human granulocyte extract	Native human calprotectin Source: human granulocyte extract
Calibrators	6 levels: Target values: 0, 50, 200, 500, 1000, 2000 µg/g	6 levels: Target values: 0, 50, 200, 500, 1000, 2000 µg/g
Conversion factor	N/A	N/A
Value assignment:	Calibrator values assigned using a value transfer protocol for each calibrator lot. Values are indicated in the QC datasheet.	Calibrator values assigned using a value transfer protocol for each calibrator lot. Values are indicated in the QC datasheet.
Configuration	Available as a separate BÜHLMANN fCAL® turbo Calibrator Kit.	Available as a separate BÜHLMANN fCAL® turbo Calibrator Kit.

----------This space intentionally left blank----------

Comparison of Controls

	BÜHLMANN fCAL® turbo	**BÜHLMANN fCAL® turbo (K190784)**
Indications for Use	The BÜHLMANN fCAL® turbo Control Kit, comprised of a high and low control, is intended for use with the BÜHLMANN fCAL® turbo Reagent Kit, for quality control, in the determination of fecal calprotectin levels in extracted stool samples.	The BÜHLMANN fCAL® turbo Control Kit, comprised of a high and low control, is intended for use with the BÜHLMANN fCAL® turbo Reagent Kit, for quality control, in the determination of fecal calprotectin levels in extracted stool samples.
Method	BÜHLMANN fCAL® turbo	BÜHLMANN fCAL® turbo
Analyte	Native human calprotectin Source: human granulocyte extract	Native human calprotectin Source: human granulocyte extract
Levels	2 (low and high)	2 (low and high)
Physio-chemical characteristics	Ready to use	Ready to use
Configuration	Available as a separate BÜHLMANN fCAL® turbo Control Kit	Available as a separate BÜHLMANN fCAL® turbo Control Kit

Discussion

As seen above, the only difference between the subject and predicate devices is the provision of the CALEX® Cap as an alternative sample extraction method. This technological difference does not create new questions of safety and effectiveness and the differences are addressed by the performance studies identified below.

VII. Performance Data

A. Clinical Thresholds

Calprotectin Concentration	**Interpretation**	**Follow-Up**
< 80 µg/g	Normal	None
80 µg/g ≤ x ≤ 160 µg/g	Gray-zone/Borderline	Retest within 4 – 6 weeks
> 160 µg/g	Elevated	Retest as appropriate

----------This space intentionally left blank----------

B. Precision

Single-Site Repeatability Study Results (Manual Weighing Extraction Method):

ID	Mean [µg/g]	n	Within-run (Repeatability)		Between-run		Between-day		Within-laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
S01	42.9	80	3.6	8.3%	1.2	2.7%	1.1	2.5%	3.9	9.1%
S02	98.4	80	2.5	2.6%	1.8	1.8%	2.2	2.2%	3.7	3.8%
S03	166.5	80	4.3	2.6%	0.8	0.5%	1.9	1.2%	4.8	2.9%
S04	267.6	80	3.9	1.4%	2.5	0.9%	1.8	0.7%	5.0	1.9%
S05	642.0	80	20.1	3.1%	14.9	2.3%	0.0	0.0%	25.1	3.9%
S06	1414.2	80	19.6	1.4%	11.1	0.8%	3.5	0.2%	22.8	1.6%
S07	3251.4	80	40.8	1.3%	21.4	0.7%	19.7	0.6%	50.1	1.5%
S08	5405.6	80	40.2	0.7%	56.6	1.0%	34.5	0.6%	77.5	1.4%

Multi-Site Reproducibility Study Results (Manual Weighing Extraction Method):

ID	Mean [µg/g]	n	Within-run (Repeatability)		Between-day		Between-site		Total Precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
S01	47.2	75	3.6	7.6	2.4	5.0	0.0	0.0	4.3	9.1
S02	91.1	75	3.5	3.8	3.5	3.8	2.8	3.1	5.7	6.2
S03	185.4	75	5.1	2.7	2.7	1.4	5.5	3.0	7.9	4.3
S04	276.9	75	6.4	2.3	4.5	1.6	9.7	3.5	12.5	4.5
S05	674.5	75	12.9	1.9	1.2	0.2	22.8	3.4	26.3	3.9
S06	1519.6	75	25.3	1.7	17.8	1.2	62.3	4.1	69.6	4.6
S07	3343.8	75	54.6	1.6	35.6	1.1	100.0	3.0	119.4	3.6
S08	5475.6	75	72.1	1.3	35.8	0.7	154.2	2.8	173.9	3.2

