BÜHLMANN fCAL® turbo

Calprotectin turbidimetric assay

for professional use

Reagent Kit

B-KCAL-RSET

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ENGLISH

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. The assay results can be used as an aid to diagnosis in distinguishing organic, inflammatory disease of the gastrointestinal tract (inflammatory bowel disease, IBD, e.g. Crohn's disease or ulcerative colitis, UC) from functional disease (irritable bowel syndrome, IBS) (ref. 1-7), in patients with chronic abdominal pain, above the age of four (ref. 8-9), and as an aid to IBD disease monitoring (ref. 10-12).

For laboratory use only.

PRINCIPLE OF THE ASSAY

The BÜHLMANN fCAL® turbo test is a particle enhanced turbidimetric immunoassay (PETIA) and allows quantification of calprotectin in fecal extracts on clinical chemistry analysers. Fecal samples are extracted with extraction buffer (B-CAL-EX) using the BÜHLMANN CALEX® Cap extraction device (Code: B-CALEX-C50 / B-CALEX-C200 / B-CALEX-C500) 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

REAGENT STORAGE AND STABILITY

- Do not freeze reagents! Shelf life of unopened reagents at 2-8 ˚C: see expiry date on package label.
- Shelf life of opened reagents at 2-8 ˚C: see expiry date on package label.
- On board stability: at least 60 days at up to 15 ˚C.

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 Device 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 for alternative extraction method	3 bottles 12 bottles 125 mL/bottle	B-CAL-EX3 B-CAL-EX12

Table 2

WARNINGS AND PRECAUTIONS

- This test is for *in vitro* use only, and must be handled by qualified personnel, in accordance with good laboratory practices (GLP).
- Do not mix reagents of different reagent lots or switch caps between reagents.
- The immunoparticles contain potentially infectious substances of animal origin and should be handled with due caution. Disposal of any discarded materials should be in accordance with local requirements.
- R1 contains MOPS that can be irritating to eye and skin. Handle with due caution.
- R2 contains polystyrene nanoparticles.

SPECIMEN COLLECTION AND STORAGE

Specimen transport: Stool specimens should be received by the laboratory within 3 days of collection. The specimens may be transported at room temperature (23 °C).

Specimen storage: Received stool specimens should be stored at 2-8 °C and extracted within 3 days.

Stool extraction with CALEX® Cap: Fecal samples are collected, extracted and diluted to a final concentration of 1:500 using the BÜHLMANN CALEX® Cap device (Code: B-CALEX-C50 / B-CALEX-C200 / B-CALEX-C500). The extraction is described in the instruction for use delivered with the respective extraction devices.

Important: Centrifuge the CALEX® Cap device for 10 minutes at 1000-3000 x g and continue with the assay procedure.

Extract storage: Calprotectin in extracts obtained by the BÜHLMANN CALEX® Cap is stable at room temperature for 3 days, at 2-8°C for 6 days and at -20°C for 18 months.

Alternative method: Stool specimens should be collected into plain tubes.
Manual weighing 1:50 with BÜHLMANN extraction buffer (Code: B-CAL-EX3, B-CAL-EX12), centrifugation (5 min. at 3000 x g) followed by an additional 1:10 dilution in BÜHLMANN extraction buffer. Calprotectin extract (1:50) obtained by manual weighing is stable at 2-8°C for 6 days or at -20°C for 18 months.

Important: The specimen must be collected without any chemical or biological additions in the collection device.

ASSAY PROCEDURE

Application notes / assay installation

Assay procedures for the BÜHLMANN fCAL® turbo are established on several clinical chemistry analysers. 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 (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 4 to 8 weeks according to the instrument specific application notes. Refer to the QC-data sheet provided with the BÜHLMANN fCAL® turbo Calibrator for assigned calibrator values. Contact BÜHLMANN support if calibration cannot be performed without error.

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, perform the following procedures:

- Repeat QC control measurement with fresh controls.
- Recalibrate the instrument.

Contact BÜHLMANN support if valid control values cannot be reproduced.

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

The results are calculated automatically on the clinical chemistry analyser and presented in µg/g unless otherwise stated in the corresponding clinical chemistry analyser specific application notes.

