BÜHLMANN fCAL® turbo

Calprotectin turbidimetric assay

Reagent Kit

B-KCAL-RSET

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For *In Vitro* Diagnostic Use Only

Rx Only

CLIA Complexity: High

INTENDED 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.

SUMMARY AND EXPLANATION OF THE TEST

Gastroenterologists are often faced with the diagnostic difficulty of differentiating individuals with functional gastrointestinal disorders, such as irritable bowel syndrome (IBS), from those with inflammatory bowel disease (IBD). Many symptoms are common to both conditions, whereas other clinical features such as a predominance of diarrhea and rectal bleeding will increase the likelihood of inflammatory disease. The clinical differentiation between these conditions remains problematic and may result in delayed diagnosis. Furthermore, many individuals with IBS must undergo invasive procedures (endoscopy) to rule out an organic disorder. This has significant implications for health care costs as well as exposing individuals to the inherent risks associated with invasive procedures (ref. 1-4).

Diseases included in the IBD category include Crohn´s disease (CD), ulcerative colitis (UC) and indeterminate colitis. IBD represents chronic and often disabling lifelong inflammatory conditions – frequently diagnosed in young people in their late teens and early twenties. It is estimated that nearly 1.2 million Americans are living with IBD, and the prevalence is rising (ref. 5). The main difference between CD and UC is the location and nature of the inflammatory condition. In UC, the disease is restricted to the colon, whereas in CD, inflammation may affect any part of the gastrointestinal tract – the ileocecal area being most often affected (ref. 6, 7).

The most striking difference between IBS and IBD is that the former is non-inflammatory in nature. Therefore, one possibility is to measure surrogate markers of intestinal inflammation to differentiate between the two (ref. 8, 9). Calprotectin is a calcium-binding protein found in neutrophilic granulocytes, monocytes, and macrophages, comprises up to 60% of the total cytosolic protein content of neutrophils, resists metabolic degradation, and can be measured in feces (ref. 10-12). Its use as a biomarker of intestinal inflammation has been extensively validated, showing consistently abnormal levels in the stool of individuals with IBD (ref. 2, 13-16).

PRINCIPLE OF THE ASSAY

The BÜHLMANN fCAL® turbo test is a particle enhanced turbidimetric immunoassay (PETIA) which allows for automated quantification of calprotectin in fecal extracts on clinical chemistry analyzers. Fecal samples are extracted with extraction buffer using the CALEX® Cap extraction device or a manual weighing method and applied at a final dilution of 1:500. The extracts are incubated with reaction buffer and mixed with polystyrene nanoparticles coated with calprotectin-specific antibodies (immunoparticles). Calprotectin available in the sample mediates immunoparticle agglutination. Sample turbidity, measured by light absorbance, increases with calprotectin-immunoparticle complex formation and is proportional to calprotectin concentration. The detected light absorbance allows quantification of calprotectin concentration via interpolation on an established calibration curve.

REAGENTS SUPPLIED

Reagents	Quantity	Code	Preparation
Reaction Buffer (R1) MOPS buffered saline	1 vial 35 mL	B-KCAL-R1	Ready to use
Immunoparticles (R2) Polystyrene beads coated with avian antibodies against human calprotectin	1 vial 7 mL	B-KCAL-R2	Ready to use

Table 1: Reagents supplied

REAGENT STORAGE AND STABILITY

Unopened reagents
Store at 2-8 °C. Do not use kit past expiration date printed on the labels.
On-board stability
Please refer to the corresponding instrument application note.

Table 2: Storage and stability of reagents

Do not freeze reagents!