----------This space intentionally left blank----------

Lot-to-Lot Precision Study Results (Manual Weighing Extraction Method):

ID	Mean [µg/g]	n	Within-Run (Repeatability)		Between-Day		Between-Lot		Total Precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
S1	45.2	75	3.22	7.1%	1.36	3.0%	3.70	8.2%	5.09	11.3%
S2	86.4	75	3.69	4.3%	1.19	1.4%	5.66	6.6%	6.86	7.9%
S3	175.8	75	5.04	2.9%	0.29	0.2%	9.90	5.6%	11.11	6.3%
S4	263.9	75	7.55	2.9%	0.00	0.0%	9.98	3.8%	12.52	4.7%
S5	647.4	75	15.47	2.4%	0.00	0.0%	15.28	2.4%	21.74	3.4%
S6	1460.7	75	33.66	2.3%	11.64	0.8%	41.01	2.8%	54.32	3.7%
S7	3234.5	75	71.23	2.2%	8.90	0.3%	130.29	4.0%	148.76	4.6%
S8	5303.1	75	97.42	1.8%	11.18	0.2%	163.87	3.1%	190.97	3.6%

Extraction Reproducibility (Manual Weighing Extraction Method) Study Results:

Sample	n	Mean (µg/g)	Within-Run (Repeatability)		Between-extraction		Between-day		Between-operator		Total Precision	
			SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV
S1	80	47.7	2.8	5.9	1.1	2.4	0.7	1.5	1.4	2.9	3.4	7.2
S2	80	72.3	3.8	5.2	3.9	5.4	4.2	5.8	0.0	0.0	6.8	9.5
S3	80	96.1	3.8	3.9	2.2	2.3	1.4	1.4	0.0	0.0	4.6	4.8
S4	80	170.6	4.0	2.4	2.5	1.5	8.7	5.1	0.0	0.0	9.9	5.8
S5	80	277.0	3.7	1.4	27.9	10.1	10.0	3.6	11.0	4.0	31.8	11.5
S6	80	421.1	9.8	2.3	5.9	1.4	15.3	3.6	0.0	0.0	19.1	4.5
S7	80	573.9	5.4	0.9	39.5	6.9	0.0	0.0	0.0	0.0	39.9	6.9
S8	80	1387.4	39.1	2.8	75.1	5.4	159.9	11.5	0.0	0.0	180.9	13.0
S9	80	3264.9	87.2	2.7	236.2	7.2	256.9	7.9	0.0	0.0	359.7	11.0
S10	80	3330.4	89.8	2.7	92.4	2.8	75.7	2.3	0.0	0.0	149.4	4.5

----------This space intentionally left blank----------

Extraction Reproducibility (CALEX® Cap) Study Results:

Sample	n	Mean (µg/g)	Within-Run (Repeatability)		Between-extraction		Between-day		Between-Lot		Between-operator		Total Precision	
			SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV
S1	72	42.7	3.2	7.5	4.6	10.8	0.0	0.0	2.7	6.3	0.0	0.0	6.2	14.5
S2	72	71.5	3.9	5.4	6.9	9.6	9.4	13.1	0.0	0.0	0.0	0.0	12.2	17.1
S3	72	111.3	3.3	2.9	14.2	12.7	0.0	0.0	6.8	6.1	7.8	7.0	17.9	16.1
S4	72	119.8	2.9	2.4	7.2	6.0	5.8	4.8	0.0	0.0	0.0	0.0	9.7	8.1
S5	72	213.0	3.2	1.5	27.9	13.1	0.0	0.0	0.0	0.0	9.0	4.2	29.5	13.8
S6	72	297.2	3.7	1.2	24.5	8.2	13.5	4.6	18.0	6.1	12.3	4.1	35.6	12.0
S7	72	561.2	5.5	1.0	18.6	3.3	66.1	11.8	0.0	0.0	0.0	0.0	68.9	12.3
S8	72	610.0	4.7	0.8	74.3	12.2	28.2	4.6	0.0	0.0	0.0	0.0	79.6	13.1
S9	72	940.4	12.2	1.3	152.7	16.2	34.8	3.7	0.0	0.0	97.5	10.4	184.9	19.7
S10	72	1558.4	7.8	0.5	152.0	9.8	39.9	2.6	98.6	6.3	146.2	9.4	236.4	15.2
S11	72	2041.6	27.2	1.3	150.3	7.4	133.8	6.6	88.9	4.4	10.5	0.5	221.9	10.9
S12	72	3440.0	48.7	1.4	177.7	5.2	321.5	9.3	0.0	0.0	0.0	0.0	370.5	10.8