STANDARDIZATION

The BÜHLMANN fCAL® turbo is standardized against the BÜHLMANN fCAL® ELISA. The results from both assays are highly comparable (see performance characteristics).

LIMITATIONS

- Sample carryover: Depending on clinical chemistry analyser, a minor sample carryover (0.5 %) which might cause slightly increased levels can be detected.
- Reagent R2, occasionally 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.
- Evaporation of calibrators and controls on the analyser could lead to incorrect results. Run the assay immediately after loading the analyser.
- A minor hook effect in extracts >8000 µg/g might occur. Values >8000 µg/g are rare (<0.5 %) but might be measured falsely between 1000 and 2000 µg/g. It has not been observed that the value of extracts >8000 µg/g could be determined as below 1000 µg/g.
- Patients who are taking NSAIDs regularly may have elevations in their fecal calprotectin levels.
- Fecal calprotectin values are intended as an aid to diagnosis in distinguishing organic disease from functional disease and as an aid to IBD monitoring. Results should always be interpreted in combination with other clinical and laboratory findings.
- 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. 21-22).

INTERPRETATION OF RESULTS

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. 1-7). BÜHLMANN recommends applying the same cut-off values as for the BÜHLMANN fCAL® ELISA:

Normal values below 50 µg/g

Calprotectin values below 50 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).

Elevated values between 50 and 200 µg/g

Calprotectin values between 50 and 200 µg/g can represent mild organic disease such as inflammation caused by NSAIDs, mild diverticulitis and IBD in remission phase. The low inflammatory response shown within this range may suggest repeating the measurement and performing further investigations.

Elevated values above 200 µg/g

Calprotectin values above 200 µg/g are indicative of active organic disease with inflammation in the gastrointestinal tract. Appropriate further investigative procedures by specialists are suggested.

The cut-off level suggested for adults (50 µg/g) can also be used for children aged from 4 to 17 years regardless of sex (ref. 8-9).

INTERPRETATION OF RESULTS

IBD MONITORING

The determination of fecal calprotectin is also a reliable and simple way to assist the monitoring of IBD patients (ref. 10-20).
Condensed knowledge of published cut-offs and the clinical performance studies support the following result categories:

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. 10-20).

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.

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. 10-20).
The above result categories are recommendations. It is advised that healthcare practitioners establish individual patient thresholds by determining the patient's baseline calprotectin level during disease remission.

A false negative result that is a calprotectin result below 100 µg/g category that should show a value above 300 µg/g, for a patient with endoscopic inflammation, although unlikely, may delay appropriate clinical decisions and patient treatment. Therefore, it is important that the patient remains under the care of a healthcare practitioner and reports any clinical symptoms.

Studies have shown that high calprotectin levels, above 300 µg/g, will not always indicate development of a clinical relapse (ref. 10-20). High calprotectin levels should be treated as a red flag signal and repeated. Confirmation of raised levels should prompt further investigative procedures.

PERFORMANCE CHARACTERISTICS

The presented performance characteristics have been determined on Mindray BS-380 (registered trademark of Mindray Medical International). Refer to clinical chemistry analyser specific application notes for the performance characteristics on other clinical chemistry analyser.

Detection Limits

BÜHLMANN fCAL® turbo has a Limit of Quantification (LoQ) of 19 µg/g calprotectin on the BS-380 analyser, where LoQ is defined as the lowest actual amount of an analyte that can be reliably detected and at which the total error meets accuracy requirements.

Precision

The precision of BÜHLMANN fCAL® turbo calibrated with the BÜHLMANN fCAL® turbo Calibrator kit (B-KCAL-CASET) was determined in a 20-day precision study to CLSI guideline EP5-A3. Four fecal extracts and 2 controls (B-KCAL-CONSET) were measured on the BS-380 analyser.