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents	Quantity	Code
BÜHLMANN fCAL® turbo Calibrator Kit Calibrators 1-6 for instrument calibration	1 x 6 vials 1 mL/vial	B-KCAL-CASET
BÜHLMANN fCAL® turbo Control Kit Controls low and high	3 x 2 vials 1 mL/vial	B-KCAL-CONSET
CALEX® Cap Extraction device filled with extraction buffer	50 tubes 200 tubes 500 tubes	B-CALEX-C50 B-CALEX-C200 B-CALEX-C500
Extraction Kit Extraction buffer	3 bottles 12 bottles 125 mL/bottle	B-CAL-EX3 B-CAL-EX12

Table 3: Materials required but not provided

WARNINGS AND PRECAUTIONS

- This test is for *in vitro* diagnostic use only.
- The immunoparticles contain potentially infectious substances of animal origin and should be handled in accordance with good laboratory practice (GLP) using appropriate precautions.
- R1 contains MOPS (3-(*N*-morpholino)propanesulfonic acid) (< 1%), that can be irritating to eye and skin. Handle with due caution.
- R2 contains polystyrene nanoparticles.
- R1 and R2 contain 2-methyl-2H-isothiazol-3-one, a preservative agent, which is below the allowable limits (0.01 %).

Technical precautions

- Please equilibrate reagents, controls, calibrators and samples as described in the application note.
- Do not mix reagents R1 and R2 of different reagent lots or switch caps between reagents.
- Sample carry over depends on the clinical chemistry analyzer. For more information refer to analyzer specific application note.

- Reagent R2, once frozen, cannot be used anymore. Freezing R2 will lead to reduced sensitivity and precision in low level samples and in the worst case to decreased measurement levels.
- The assay is designed for fecal extract samples prepared using the specific BÜHLMANN extraction buffer (B-CAL-EX). Application of other extraction buffers could lead to incorrect results.
- Ensure that samples have no bubbles prior to running the test.
- Evaporation of calibrators and controls on the analyzer could lead to incorrect results. Run the assay immediately after loading the analyzer.

SPECIMEN COLLECTION AND STORAGE

Collection of less than 1 g (1 mL) of stool sample is required. The extraction procedure requires 50-100 mg of stool sample.
Collect stool samples into plain tubes and store them refrigerated at 2-8 °C until ready for transport to the laboratory.
Important: The sample must be collected in empty collection devices; no chemical or biological additives should be added to the sample.

Specimen transport

Stool specimens should be received for processing by the laboratory within 3 days of collection. Stool specimens may be shipped at room temperature or on cold packs.

Specimen storage

Stool specimens should be refrigerated at 2-8 °C and extracted within 3 days of receipt at the laboratory. Do not store samples at elevated temperatures.

STOOL SAMPLE PREPARATION AND EXTRACT STORAGE

CALEX® Cap

Follow the instruction for use provided with the CALEX® Cap device kit (code: B-CALEX-C50, B-CALEX-C200, B-CALEX-C500). Fecal sample extracts prepared using the CALEX® Cap device will have a final dilution of 1:500 and are ready to use.

Liquid stool samples can be pipetted directly into the CALEX® Cap device. Unscrew the blue cap and pipet 10 µL of stool sample into the device. Recap the CALEX® Cap device and proceed with vortexing step according to the extraction procedure described and illustrated in the instruction for use delivered with the CALEX® Cap device.

Important: Centrifuge the CALEX® Cap device for 10 minutes at 1000 - 3000 g prior to running the BÜHLMANN fCAL® turbo procedure.

CALEX® Cap extracts can be kept at room temperature for up to 2 hours, and after centrifugation, at 2-8°C for up to 3.5 days (84 hours). For longer storage freeze CALEX® Cap extracts at -20°C.

CALEX® Cap extracts can be subjected to four freeze-thaw cycles. Allow frozen extracts to equilibrate to room temperature for up to 2 hours before measurement. Prior to measurement, CALEX® Cap extract should be vortexed thoroughly for 10 seconds and centrifuged for 10 minutes at 1000 - 3000 g.

Please note that extracts can be stored and frozen directly within the CALEX® Cap.

Extraction Kit

For manual weighing extraction method follow the instruction for use provided with the Extraction Kit (code: B-CAL-EX3, B-CAL-EX12). Fecal sample extracts prepared using the Extraction Kit will have a final dilution of 1:50. Dilute the stool extracts 1:10 in BÜHLMANN extraction buffer, provided in the Extraction Kit, (e.g. 50 µL extract and 450 µL extraction buffer) prior to running the BÜHLMANN fCAL® turbo procedure.

Fecal calprotectin in extracts (1:50) obtained by manual weighing is stable at 2-8 °C for up to 7 days. For longer storage, freeze the extracts at -20 °C. Frozen extracts are stable for a period of 2 months.

