Quantum Blue® fCAL extended

Quantitative Lateral Flow Assay

For *In Vitro* Diagnostic Use

LF-CALE25 25 tests

Release date: 2022-07-13
Version A4

Manufacturer

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ENGLISH

INTENDED USE

The BÜHLMANN Quantum Blue® fCAL extended is an *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, specifically Crohn's disease or ulcerative colitis, UC) from functional disease (irritable bowel syndrome, IBS) (ref. 1-7), in patients with chronic abdominal pain and as an aid to IBD disease monitoring (ref. 7-18).

For laboratory use only.

PRINCIPLE OF THE ASSAY

The test is designed for the selective measurement of calprotectin antigen by sandwich immunoassay. A monoclonal capture antibody (mAb) highly specific for calprotectin is coated onto the test membrane. A second monoclonal detection antibody conjugated to gold colloids is deposited onto the conjugate release pad and released into the reaction system after addition of the extracted and diluted stool sample. The calprotectin / anti-calprotectin gold conjugate binds to the anti-calprotectin antibody coated on the test membrane (test line) and the remaining free anti-calprotectin gold conjugate binds to the goat anti-mouse antibody coated on the test membrane (control line). The signal intensities of the test line (T) and the control line (C) are measured quantitatively by the BÜHLMANN Quantum Blue® Reader.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Comments
Test Cassette	25 pieces	B-LFCALUS-TC	vacuum-sealed in a foil pouch
Extraction Buffer	1 bottle 125 mL	B-CAL-EX	Ready to use
Controls Low* / High*	2 vials 0.5 mL	B-CALE-CONSET	Ready to use
RFID Chip Card	1 piece	B-CALE-RCC	**White** plastic card
RFID Chip Card	1 piece	B-CALE-RCC720	**Green** plastic card
Barcode Card	1 piece	B-CALE-BCC	2D Barcode plastic card

Table 1

* The controls contain lot specific amounts of native human calprotectin. Refer to the additional QC data sheet for actual concentrations.

CHECK YOUR TEST KIT

BÜHLMANN products have been manufactured with the greatest of care and all possible efforts have been taken to ensure completeness of this test kit and its performance. Nevertheless, we advise you to verify your test kit for the condition of the test cassette and its pouch based on the following criteria:

- Expiration date
- The fault-free condition of the pouch (e.g. absence of any perforation that could be caused by improper handling).
- The fault-free condition of the test cassette (e.g. absence of scratches on the analytical membrane).

Should one of the test cassettes not fulfil the criteria mentioned above, please use another test cassette.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened reagents	
Store at 2-8 °C. Do not use the reagents beyond the expiration date printed on the labels.	
Opened reagents	
Test Cassette	Test cassettes removed from the foil pouch must be used within 4 hours.
Extraction Buffer	Store for up to 6 months at 2-8 °C after opening.
Controls Low / High	Store for up to 6 months at 2-8 °C after opening.

Table 2

REAGENTS & MATERIALS SUPPLIED SUPPLEMENTARY

Fecal extraction devices

Fecal extraction devices described in table 3 are not delivered with the kit. The selected extraction devices must be ordered separately.

Extraction device Kits	Quantity	Code
CALEX® Cap device	Packages of 50, 200 or 500 tubes available, filled with 5 mL extraction buffer Ready to use	B-CALEX-C50 B-CALEX-C200 B-CALEX-C500
Smart-Prep	50 tubes consisting of spatulas and base caps	B-CAL-RD

Table 3

MATERIALS REQUIRED BUT NOT PROVIDED

- Vortex mixer for stool extraction
- Precision pipettes with disposable tips: 10-100 µL, 100-1000 µL and 250-2500 µL
- Centrifuge
- 5 mL polypropylene or polystyrene tubes for dilution of the extracts
- Timer (optional)
- Quantum Blue® Reader available from BÜHLMANN (order code: BI-POCTR-ABS)
- Soft tissues or blotting paper

PRECAUTIONS

Safety precautions

- The controls of this test contain components of human origin. Although tested and found negative for HBV surface antigen, HCV and HIV1/2 antibodies, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practices (GLP) using appropriate precautions.
- The extraction buffer and controls of this kit contain 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).

- Patient specimens should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practice (GLP) using appropriate precautions.
- Reagents: 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.
- Reagents and chemicals have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

Technical precautions

Kit components

- The test must be performed at room temperature (18-28 °C).
- All reagents and test samples must be equilibrated to room temperature (18-28 °C) before starting the assay.
- Once equilibrated to room temperature remove the test cassette from the foil pouch. Allow the test cassette to equilibrate in the laboratory environment for at least 2 minutes before starting the assay.
- Mix well (vortex) the reagents before use.
- Kit components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.
- The assay is designed for fecal extracts prepared using the extraction buffer provided in the kit or with the CALEX® Cap device. The use of other extraction buffers could lead to incorrect results.
- Do not disassemble the test cassettes.
- Handle the test cassettes with care. Do not contaminate the sample loading port or read-out window via skin contact, other liquids, etc. (figure 1D).
- Ensure a flat, horizontal position of the test cassette while performing the assay.
- Test cassettes cannot be re-used.