C. Linearity

Study procedures were performed using two dilution series. For each dilution series, a stool specimen extract with a calprotectin concentration above the anticipated upper limit of the analytical measuring range was combined with a stool specimen extract with a calprotectin concentration below the anticipated lower limit of the analytical measuring range, in various mixing ratios covering the range; each dilution was tested in four (4) replicates. Results of the linear regression analyses are presented in the table below.

Dilution Series	Measuring Range [µg/g]	Linear regression parameters		
		Intercept (95% C.I.)	Slope (95% C.I.)	R^2
1	37.6 – 12,216.0	5.7 (1.6, 16.9)	1.057 (1.044, 1.075)	0.9983
2	33.5 – 13,339.5	3.8 (-0.4, 13.3)	1.031 (1.014, 1.042)	0.9984

The data supports the following claims for analytical measuring range:

- Direct analytical measuring range: 30 – 2000 µg/g
- Measuring range with automatic dilution: 30 – 10,000 µg/g

D. High Dose Hook Effect

No high dose hook effect at theoretical concentrations up to 45,715 µg/g.

E. Accuracy/Recovery

Sample No	7226	7228	7238	7236	7244	7234	7246
Baseline result [µg/g]	44.10	65.45	116.43	138.48	230.88	510.78	1076.33
Expected post-spike result [µg/g]	101.04	122.39	173.37	195.42	458.65	738.55	1304.10
Observed post-spike result [µg/g]	94.55	114.53	170.23	186.93	453.10	753.18	1309.28
Total recovery [%]	**93.6%**	**93.6%**	**98.2%**	**95.7%**	**98.8%**	**102.0%**	**100.4%**

F. Analytical Sensitivity

Results of the analytical sensitivity studies support a claimed direct measuring range of 30 – 2000 µg/g and a measuring range of 30 – 10,000 µg/g with automatic dilution.

LoB = 16.7 µg/g
LoD = 23.7 µg/g
LoQ = 30 µg/g

G. Interfering Substances

Study procedures were performed using stool specimen extracts with the following approximate calprotectin concentrations: 30 µg/g, 100 µg/g, 300 µg/g, and 550 µg/g. The following analytes, pharmaceuticals, and nutritional supplements did not interfere with the BÜHLMANN fCAL® turbo:

Trade name	Active component	Solvent	Concentration mg/50 mg stool
gyno-Tardyferon	Iron (II) sulfate	HCl/NaOH	0.11
Prednisone	Prednisone	DMSO	0.31
Imurek	Azathioprine	DMSO	0.19
Salofalk	Mesalamine; 5-ASA	DMSO	5.21
Agopton	Lansoprazole	Dimethylformamide	0.18
Asacol	Mesalamine; 5-ASA	DMSO	2.50
Vancocin	Vancomycin	H_2Odd	2.00
Sulfamethoxazole	Sulfamethoxazole	DMSO	1.60
Trimethoprim	Trimethoprim lactate	DMSO/Exbuffer	0.35
Ciproxine	Ciprofloxacin	solvent from manufacturer/H2Odd	1.25
Vitamin E	DL-α Tocopherol Acetate	H_2O + Tween	0.30
Bion 3	Multivitamin	HCl/NaOH	1.06
Hemoglobin	Hemoglobin	H_2Odd	1.25

The following enteropathological microorganisms did not interfere with the BÜHLMANN fCAL® turbo when added to stool extracts at the given cell counts:

Microorganism	Concentration (cfu/mL)
Escherichia coli	3.3×10^7
Salmonella enterica subsp. enterica	9.0×10^7
Klebsiella pneumoniae subsp. pneumonia	5.3×10^7
Citrobacter freundii	12.9×10^7
Shigella flexneri	5.0×10^7
Yersinia enterocolitica subsp. enterocolitica	9.8×10^7

H. Method Comparison

A total of 248 clinical study samples, prepared using the manual weighing extraction method, were tested using the BÜHLMANN fCAL® turbo and the predicate device (BÜHLMANN fCAL® ELISA assay); valid results within the linear measuring range for both assays were obtained for 220 of these samples. Results were analyzed by Passing-Bablok regression analysis.

