BÜHLMANN fCAL® turbo

Calprotectin turbidimetric assay
for professional use

Reagent Kit

B-KCAL-RSET
Version A6

For *In Vitro* Diagnostic Use

Manufacturer

BÜHLMANN Laboratories AG
Baselstrasse 55
4124 Schönenbuch
Switzerland
Tel.: +41 61 487 1212
Fax: +41 61 487 1234
info@buhlmannlabs.ch

www.buhlmannlabs.ch/support/downloads/

INTENDED USE

The BÜHLMANN fCAL® turbo is an automated *in vitro* diagnostic test for the quantitative determination of calprotectin in human stool specimens intended as an aid in the assessment of intestinal mucosal inflammation (ref. 1-3). The assay results can be used as an aid to diagnosis in distinguishing organic, inflammatory disease of the gastrointestinal tract (inflammatory bowel disease, IBD, specifically Crohn's disease (CD) or ulcerative colitis (UC)) from functional disease (irritable bowel syndrome, IBS) (ref. 4-10), in patients with chronic abdominal pain and as an aid to IBD disease monitoring (ref. 10-22).

The BÜHLMANN fCAL® turbo assay is intended to be run on clinical chemistry analyzers. For laboratory use only.

***Disclaimer for South Korea**: IBD monitoring claim does not apply. For Korean instruction for use please contact your local distributor.*

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 or manual extraction 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
Store for up to 90 days at 2-15 °C.

Table 2: Storage and stability of reagents

Do not freeze reagents!

MATERIALS REQUIRED BUT NOT PROVIDED

- The reagents described below are not delivered with the kit and must be ordered separately:

Reagents	Quantity	Code
BÜHLMANN fCAL® turbo Calibrator Kit Calibrators 1-6 for establishment of six point calibration curve	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 with the kit

- General laboratory equipment
- Clinical chemistry analyzer
- Centrifuge

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.
- R2 contains polystyrene nanoparticles.
- This kit contains components classified in accordance with the Regulation (EC) No. 1272/2008: 2-methyl-4-isothiazolin-3-one hydrochloride (conc. ≥ 0.0015%), thus the reagents may cause allergic skin reactions (H317).
- Avoid contact of reagents with the skin, eyes or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, irritation can occur.

Technical precautions

- Please equilibrate reagents, controls, calibrators and samples as described in the application note.
- Evaporation of calibrators and controls on the analyzer could lead to incorrect results. Run the assay immediately after loading the analyzer.
- Do not mix reagents R1 and R2 of different reagent lots or switch caps between reagents.
- Reagent R2, once frozen, cannot be used anymore.
- The assay is designed for fecal extract samples prepared using the specific BÜHLMANN extraction buffer.
- Ensure that samples have no bubbles prior to running the test.
- Sample carry over depends on the clinical chemistry analyzer. For more information refer to analyzer specific application note.

SPECIMEN COLLECTION AND STORAGE

For the extraction procedure, less than 1 g of native stool specimen is required. Collect stool specimen into plain tubes.
Important: The specimen must be collected without any chemical or biological additives.

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 refrigerated.

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 EXTRACTION AND EXTRACT STABILITY

CALEX® Cap

Follow the instruction for use provided with the CALEX® Cap kit. Fecal sample extracts prepared using the CALEX® Cap will have a final dilution of 1:500 and are ready to use.

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

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

Fecal calprotectin in extracts obtained by the CALEX® Cap is stable at room temperature (23 °C) for 7 days, at 2-8 °C for 15 days and at -20 °C for up to 23 months.

CALEX® Cap extracts can be frozen directly and stored within the CALEX® Cap. Extracts can be subject to four freeze-thaw cycles. Prior to measurement, allow frozen extracts to equilibrate to room temperature, vortex thoroughly for 10 seconds and centrifuge according to the instruction for use of the assay.

Extraction Kit

For manual extraction follow the instruction for use provided with the Extraction Kit. 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 extraction is stable at 2-8 °C for 7 days or at -20 °C for up to 36 months.

PROCEDURE

Application notes / assay installation

Assay procedures for the BÜHLMANN fCAL® turbo are established on several clinical chemistry analyzers. Validated application notes describing installation and analysis on specific instruments are available from BÜHLMANN upon request. 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 is used to establish a six point calibration 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. Contact BÜHLMANN support if calibration cannot be performed without error.