PROCEDURE

Application notes / assay installation

The assay procedure for the BÜHLMANN fCAL® turbo has been established on the Roche cobas® c501/502 platforms and will be expanded to other platforms over time. The list below outlines the clinical chemistry analyzer models which are validated for the BÜHLMANN fCAL® turbo:

- Roche cobas® c501/502
- Ortho Vitros® 5600
- Siemens ADVIA® XPT
- Beckmann Coulter AU 480
- Abbott Architect® c4000

Application notes describing installation and analysis on all validated clinical chemistry analyzers are available from BUHLMANN Diagnostics Corp, BDC at (844) 300-9799 (Mon-Fri 8:00AM-5:00PM EST). Corresponding instrument manuals must be considered for instrument setup, maintenance, operation and precautions.

Reagent preparation

The reagents supplied are ready to use. Mix gently before loading onto the instrument. The reagent bottles may fit directly into the instrument, unless otherwise stated in the application note.

Establishment of the calibration curve

The BÜHLMANN fCAL® turbo Calibrator Kit (Code: B-KCAL-CASET) is used to establish a six point standard curve according to the instrument manual. Calibrator values are lot-specific. A new calibration must be performed for each new calibrator and reagent lot. Otherwise, calibration should be performed every one to two months according to the instrument specific application notes. Refer to the QC-data sheet provided with the BÜHLMANN fCAL® turbo Calibrator Kit for assigned calibrator values. If calibration cannot be performed without error, contact BUHLMANN Diagnostics Corp, BDC at (844) 300-9799 (Mon-Fri 8:00AM-5:00PM EST).

QC controls

The BÜHLMANN fCAL® turbo Control Kit, (Code: B-KCAL-CONSET) must be assayed each day before running patient fecal sample extracts to validate the calibration curve. The controls have assigned value ranges indicated on the QC-data sheet supplied with each lot of the BÜHLMANN fCAL® turbo Control Kit. The control measurements must be within the indicated value ranges to obtain valid results for patient fecal sample extracts.
If the control values are not valid, repeat QC control measurement with fresh controls. If control values remain invalid, recalibrate the instrument. If valid control values cannot be reproduced, after performing the steps described above, contact BUHLMANN Diagnostics Corp, BDC at (844) 300-9799 (Mon-Fri 8:00AM-5:00PM EST).

Patient fecal sample extract measurement

Once a calibration curve is established and validated with the QC controls, patient fecal extracts may be measured. Perform patient fecal extract measurement according to the application note and instrument manual.

Results

Results are calculated automatically on the clinical chemistry analyzer and presented in µg/g unless otherwise stated in the corresponding clinical chemistry analyzer-specific application notes.

LIMITATIONS

- Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.
- False negative results could occur in patients who have granulocytopenia due to bone marrow depression.
- Some patients taking non-steroidal anti-inflammatory drugs (NSAID) will have elevations in their fecal calprotectin levels.
- Results may not be clinically applicable to children less than 4 years of age who have mildly increased fecal calprotectin levels.
- Patients with IBD fluctuate between active (inflammatory) and inactive stages of the disease. These stages must be considered when interpreting results of the fecal calprotectin assay.

CLINICAL THRESHOLDS

Calprotectin concentration	**Interpretation**	**Follow-up**
< 80 µg/g	Normal	None
80 - 160 µg/g	Gray-zone/Borderline	Follow-up within 4 – 6 weeks
> 160 µg/g	Elevated	Repeat as needed

Table 4: BÜHLMANN fCAL® turbo diagnostic ranges.

Calprotectin values below 80 µg/g

Fecal calprotectin values <80 µg/g are not indicative of active inflammation in the gastrointestinal tract. Low fecal calprotectin levels (calprotectin <80 µg/g) can be used in conjunction with the patient's clinical symptoms, medical history, and other clinical and laboratory findings to determine the need for additional diagnostic work-up. Specifically, for patients with a clinical and laboratory presentation suggesting a non-inflammatory disorder such as IBS fecal calprotectin values of <80 µg/g can be used to support a decision to defer invasive testing.