Slope (95% CI)	Intercept (µg/g) (95% CI)	Bias at 80 µg/g (95% CI)	Bias at 160 µg/g (95% CI)	Correlation *r*
1.025 (0.990, 1.058)	-4.5 (-8.7, 0.3)	-3.1% (-7.2%, 0.5%)	-0.3% (-2.4%, 2.7%)	0.972

Frequency counts of BÜHLMANN fCAL® turbo test results and corresponding BÜHLMANN fCAL® ELISA assay results within each of the diagnostic ranges of these tests are provided below.

		# in BÜHLMANN fCAL® ELISA assay range (µg/g)			
		< 80	**80 - 160**	**> 160**	**Total**
# in fCAL turbo range (µg/g)	**< 80**	84	10	0	94
	80 - 160	8	41	6	55
	> 160	0	7	92	99
Total		92	58	98	248

Estimates of positive percent agreement (PPA) and negative percent agreement (NPA) between the BÜHLMANN fCAL® turbo results and corresponding BÜHLMANN fCAL® ELISA assay results, using both sets of assay cutoffs, with respect to IBD subjects, IBS subjects, other GI subjects, normal subjects, and all subjects combined are shown in the table below.

Subgroup	Metric	Estimate	95% C.I.
IBD	PPA (lower cutoff)	68/70 = 97.1%	[90.1%, 99.7%]
	NPA (lower cutoff)	6/7 = 85.7%	[42.1%, 99.6%]
	PPA (upper cutoff)	52/56 = 92.9%	[82.7%, 98.0%]
	NPA (upper cutoff)	19/21 = 90.5%	[69.6%, 98.8%]
IBS	PPA (lower cutoff)	28/32 = 87.5%	[71.0%, 96.5%]
	NPA (lower cutoff)	31/33 = 93.9%	[79.8%, 99.3%]
	PPA (upper cutoff)	12/13 = 92.3%	[64.0%, 99.8%]
	NPA (upper cutoff)	49/52 = 94.2%	[84.1%, 98.8%]
Other GI	PPA (lower cutoff)	20/21 = 95.2%	[76.2%, 99.9%]
	NPA (lower cutoff)	16/16 = 100%	[79.4%, 100%]
	PPA (upper cutoff)	13/14 = 92.9%	[66.1%, 99.8%]
	NPA (upper cutoff)	23/23 = 100%	[85.2%, 100%]
Normal	PPA (lower cutoff)	30/33 = 90.9%	[75.7%, 98.1%]
	NPA (lower cutoff)	31/36 = 86.1%	[70.5%, 95.3%]
	PPA (upper cutoff)	15/15 = 100%	[78.2%, 100%]
	NPA (upper cutoff)	52/54 = 96.3%	[87.3%, 99.5%]
All subjects	**PPA (lower cutoff)**	**146/156 = 93.6%**	**[88.5%, 96.9%]**
	NPA (lower cutoff)	**84/92 = 91.3%**	**[83.6%, 96.2%]**
	PPA (upper cutoff)	**92/98 = 93.9%**	**[87.1%, 97.7%]**
	NPA (upper cutoff)	**143/150 = 95.3%**	**[90.6%, 98.1%]**

I. Extraction Method Comparison

A total of 241 clinical study samples were extracted using the CALEX® Cap and manual weighing extraction methods and tested using the BÜHLMANN fCAL® turbo. Valid results within the linear measuring range were obtained for 202 of these samples using both extraction methods.

Results were analyzed by Passing-Bablok regression analysis.

Slope (95% CI)	Intercept (µg/g) (95% CI)	Bias at 80 µg/g (95% CI)	Bias at 160 µg/g (95% CI)	Correlation *r*
1.149 (1.100, 1.201)	-8.3 (-17.1, -2.0)	4.6% (-4.3%, 9.1%)	9.7% (4.2%, 13.8%)	0.921

Frequency counts of BÜHLMANN fCAL® turbo test results using the CALEX® Cap and manual weighing extraction methods within each of the BÜHLMANN fCAL® turbo diagnostic ranges are provided below.