QC controls

The BÜHLMANN fCAL® turbo Control Kit, 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 measurement with fresh controls. If control values remain invalid, recalibrate the assay. If valid control values cannot be reproduced, after performing the steps described above, contact BÜHLMANN support.

Patient fecal sample extract measurement

Once a calibration curve is established and validated with the 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.

STANDARDIZATION AND METROLOGICAL TRACEABILITY

There are no internationally or nationally recognized reference materials or reference measurement procedures for the calprotectin analyte in stool specimen. The BÜHLMANN fCAL® turbo is standardized against an internally established reference material and values of controls and calibrators are assigned according to a value transfer protocol (ref. 23, 24) to guarantee metrological traceability. The 95% confidence interval of the combined uncertainty of product calibrators was determined as lower than 3.7%, the combined uncertainty of the controls lower than 6.9%.

LIMITATIONS

- Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.
- For IBD disease monitoring, multiple fecal calprotectin measurements performed at up to 4 weeks intervals have been suggested to have best diagnostic accuracy in predicting clinical relapse in patients (ref. 25-26).
- Intake of non-steroidal anti-inflammatory drugs (NSAID) may lead to elevated fecal calprotectin levels.
- Results may not be clinically applicable to children less than 4 years of age who have mildly increased fecal calprotectin levels (ref. 27-30).

INTERPRETATION OF RESULTS

I. Distinguishing organic disease from functional gastrointestinal disease

Determination of fecal calprotectin levels can be used as a reliable and simple aid in distinguishing organic from functional gastrointestinal diseases (ref. 4-10). BÜHLMANN recommends applying the same cut-off values as for the BÜHLMANN fCAL® ELISA:

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

The result categories are based on data from clinical studies performed by BÜHLMANN and are BÜHLMANN's recommendations. All test results should be interpreted in conjunction with information available from the patient's clinical symptoms, medical history, and other clinical and laboratory findings:

Calprotectin values below 80 µg/g

Fecal calprotectin values < 80 µg/g are not indicative of inflammation in the gastrointestinal tract. Patients with low calprotectin levels are not likely to be in need of invasive procedures to determine the inflammation cause (ref. 4).

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.

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. Appropriate further investigative procedures by specialists are suggested to achieve an overall clinical diagnosis.

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 295 patients and extracted using the CALEX® Cap. One hundred and twenty seven (127) patients had a final diagnosis of IBD (Crohn's disease, ulcerative colitis or indeterminate colitis), 103 patients suffered from IBS and 65 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.

The optimal cut-off combination for these patient pools could be defined by ROC analysis at 80 µg/g and 160 µg/g calprotectin (table 6 and 8), which is slightly more stringent than a combination of a more sensitive lower cut off of 50 µg/g with lower performance in specificity, and an upper cut off 200 µg/g with slightly lower sensitivity (table 7 and 9).

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	11 (8.7%)	8 (6.3%)	108 (85.0%)	127 (100%)
IBS	75 (72.8%)	11 (10.7%)	17 (16.5%)	103 (100%)
Other GI	42 (64.6%)	8 (12.3%)	15 (23.1%)	65 (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)	91.3% (85.0%, 95.6%)	85.0% (77.6%, 90.7%)
Specificity (95% CI)	69.6% (62.1%, 76.5%)	81.0% (74.2%, 86.6%)
PPV (95% CI)	69.5% (61.9%, 76.3%)	77.1% (69.3%, 83.8%)
NPV (95% CI)	91.4% (85.1%, 95.6%)	87.7% (81.5%, 92.5%)
ROC AUC (95% CI)	0.912 (0.878, 0.946)	

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. non-IBD	Clinical decision point	
	50 µg/g	200 µg/g
Sensitivity (95% CI)	94.5% (89.0%, 97.8%)	80.3% (72.3%, 86.8%)
Specificity (95% CI)	62.5% (54.7%, 69.8%)	85.7% (79.5%, 90.6%)
PPV (95% CI)	65.6% (58.2%, 72.4%)	81.0% (73.0%, 87.4%)
NPV (95% CI)	93.8% (87.5%, 97.5%)	85.2% (78.9%, 90.2%)

Table 7: Clinical performance characteristics of the BÜHLMANN fCAL® turbo in discriminating IBD from non-IBD – IBS and other GI-related disorders, at 50 µg/g and 200 µg/g clinical decision points