Test procedure

- Read the instructions carefully prior to carrying out the assay. Assay performance will be adversely affected, if reagents are incorrectly diluted, handled or stored under conditions other than those as detailed in this instruction for use.
- Please note that there are two generations of readers: The Quantum Blue® Reader 2nd Generation with serial numbers between 1000 and 3000 (QB2) and Quantum Blue® Reader 3rd Generation with serial numbers above 3000 (QB3G).
- The QB2 must be switched on and programmed for the Quantum Blue® fCAL extended assay. Load the assay method using the RFID chip card (B-CALE-RCC or B-CALE-RCC720) before starting the assay (see Quantum Blue® Reader manual).
- The QB3G must be switched on and programmed for the Quantum Blue® fCAL extended assay either by using the barcode card (B-CALE-BCC) or by selecting from the test menu (Fast Track Mode only). For more information please refer to the Quantum Blue® Reader manual.
- Use the RFID chip card (QB2) / barcode card (QB3G) in order to change lot-specific test parameters.
- Patient samples that are not properly handled may cause inaccurate results.
- In order to receive reliable and quantitative results it is important to homogenize the stool sample entirely in the extraction buffer within the extraction device.
- When using BÜHLMANN Smart-Prep, it is important to centrifuge the extracts before storage. Centrifuge the tubes for 5 minutes at 3000 x g. After centrifugation the supernatant must be transferred into a fresh storage tube.

SPECIMEN COLLECTION, STORAGE, STABILITY

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. The specimens may be transported 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.

Extract stability

Fecal calprotectin extracts obtained with the CALEX® Cap device are stable at room temperature (23 °C) for 7 days and at 2-8 °C for up to 15 days. For longer storage, freeze extracts at -20 °C. Frozen extracts are stable for a period of up to 23 months.
CALEX® Cap extracts can be stored and frozen directly within the CALEX® Cap device. Extracts can be subject to four freeze-thaw cycles. Prior to measurement, allow frozen extracts to equilibrate to room temperature. For re-use / re-measurement of the extracts see step 2 under the chapter assay procedure.
Fecal calprotectin extracts obtained by BÜHLMANN Smart-Prep are stable at 2-8 °C for ≤ 7 days or at -20 °C for 36 months.

ASSAY PROCEDURE

The assay procedure consists of three steps:

1. **Extraction of stool samples**

   The extraction is described in the instruction for use delivered with the respective extraction devices.

   CALEX® Cap device: 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.

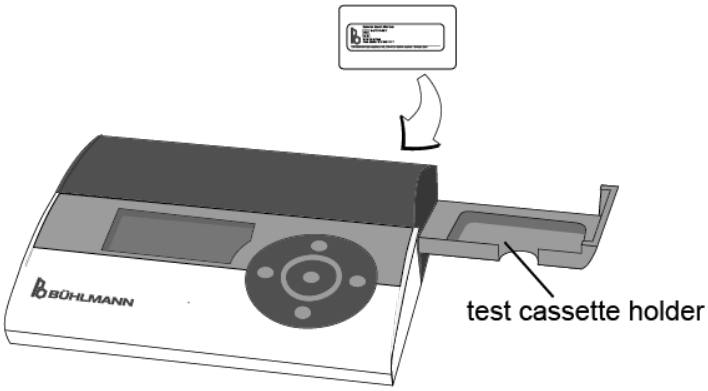
2. Sample processing

- Smart-Prep: Let the stool extract settle for 10 minutes after extraction. Dilute the supernatant 1:10 with extraction buffer (e.g. 50 µL stool extract and 450 µL extraction buffer) and mix well. Let the samples equilibrate for at least 5 minutes at 18-28 °C prior to proceeding to the next step (step no. 3).
- CALEX® Cap device: After extraction, let the stool extract settle for 10 minutes with the white head of the device down. Unscrew the blue cap. The supernatant can be used without further dilution in the lateral flow assay.

3. Lateral flow assay procedure and readout

QB2

Two alternative methods can be loaded from the respective RFID chip card: B-CALE-RCC720 (with internal timer) or B-CALE-RCC (without internal timer). Select one of the RFID chip cards before starting the experiments. Load the test method from the RFID chip card on the Quantum Blue® Reader.



QB3G

Two different modes of operation are available from BÜHLMANN to measure samples with the QB3G: Fast Track Mode or Fail Safe Mode. Before starting the assay, please inform yourself in which operation mode your reader is working.
The test method can be loaded from the barcode card (Fast Track and Fail Safe Mode) or, if previously used, selected from the test menu (Fast Track Mode only). Measurements can be performed with or without an internal timer in the Fast Track Mode. Measurements in the Fail Safe Mode can be performed with internal timer only.
Follow the instructions provided on the screen of the QB3G. You may also refer to the QB3G Quick Guides for the Fast Track and Fail Safe Mode.