		# of CALEX® Cap results			
		< 80	**80 - 160**	**> 160**	**Total**
# of manual weighing results	**< 80**	71	8	0	79
	80 - 160	3	30	4	37
	> 160	0	3	122	125
Total		74	41	126	241

Estimates of positive percent agreement (PPA) and negative percent agreement (NPA) between the BÜHLMANN fCAL® turbo results using the CALEX® Cap extraction method and corresponding results using the manual weighing extraction method, using both sets of assay cutoffs are shown in the table below.

Metric	Estimate	95% C.I.
PPA (lower cutoff)	159/162 = 98.1%	[94.7%, 99.6%]
NPA (lower cutoff)	71/79 = 89.9%	[81.0%, 95.5%]
PPA (upper cutoff)	122/125 = 97.6%	[93.1%, 99.5%]
NPA (upper cutoff)	112/116 = 96.6%	[91.4%, 99.1%]

----------This space intentionally left blank----------

J. Clinical Sensitivity/Specificity (Manual Weighing Extraction Method)

IBD vs. IBS:

Borderline Values Considered Positive		**Clinical Diagnosis**		**Total**
		IBD	**IBS**	
BÜHLMANN fCAL® turbo	**Positive**	123	31	154
	Negative	12	99	111
	Total	135	130	265
Sensitivity = 91.1%; 95% C.I. (85.0%, 95.3%)				
Specificity = 76.2%; 95% C.I. (67.9%, 83.2%)				
PPV = 79.9%; 95% C.I. (72.7%, 85.9%)				
NPV = 89.2%; 95% C.I. (81.9%, 94.3%)				

Borderline Values Considered Negative		**Clinical Diagnosis**		**Total**
		IBD	**IBS**	
BÜHLMANN fCAL® turbo	**Positive**	108	16	124
	Negative	27	114	141
	Total	135	130	265
Sensitivity = 80.0%; 95% C.I. (72.3%, 86.4%)				
Specificity = 87.7%; 95% C.I. (80.8%, 92.8%)				
PPV = 87.1%; 95% C.I. (79.9%, 92.4%)				
NPV =80.9%; 95% C.I. (73.4%, 87.0%)				

IBD vs. non-IBD:

Borderline Values Considered Positive		**Clinical Diagnosis**		**Total**
		IBD	**Non-IBD**	
BÜHLMANN fCAL® turbo	**Positive**	123	52	175
	Negative	12	150	162
	Total	135	202	337
Sensitivity = 91.1%; 95% C.I. (85.0%, 95.3%)				
Specificity = 74.3%; 95% C.I. (67.7%, 80.1%)				
PPV = 70.3%; 95% C.I. (62.9%, 76.9%)				
NPV = 92.6%; 95% C.I. (87.4%, 96.1%)				

----------This space intentionally left blank----------

Borderline Values Considered Negative		Clinical Diagnosis		Total
		IBD	IBS	
BÜHLMANN fCAL® turbo	Positive	108	30	138
	Negative	27	172	199
	Total	135	202	337
Sensitivity = 80.0%; 95% C.I. (72.3%, 86.4%)				
Specificity = 85.1%; 95% C.I. (79.5%, 89.8%)				
PPV = 78.3%; 95% C.I. (70.4%, 84.8%)				
NPV =86.4%; 95% C.I. (80.9%, 90.9%)				

K. *Expected Values/Reference Range:*

Stool samples, prepared using the manual weighing extraction method, were obtained from 141 apparently healthy normal adults (> 21 years of age) with no symptoms or signs of gastrointestinal disease and were. The test results were categorized by the assay cut-offs below.

	Calprotectin level by BÜHLMANN fCAL turbo			Total
	< 80 µg/g	80 - 160 µg/g	> 160 µg/g	
Number of subjects (%)	106 (75.2%)	18 (12.8%)	17 (12.1%)	141 (100%)

VIII. Conclusion

The information provided above supports that the BÜHLMANN fCAL® turbo and CALEX® Cap are as safe and effective as the predicate device. Verification and validation studies support that the use of the CALEX® Cap to prepare stool sample extracts does not raise any new questions of safety and effectiveness. Therefore, it is concluded that the BÜHLMANN fCAL® turbo and CALEX® Cap are substantially equivalent to the predicate device.

----------This space intentionally left blank----------