IBD vs. IBS	Clinical decision point	
	80 µg/g	160 µg/g
Sensitivity (95% CI)	91.3% (85.0%, 95.6%)	85.0% (77.6%, 90.7%)
Specificity (95% CI)	72.8% (63.2%, 81.1%)	83.5% (74.9%, 90.1%)
PPV (95% CI)	80.6% (73.1%, 86.7%)	86.4% (79.1%, 91.9%)
NPV (95% CI)	87.2% (78.3%, 93.4%)	81.9% (73.2%, 88.7%)
ROC AUC (95% CI)	0.925 (0.892, 0.958)	

Table 8: Clinical performance characteristics of the BÜHLMANN fCAL® turbo in discriminating IBD from IBS at 80 µg/g and 160 µg/g clinical decision points

IBD vs. IBS	Clinical decision point	
	50 µg/g	200 µg/g
Sensitivity (95% CI)	94.5% (89.0%, 97.8%)	80.3% (72.3%, 86.8%)
Specificity (95% CI)	67.0% (57.0%, 75.9%)	88.3% (80.5%, 93.8%)
PPV (95% CI)	77.9% (70.5%, 84.2%)	89.5% (82.3%, 94.4%)
NPV (95% CI)	90.8% (81.9%, 96.2%)	78.4% (69.9%, 85.5%)

Table 9: Clinical performance characteristics of the BÜHLMANN fCAL® turbo in discriminating IBD from IBS at 50 µg/g and 200 µg/g clinical decision points

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

II. IBD monitoring

Disclaimer for South Korea: *IBD monitoring claim does not apply. For Korean instruction for use please contact your local distributor.*

Clinical thresholds and evaluation

The determination of fecal calprotectin is a reliable and simple way to assist the monitoring of IBD patients (ref. 10-22).
Correlation of calprotectin levels and the inflammatory status of patient's intestinal mucosa, according to endoscopic evaluations, was determined in three independent studies using BÜHLMANN calprotectin tests (table 10). The diagnostic value of calprotectin in predicting clinical remission and relapse, according to patient`s symptoms, clinical activity indices, unplanned need for therapy escalation, hospitalization or emergency was determined in three studies using BÜHLMANN calprotectin tests (table 11).

Calprotectin[1] vs IBD activity determined by endoscopic findings	**Study 1 Spain (ref. 12)**	**Study 2 Spain (ref. 13)**	**Study 3 Poland (ref. 14)**
Patient number and demographics	89 (CD[2]) Ages: 32-58 44% male	123 (UC[3]) Ages: 18-85 66.4% male	57 (CD[2]) Mean age: 35.3 48% male
Cut-off	272 µg/g	280 µg/g	238.5 µg/g
NPV	98%	86%	88%
PPV	76%	80.3%	73%

Table 10: Correlation of calprotectin levels with IBD disease activity determined by endoscopic evaluations

[1] Results for studies 1 and 2 were obtained with the BÜHLMANN lateral flow assays (Quantum Blue® fCAL and Quantum Blue® fCAL high range). Results in study 3 were obtained with the BÜHLMANN fCAL® ELISA and Quantum Blue® fCAL high range).
[2] CD = Crohn`s disease patients
[3] UC = Ulcerative Colitis patients

Calprotectin[1] vs future clinical remission or relapse	Study 4 UK (ref. 15)	Study 5 Spain (ref. 16)	Study 6 Spain (ref. 17)
Patient number and demographics	92 (CD[2]) 38% male	30 (CD[2]) adalimumab therapy Ages: 24-64 43.3% male	33 (CD[2]) 20 (UC[3]) infliximab therapy Ages: 18-68 47.2% male
Follow-up time after calprotectin measurement	12 months	4 months	12 months
Patients in clinical relapse after follow-up	11%	30%	23%
Cut-off	240 µg/g	204 µg/g	160 µg/g
NPV	96.8%	100%	96.1%
PPV	27.6%	75%	68.7%

Table 11: Determination of diagnostic value of calprotectin in predicting clinical remission and relapse of IBD disease

[1] Results for study 4 were obtained with the BÜHLMANN fCAL® ELISA. Results for studies 5 & 6 were obtained with the BÜHLMANN lateral flow assays (Quantum Blue® fCAL and Quantum Blue® fCAL high range).