Fecal extracts	Mean value (µg/g)	Within-Run CV (%)	Between-Day CV (%)	Between-Run CV (%)	Total CV (%)
Low	52	3.91	3.10	3.60	6.15
Medium low	84	1.99	3.98	0.87	4.54
Medium high	418	0.64	0.15	1.54	1.67
High	2024	0.32	1.79	1.12	2.14
Control low	74	4.43	5.24	0.51	6.88
Control high	259	0.62	2.48	1.52	2.97

Table 3 : Precision results

Linearity

BÜHLMANN fCAL® turbo calibrated with the BÜHLMANN fCAL® turbo Calibrator kit (B-KCAL-CASET) is linear within the range of 21-2058 µg/g on the BS-380 analyser. Concentrations outside this range will be diluted by the analyser-specific protocol 1:10 and retested.

Hook Effect

For the BÜHLMANN fCAL® turbo no hook effect is observed for calprotectin concentrations up to 8000 µg/g.

Interference

No interference is detected with Haemoglobin (0.05 g/L), Triglycerides (Intralipid®) (1 g/L) or Bilirubin (0.5 mg/L) on the BS-380 analyser. As samples for BÜHLMANN fCAL® turbo are extracted at high dilution (1:500) interfering concentrations lower than standard were used. No interference was observed with standard, orally-administered drugs and food additives prescribed for gastrointestinal complaints. The interference study was designed in accordance with the CLSI guideline EP07-A2.

Method Comparison

Results obtained with BÜHLMANN fCAL® turbo on the BS-380 analyser were compared with those obtained by the BÜHLMANN fCAL® ELISA by Passing-Bablok regression. The study included 112 fecal extracts in the range of 30-1800 µg/g (Fig. 1).

SHIPPING DAMAGE

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

REACH

None of the materials and reagents in the kit require a Safety Data Sheet (MSDS) according to CLP-Regulation (EC) No 1272/2008 and directive EC 1907/2006 (REACH).

APPENDIX I

TABLES AND FIGURES

	Linear Regression (turbo vs. ELISA)		**Altman Bland analysis** (turbo vs. ELISA)		
N	Slope	Coefficient of determination (R^2)	Bias (%)	Lower LoA*	Upper LoA*
112	1,05	0,91	-2.5	-47.8 %	42.9 %

Table 4 : Method comparison results
*LoA = Limits of agreement

Method comparison

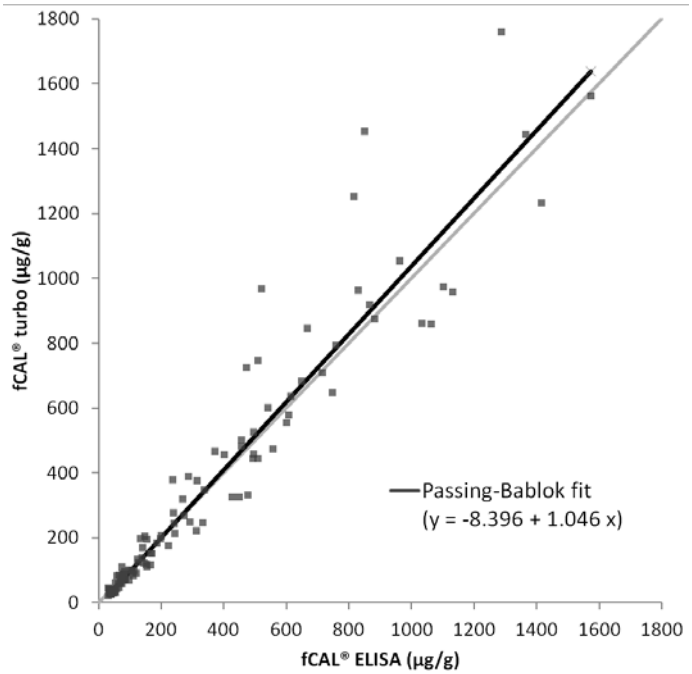

Fig. 1: Scatterplot of 112 samples included in the method comparison analysis.

APPENDIX II

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APPENDIX III

SYMBOLS

Symbol	Explanation
	Expiration date
REF	Catalogue Number
LOT	Lot number
IVD	*In Vitro* Diagnostic Medical Device

Symbol	Explanation
	Consult Instructions for Use
	Manufacturer
	Temperature limitations

CE

IVD