Calprotectin values between and equal to 80 and 160 µg/g

Mid-fecal calprotectin levels between and equal to 80 and 160 µg/g, also called gray-zone levels, are not directly indicative of an active inflammation requiring immediate follow-up with invasive testing. However, the presence of inflammation cannot be excluded. Re-evaluation of fecal calprotectin levels after 4 to 6 weeks is recommended to determine the inflammatory status. This decision should be made by the clinician in conjunction with the patient's clinical symptoms, medical history, and other clinical and laboratory findings.

Calprotectin values greater than 160 µg/g

Fecal calprotectin values >160 µg/g are indicative of neutrophil infiltrate in the gastrointestinal tract; therefore, this may signal the presence of active inflammatory disease. Elevated fecal calprotectin levels (calprotectin >160 µg/g) can be used in conjunction with the patient's clinical symptoms, medical history, and other clinical and laboratory findings to determine the need for further investigative procedures, including invasive procedures performed by specialists, to achieve an overall clinical diagnosis, in particular of IBD.

CLINICAL EVALUATION

The ability of the BÜHLMANN fCAL® turbo to discriminate between patients with IBD and other non-inflammatory GI disorders, including IBS, was evaluated using clinical samples collected from 337 adult and pediatric patients. One hundred and thirty five patients (135) had a final diagnosis of IBD (Crohn's disease, ulcerative colitis or indeterminate colitis), 130 patients suffered from IBS and 72 patients presented with abdominal pain and/or diarrhea, or other GI-related non-inflammatory conditions. Final diagnosis was supported by endoscopic as well as other clinical findings.

Final diagnosis	Distribution of patients results in numbers (percent) within BÜHLMANN fCAL® turbo diagnostic ranges.			
	< 80 µg/g	80 - 160 µg/g	> 160 µg/g	Total
IBD	12 (8.9%)	15 (11.1%)	108 (80%)	135 (100%)
IBS	99 (76.2%)	15 (11.5%)	16 (12.3%)	130 (100%)
Other GI	51 (70.8%)	7 (9.7%)	14 (19.4%)	72 (100%)

Table 5: Distribution of patients results within BÜHLMANN fCAL® turbo diagnostic ranges.

IBD vs. non-IBD	Clinical decision point			
	80 µg/g		160 µg/g	
Sensitivity (95% CI)	91.1%	(85.0%, 95.3%)	80.0%	(72.3%, 86.4%)
Specificity (95% CI)	74.3%	(67.7%, 80.1%)	85.1%	(79.5%, 89.8%)
PPV (95% CI)	70.3%	(62.9%, 76.9%)	78.3%	(70.4%, 84.8%)
NPV (95% CI)	92.6%	(87.4%, 96.1%)	86.4%	(80.9%, 90.9%)
ROC AUC (95% CI)	0.916	(0.884, 0.947)		

Table 6: Clinical performance characteristics of the BÜHLMANN fCAL® turbo in discriminating IBD from non-IBD – IBS and other GI-related disorders, at 80 µg/g and 160 µg/g clinical decision points

IBD vs. IBS	Clinical decision point			
	80 µg/g		160 µg/g	
Sensitivity (95% CI)	91.1%	(85.0%, 95.3%)	80.0%	(72.3%, 86.4%)
Specificity (95% CI)	76.2%	(67.9%, 83.2%)	87.7%	(80.8%, 92.8%)
PPV (95% CI)	79.9%	(72.7%, 85.9%)	87.1%	(79.9%, 92.4%)
NPV (95% CI)	89.2%	(81.9%, 94.3%)	80.9%	(73.4%, 87.0%)
ROC AUC (95% CI)	0.929	(0.898, 0.960)		

Table 7: Clinical performance characteristics of the BÜHLMANN fCAL® turbo in discriminating IBD from IBS at 80 µg/g and 160 µg/g clinical decision points

CI – confidence interval
PPV – positive predictive value
NPV – negative predictive value
ROC AUC – area under receiver operating characteristic curve

REFERENCE RANGE

Stool samples were obtained from 141 apparently healthy normal adults (> 21 years of age) with no symptoms or signs of gastrointestinal disease. The test results were categorized by the assay cut-offs.