[2] CD = Crohn`s disease patients

[3] UC = Ulcerative Colitis patients

The result categories shown are recommendations and their establishment is based on condensed knowledge of published cut-offs and clinical performance studies. It is advised that healthcare practitioners establish individual patient thresholds by determining the patient's baseline calprotectin level during disease remission.

Calprotectin values below 100 µg/g

Fecal calprotectin levels below 100 µg/g can reliably indicate patients, with low risk of clinical relapse, in endoscopic remission for whom invasive endoscopic procedures can be avoided (ref. 10-22).

Calprotectin values between 100 – 300 µg/g

Fecal calprotectin levels between 100 – 300 µg/g may indicate the necessity of tighter control in the following period to assess disease development tendencies.

Calprotectin values above 300 µg/g

Fecal calprotectin levels above 300 µg/g should be repeated and, if raised levels are confirmed, prompt further investigative procedures (ref. 10-22).

PERFORMANCE CHARACTERISTICS

The presented performance characteristics have been established on a Roche cobas® 6000 c501 instrument. Refer to clinical chemistry analyzer specific application notes for the performance characteristics on other clinical chemistry analyzers.

Method comparison – fCAL® turbo CALEX® Cap vs fCAL® ELISA CALEX® Cap

The method comparison study was performed according to the CLSI guideline EP09-A3. One hundred and ninety nine (199) clinical samples were measured using one lot of BÜHLMANN fCAL® turbo over 18 days in one calibration cycle. Reference values, with a final calprotectin concentration interval of 30.3 – 1672.5 µg/g, were established with the BÜHLMANN fCAL® ELISA. Samples were extracted using the CALEX® Cap. Single determinations from CALEX® Cap extracts were performed in both methods. Bias was determined using Passing-Bablok linear regression and Bland-Altman analysis.

Bland-Altman Analysis			Passing-Bablok Regression Analysis				
Mean bias (95% CI)	**Lower LoA (95% CI)**	**Upper LoA (95% CI)**	**Slope (95% CI)**	**Intercept [µg/g] (95% CI)**	**Bias at 80 µg/g (95% CI)**	**Bias at 160 µg/g (95% CI)**	**r**
0.7% (-2.6%, 4.0%)	-46.0% (-51.6%, -40.3%)	47.3% (41.6%, 53.0%)	1.139 (1.104, 1.172)	-18.3 (-24.4, -13.2)	-9.0% (-15.1%, -3.1%)	2.4% (-1.2%, 5.4%)	0.982

Method comparison – fCAL® turbo CALEX® Cap vs fCAL® ELISA manual extraction

The method comparison study was performed according to the CLSI guideline EP09-A3. One hundred and sixty eight (168) clinical samples were extracted using three lots of the CALEX® Cap and measured using one lot of BÜHLMANN fCAL® turbo over 18 days in one calibration cycle. Reference values, with a final calprotectin concentration interval of 30.5 – 1573.8 µg/g, were established using the manual extraction method and extract measurement with the BÜHLMANN fCAL® ELISA. Extracts were measured in single determinations in both methods. Bias was determined using Passing-Bablok linear regression and Bland-Altman analysis.

Bland-Altman Analysis			Passing-Bablok Regression Analysis				
Mean bias (95% CI)	**Lower LoA (95% CI)**	**Upper LoA (95% CI)**	**Slope (95% CI)**	**Intercept [µg/g] (95% CI)**	**Bias at 80 µg/g (95% CI)**	**Bias at 160 µg/g (95% CI)**	**r**
11.1% (5.5%, 16.6%)	-60.7% (-70.3%, -51.2%)	82.8% (73.3%, 92.4%)	1.336 (1.265, 1.429)	-31.7 (-44.1, -19.4)	-6.0% (-16.4%, 7.1%)	13.8% (8.1%, 23.2%)	0.955

Reproducibility (Multisite precision evaluation study): 3.2 – 9.1% CV
Reproducibility was established according to the CLSI guideline EP05-A3 using a 3 laboratory sites x 5 days x 5 replicates study design. Eight pooled stool specimen extracts with calprotectin concentrations ranging from 47.2 – 5475.6 µg/g were tested.

Between-lot precision: 2.4 – 8.2% CV
Between-lot precision was established according to the CLSI guideline EP05-A3 using a 3 lots x 5 days x 5 replicates study design. Eight pooled stool specimen extracts with calprotectin concentrations ranging from 45.2 – 5303.1 µg/g were tested.

Repeatability: 0.7 – 8.3% CV

Within-laboratory precision: 1.4 – 9.1% CV
Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3 using the standardized 20 days x 2 runs x 2 replicates study design. Eight pooled stool specimen extracts with calprotectin concentrations ranging from 42.9 – 5405.6 µg/g were tested.

Extraction reproducibility – CALEX® Cap: 8.1 – 19.7% CV
The extraction reproducibility was established according to the CLSI guideline EP05-A3 using a 2 days x 2 operators x 3 CALEX® Cap lots x 2 extractions x 3 replicates study design. Twelve clinical stool specimens, including specimens with solid, semi-solid and liquid consistency, with calprotectin concentrations in the range of 42.7 – 3440.0 µg/g, were tested.

Accuracy / Recovery: 93.6 – 102% CV
Seven stool specimen extracts from clinical samples with calprotectin levels ranging from 44.1 – 1076.3 µg/g 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.

Sample carry-over
The sample carry-over was established according to the CLSI guideline EP10-A2. No statistically significant carry-over with the BÜHLMANN fCAL® turbo test on Roche cobas® 6000 c501 instrument was detected.

Limit of Detection (LoD): 23.7 µg/g
The LoD was established according to the CLSI guideline EP17-A2 and with proportions of false positives (α) less than 5% and false negatives (β) less than 5% based on 120 determinations, with 60 blank and 60 low level replicates; and a **LoB of 16.7 µg/g**.

Limit of Quantitation (LoQ): 23.7 µg/g
The LoQ was established according to the CLSI guideline EP17-A2, based on 90 determinations and a precision goal of 20% CV. The LoQ estimate was found below that of the LoD and therefore is indicated as equal to the estimated LoD.

Linearity range: 15.6 – 12216 µg/g
The linear range of the BÜHLMANN fCAL® turbo was determined according to the CLSI guideline EP06-A. Samples with a concentration over 2000 µg/g were diluted automatically 1:10 by the analyzer. A maximum deviation from linearity of 10% was allowed. For values below 75 µg/g an absolute difference of less than 7.5 µg/g was allowed.

High Dose Hook Effect
Samples with theoretical concentrations of up to 45715 µg/g can be measured without limiting the measuring range of the assay.

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 the following substances [Concentration in mg/ 50 mg stool]; gyno-Tardyferon (0.11), Prednisone (0.31), Imurek (0.19); Salofalk (5.21), Asacol (2.50), Agopton (0.18), Vancocin (2.00), Sulfamethoxazole (1.6), Trimethoprim (0.35), Ciproxine (1.25), Vitamin E (0.30), Bion 3 (1.06), Hemoglobin (1.25).

No interference was detected with the following enteropathological microorganisms [Concentration in colony forming units (CFU)/ mL stool extract]; *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).

REFERENCES

1. Nilsen T et al.: J Clin Lab Anal 2017 ; 31(4). doi: 10.1002/jcla.22061.
2. Mandic-Havelka A et al.: Clin Lab. 2017 ; 63(5):907-913.
3. Noebauer B et al.: Biochem Med (Zagreb) 2017 ; 27(3):030710.
4. Fagerhol MK: Lancet 2000; 356, 1783-4.
5. Tibble JA et al.: Gut 2000; 47, 506-13.
6. Tibble JA et al.: Gastroenterol 2002; 123, 450-60.
7. Jahnsen J et al.: Tidsskr Nor Legeforen 2009; 129(8), 743-5.
8. Manz M. et al.: BMC Gastroenterology 2012; 12, 5.
9. Pavlidis P. et al.: Scand J Gastroenterol. 2013; 48(9), 1048-54.
10. Konikoff MR and Denson LA: Inflamm Bowel Dis 2006; 12(6), 524-34.
11. Lin et al.: Inflamm Bowel Dis 2014; 20: 1407-15.
12. Lobatón T et al.: J Crohns Colitis 2013, 641-51.
13. Lobatón T et al.: Inflamm Bowel Dis 2013; 19(5), 1034-42.
14. Moniuszko A et al.: Polish Arch. Intern. Med. 127, 312-318 (2017).
15. Naismith GD et al.: J Crohns Colitis 2014; 8, 1022-9.
16. Ferreiro-Iglesias R et al.: Scand J Gastroenterol 2016, 23, 1-6.
17. Ferreiro-Iglesias R1 et al.: J Clin Gastroenterol 2016; 50(2),147-51.
18. Guardiola J. et al.: Clinical Gastroenterology & Hepatology 2014; 12(11) 1865-70.
19. Lasson A et al.: United European Gastroenterol J 2015, 3(1) 72-9.
20. Bressler B et al.: Can J Gastroenterol Hepatol 2015, 29(7), 369-72.
21. Peyrin-BL et al.: Am J Gastroenterol 2015, 110, 1324-38.
22. Ricciuto A et al.: Crit Rev Clin Lab Sci. 2019; 56(5):307-320.
23. Blirup-Jensen et al.: Clin Chem Lab Med 2001; 39, 1110-22.
24. Blirup-Jensen et al.: Clin Chem Lab Med 2008; 46, 1470-9.
25. Molander P et al.: Journal of Crohn's and Colitis 2015, 33-40.
26. De Vos M et al.: Inflamm Bowel Dis. 2013; 19, 2111-2117.
27. Fagerberg UL et al.: J Pediatr Gastroenterol Nutr 2005; 40, 450-5.
28. Li F. et al.: PLoS ONE 10(3) (2015).
29. Zhu Q. et al.: PLoS ONE 11 (3) (2016).
30. Peura S. et al.: Scand J Clin Lab Invest 2018; 78(1-2): 120-124.