	Distribution of results within BÜHLMANN fCAL® turbo diagnostic ranges.			
	< 80 µg/g	**80 – 160 µg/g**	**> 160 µg/g**	**Total**
Number of subjects (%)	106 (75.2%)	18 (12.8%)	17 (12.1%)	141 (100%)

Table 8: Distribution of healthy subjects results within BÜHLMANN fCAL® turbo diagnostic ranges.

PERFORMANCE CHARACTERISTICS

The presented performance characteristics have been established on a Roche cobas® 6000 c501 instrument using stool extracts obtained by manual weighing. Refer to clinical chemistry analyzer specific application notes for the performance characteristics on other clinical chemistry analyzer.

Method comparison - BÜHLMANN fCAL® turbo vs. fCAL ELISA

The method comparison study was performed according to the CLSI guideline EP09-A3. Two hundred forty eight (248) clinical samples were measured according to the instructions for use with the BÜHLMANN fCAL® turbo and with BÜHLMANN fCAL® ELISA assay, with 220 samples yielding results within the measuring range for both tests. Measurements were performed over eighteen days using three reagent lots.

Bland-Altman Analysis			**Passing-Bablok Regression Analysis**				
Mean bias (95 % CI)	**Lower LoA (95 % CI)**	**Upper LoA (95 % CI)**	**Slope (95 % CI)**	**y-Intercept (95 % CI)**	**Bias at 80 µg/g (95 % CI)**	**Bias at 160 µg/g (95 % CI)**	**r**
-0.5 % (-3.6%, 2.7%)	-46.5 % (-51.8%, -41.1%)	45.5 % (40.2%, 50.9%)	1.025 (0.990, 1.058)	-4.5 µg/g (-8.7, 0.3)	-3.1 % (-7.2%, 0.5%)	-0.3 % (-2.4%, 2.7%)	0.972

Table 9: Summary of Bland-Altman and Passing-Bablok regression analyses for the method comparison between BÜHLMANN fCAL® turbo and BÜHLMANN fCAL® ELISA assay.

Method comparison – CALEX® Cap vs. manual weighing extraction

The method comparison study was performed according to the CLSI guideline EP09-A3. Two hundred forty one (241) clinical samples, extracted using the CALEX® Cap device and manual weighing and extraction method, were measured according to the instructions for use with the BÜHLMANN fCAL® turbo, with 202 samples yielding results within the measuring range of the assay. Measurements were performed over eighteen days using three CALEX® Cap device lots

Bland-Altman Analysis			Passing-Bablok Regression Analysis				
Mean bias (95 % CI)	Lower LoA (95 % CI)	Upper LoA (95 % CI)	Slope (95 % CI)	y-Intercept (95 % CI)	Bias at 80 µg/g (95 % CI)	Bias at 160 µg/g (95 % CI)	r
7.7% (3.5%, 12.0%)	-51.9% (-59.1%, 44.6%)	67.4% (60.1%,74.6%)	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

Table 10: Summary of Bland-Altman and Passing-Bablok regression analyses for the method comparison between CALEX® Cap extraction and manual weighing extraction method.

Reproducibility (Multisite precision evaluation study)

Reproducibility was established according to the CLSI guideline EP05-A3 by performing measurements at three laboratory sites. Eight pooled stool specimen extracts with calprotectin concentrations covering the measuring range of the test and clinical decision points were tested over five days, in one run per day, with five results generated per run. One reagent lot was used in the study.

ID	Mean [µg/g]	n	Within-run (Repeatability)		Between-day		Between-site		Total (Reproducibility)	
			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%

Table 11: Reproducibility study results – within-run, between-day, between-site and total precision estimates.

Between-lot precision

Between-lot precision was established according to the CLSI guideline EP05-A3. Eight pooled stool extracts with calprotectin concentrations covering the measuring range of the test and clinical decision points were tested over five days, in one run per day, with five results generated per run. Three reagent lots were used in the study.

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%

Table 12: Between-lot precision study results

Within-laboratory precision

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3. Eight pooled stool specimen extracts with calprotectin concentrations covering the measuring range of the test and clinical decision points were tested over 20 days, in two runs per day, with two results generated per run. One reagent lot was used in the study.

ID	Mean [µg/g]	n	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%

Table 13: Within-laboratory precision study results

Extraction reproducibility – CALEX® Cap

The extraction reproducibility was established according to the CLSI guideline EP05-A3. Twelve clinical stool specimens, selected to reflect different stool consistencies: solid, semi-solid and liquid, with calprotectin concentrations covering the measuring range of the test and clinical decision points, were extracted in duplicates by two operators on two days using three CALEX® Cap device lots. Each stool extract was tested in three replicates using one reagent lot of the BÜHLMANN fCAL® turbo.

ID	Mean [µg/g]	n	Within-run		Between-extraction		Between-day		Between-lot		Between-operator		Total precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
S1	42.7	72	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	71.5	72	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	111.3	72	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	119.8	72	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	213.0	72	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	297.2	72	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	561.2	72	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	610.0	72	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	940.4	72	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	1558.4	72	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	2041.6	72	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	3440.0	72	48.7	1.4	177.7	5.2	321.5	9.3	0.0	0.0	0.0	0.0	370.5	10.8

Table 14: CALEX® Cap extraction reproducibility study results.

Extraction reproducibility – manual weighing

Extraction reproducibility was established according to the CLSI guideline EP05-A3. Ten clinical stool specimens, selected to reflect different stool consistencies: solid, semi-solid and liquid, with calprotectin concentrations covering the measuring range of the test and clinical decision points, were extracted four times each by two operators on two days using manual weighing. Each stool extract was tested in five replicates using one reagent lot of the BÜHLMANN fCAL® turbo.

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

Table 15: Manual weighing extraction reproducibility study results.

Accuracy / Recovery

Seven stool specimen extracts from clinical left over samples with calprotectin levels spanning the measuring range of the test were spiked with 56.9 µg/g or 227.8 µg/g calprotectin in calibrator material. Spiking was performed at 10 % of the specimen extract volume. “Baseline” samples were spiked with the corresponding volume of analyte-free specimen. “Baseline” and “baseline + spike” samples were measured in four replicates with one reagent lot.

ID	Mean baseline [µg/g]	Expected baseline + spike [µg/g]	Observed baseline + spike [µg/g]	Recovery rate [%]
#1	44.1	101.0	94.6	93.6%
#2	65.5	122.4	114.5	93.6%
#3	116.4	173.4	170.2	98.2%
#4	138.5	195.4	186.9	95.7%
#5	230.9	458.6	453.1	98.8%
#6	510.8	738.5	753.2	102.0%
#7	1076.3	1304.1	1309.3	100.4%

Table 16: Accuracy/recovery study results

Sample carry-over

The sample carry-over was established according to the CLSI guideline EP10-A3. Low, medium and high sample pools were tested over 5 days, in one run per day, with three results generated per sample pool per run. One reagent lot was used in the study. No statistically significant sample carry-over with the BÜHLMANN fCAL® turbo test on Roche cobas® 6000 c501 instrument was detected.

	Day 1 [%]	Day 2 [%]	Day 3 [%]	Day 4 [%]	Day 5 [%]	Mean [%]
Adjusted carry-over coefficient	**0.80**	**-0.13**	**0.64**	**0.17**	**-0.70**	**0.16**

Table 17: Sample carry-over study results

Limit of Blank (LoB): 16.7 µg/g

The LoB was established according to the CLSI guideline EP17-A2 with four negative (extraction buffer) samples. The samples were measured over three days in five replicates each day to produce 60 blank values. The LoB was calculated using non-parametric analysis. The study was performed independently with two reagent lots, taking the higher estimate obtained with one lot as the claimed LoB value.

Limit of Detection (LoD): 23.7 µg/g

The LoD was established according to the CLSI guideline EP17-A2 with four stool specimen extracts with concentrations corresponding to 1-5 times the LoB value. The samples were measured over three days in five replicates each day to produce 60 values. The LoD was calculated using parametric analysis. The study was performed independently with two reagent lots, taking the higher estimate obtained with one lot as the claimed LoD value.

Limit of Quantitation (LoQ): 30 µg/g

The LoQ was established according to the CLSI guideline EP17-A2 with six low level stool specimen extracts. The samples were measured over three days in five replicates each day to produce a total of 15 replicates per sample. The study was performed independently with two reagent lots. The LoQ was defined as the lowest calprotectin concentration, which can be determined with a precision of below 20 % CV.