CHANGELOG

Date	Version	Change
2025-09-01	A6	Precising the *Intended Use* by adding the test platform. Revision of chapters *Reagent Storage and stability*, *Materials required but not provided, Warnings and Precautions, Clinical thresholds and evaluation (Table 10)* and *References.* Update to chapter *Performance Characteristics* (subchapters *Method comparison* and *Linearity Range*).

INCIDENT REPORTING IN EU MEMBER STATES

If any serious incident in relation to this device has occurred, please report without delay to the manufacturer and competent authority of your Member State.

SHIPPING DAMAGE

Please notify your distributor, if this product was received damaged.

SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1.
For definition of symbols see the symbol glossary at:
www.buhlmannlabs.ch/support/downloads/
In addition the following symbols and signs are used:

EN: electronic instruction for use available in different languages at:/ **BG**: електронни инструкции за употреба на различни езици на адрес:/ **CS**: elektronický návod k použití dostupný v různých jazycích na adrese:/ **DA**: elektronisk brugsanvisning på forskellige sprog på:/ **DE**: elektronische Gebrauchsanweisung in verschiedenen Sprachen verfügbar unter:/ **EL**: ηλεκτρονικές οδηγίες χρήσης διαθέσιμες σε διάφορες γλώσσες στη διεύθυνση:/ **ES**: instrucciones de uso electrónicas disponibles en diferentes idiomas en:/ **ET**: elektrooniline kasutusjuhend, mis on saadaval erinevates keeltes aadressil:/ **FR**: un mode d'emploi électronique disponible en différentes langues à l'adresse:/ **HU**: különböző nyelveken elérhető elektronikus használati utasítás a következő címen:/ **IT**: istruzioni elettroniche per l'uso disponibili in diverse lingue su:/ **LT**: elektroninės naudojimo instrukcijos įvairiomis kalbomis:/ **LV**: dažādās valodās pieejama elektroniska lietošanas instrukcija:/ **NO**: elektronisk instruksjon for bruk tilgjengelig på forskjellige språk på:/ **PL**: elektroniczna instrukcja obsługi dostępna w różnych językach na stronie:/ **PT**: instrução electrónica para utilização disponível em diferentes línguas em:/ **RO**: instrucțiuni electronice de utilizare disponibile în diferite limbi la adresa:/ **SK**: elektronický návod na použitie dostupný v rôznych jazykoch na:/ **SL**: elektronska navodila za uporabo so na voljo v različnih jezikih na:/ **SR**: elektronsko uputstvo za upotrebu dostupno na različitim jezicima na:/ **SV**: elektronisk bruksanvisning på olika språk på följande adress:/ **KR**: 다양한 언어로 사용 가능한 전자 지침

www.buhlmannlabs.ch/support/downloads/

Parts of the kits and pre-analytical procedures are patent protected by EP2947459(B1); US10620216(B2); AU2015261919(B2); JP6467436(B2)

CE 0123