Linearity

The linear range of the BÜHLMANN fCAL® turbo was determined according to the CLSI guideline EP06-A. To demonstrate linearity, two dilution series, with at least 17 different calprotectin levels, covering the expected measuring range of the test, were generated by blending low and high specimen extracts. To demonstrate accuracy of automated dilution relative to manual dilution, the relative difference between test results obtained for six high level samples (> 2000 µg/g) diluted 1:10 with deionized water either manually by the operator or automatically by the instrument was calculated. Each sample was tested in four replicates after each dilution method, using one reagent lot. The linear range was defined as the concentration interval in which coefficients of the non-linear, polynomial, fits were determined as not significant or as the concentration interval in which the deviation of the polynomial fit from linearity was below 10 %. For values below 75 µg/g an absolute difference of less than 7.5 µg/g was allowed.

Dilution Series	Measuring range tested	Linear regression parameters			Linear range
		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	37.6 – 12,216.0
2	33.5 – 13,339.5	3.8 (-0.4, 13.3)	1.031 (1.014, 1.042)	0.9984	33.5 – 13,339.5

Table 18: Linearity study results

ID	Mean result, automatic dilution [µg/g]	Mean result, manual dilution [µg/g]	Relative difference
14	2869.7	2846.5	0.8%
22	3359.7	3267.0	2.8%
7623	7846.6	7440.8	5.5%
8073	4260.7	4188.0	1.7%
8074	5034.7	4876.3	3.2%
S08	5215.8	5065.3	3.0%

Table 19: Relative difference of test results of manually and automatically diluted samples.

High Dose Hook Effect

No high dose hook effect was observed for samples with theoretical concentrations up to 45,715 µg/g. The presence of a high dose hook effect was tested on one lot.

Interfering substances

The susceptibility of the BÜHLMANN fCAL® turbo assay to oral pharmaceuticals, nutritional supplements, hemoglobin as well as enteropathological microorganisms was assessed according to the CLSI guideline EP07-A2. Bias in results exceeding 10 % was considered interference. No interference was detected with substances, listed in Table 20, up to the indicated concentrations. No interference was detected with enteropathological microorganisms, listed in Table 21, up to the indicated amounts of colony forming units (CFU) per mL of stool specimen extract.

Trade Name	Active Component	Concentration in mg/50 mg stool
gyno-Tardyferon	Iron (II) sulfate (contains 0.35 mg folic acid)	0.11
Prednisone	Prednisone	0.31
Imurek	Azathioprine	0.19
Salofalk	Mesalamine; 5-ASA	5.21
Agopton	Lansoprazole	0.18
Asacol	Mesalamine; 5-ASA	2.50
Vancocin	Vancomycin	2.00
Sulfamethoxazole	Sulfamethoxazole	1.60
Trimethoprim	Trimethoprim lactate	0.35
Ciproxine	Ciprofloxacin	1.25
Vitamin E	DL-α-Tocopherol Acetate	0.30
Bion 3	3 probiotics (10^7 CFU): lactobacillus gasseri PA16 / 8, bifidobacterium bifidum MF 20/5, bifidobacterium longum SP07 / 3, 12 vitamins: A (800 µg), B1 (1.4 mg), B2 (1.6 mg), B6 (2 mg), B12 (1 µg), C (60 mg), D (5 µg), E (10 mg), biotin (150 µg), folic acid (200 µg), niacin (18 mg), pantothenic acid (6 mg) and 7 minerals: iodine (100 µg), iron (5 mg), zinc (5 mg), selenium (30 µg), chromium (25 µg), manganese (1.2 mg), molybdenum (25 µg)	1.06
Hemoglobin	Hemoglobin	1.25

Table 20: Interfering substances: oral pharmaceuticals, nutritional supplements, hemoglobin

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

Table 21: Interfering substances: enteropathological microorganisms

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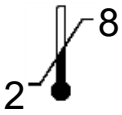
SYMBOLS KEY

	Expiration Date
	Consult Instructions for Use
	Manufacturer
	Catalogue Number
	*In Vitro* Diagnostic Medical Device
	Lot Number
	Temperature Limitations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