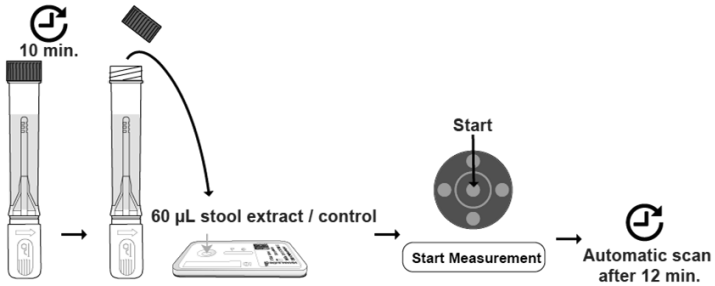
3.1. Method with internal timer

QB2: use the green RFID chip card B-CALE-RCC720

QB3G (Fast Track Mode): when prompted by the QB3G to skip the incubation time, select “NO”

QB3G (Fail Safe Mode): default setting

- Unpack the test cassette and equilibrate it for at least 2 minutes in the laboratory environment.
- Add 60 µL of stool extract onto the sample loading port of the test cassette.
- Load the test cassette onto the test cassette holder of the reader.
- Close the test cassette holder and start the measurement by pressing the start button on the QB2 or the “Start Measurement” option on the QB3G.
- The scan starts automatically after 12 minutes (720 seconds).
- For low / high controls: Repeat step 3.1 with 60 µL controls instead of stool extract.



3.2. Method without internal timer

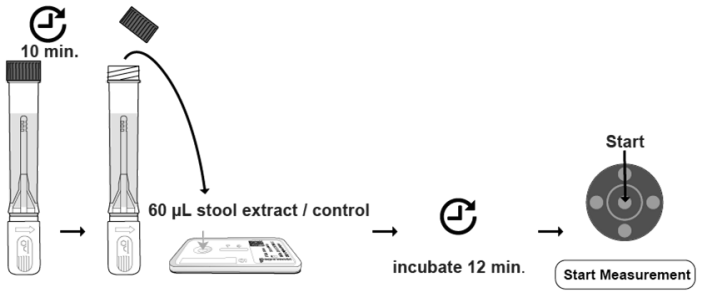
QB2: Use the white RFID chip card B-CALE-RCC

QB3G (Fast Track Mode): when prompted by the QB3G to skip the incubation time, select “YES”

QB3G (Fail Safe Mode): option not available

- Unpack the test cassette and equilibrate it for at least 2 minutes in the laboratory environment.
- Add 60 µL of stool extract onto the sample loading port of the test cassette.
- Incubate for 12 minutes +/- 1 minute (set a timer manually).
- Load the test cassette onto the test cassette holder of the reader.

- Scan the test cassette with the Quantum Blue® Reader immediately by pressing the start button on the QB2 or the “Start Measurement” option on the QB3G.
- For low / high controls: Repeat step 3.2 with 60 µL of controls instead of stool extract.



Remark: Please refer to your Quantum Blue® Reader manual to learn about the basic functions and how to initialize and operate the Quantum Blue® Readers, especially how to select test methods and how to load lot-specific parameters from the RFID chip card (QB2) / barcode card (QB3G) on the Quantum Blue® Reader. Ensure the correct insertion of the test cassette into the Quantum Blue® Reader, with the read-out window first (figure 1D).

QUALITY CONTROL

- If the performance of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing ii) expiration dates of reagents and iii) storage and incubation conditions.
- The result of the self-test of the Quantum Blue® Reader performed at startup has to be valid.

VALIDATION OF RESULTS

- For a valid test result, the control line (C) must be visible in any case (see figures 1A and 1B). It is used as functional test control only and cannot be used for the interpretation of the test line (T). If the test line (T) is not detectable after 12 minutes of incubation time (figure 1A), the concentration of calprotectin present in the stool sample is below the detection limit. If a test line (T) is detectable after 12 minutes of incubation time (figure 1B), the calprotectin concentration present in the stool sample is calculated by the Quantum Blue® Reader.
- If only the test line (T) is detectable after 12 minutes of incubation time (figure 1C), the test result is invalid and the assay has to be repeated using another test cassette.
- If neither the control line (C) nor the test line (T) are detectable after 12 minutes of incubation time (figure 1D), the test result is invalid and the assay has to be repeated using another test cassette.
- As the Quantum Blue® Reader allows a quantitative evaluation of the test (T) and control (C) lines, an additional validity check of the control line (C) is undertaken. If the signal intensity of the control line (C) is below a threshold after 12 minutes of incubation time, the test result is also invalid and the assay has to be repeated using another test cassette.

STANDARDIZATION

- There are no internationally or nationally recognized reference materials or reference measurement procedures for the calprotectin analyte in stool specimen. The Quantum Blue® fCAL extended is standardized with the BÜHLMANN fCAL® ELISA (order code: EK-CAL), which is standardized using internal reference material.
- The Quantum Blue® Reader uses a lot-specific standard curve to calculate the calprotectin concentration. The 95 % confidence interval of the combined uncertainty of the product calibrator is lower than 20.0 %, the combined uncertainty of the controls lower than 30.0 %.
- The assay range is between 30 and 1000 µg/g.
- To receive quantitative results for calprotectin concentration between 850-1800 µg/g, high samples reading above 850 µg/g can be re-tested with the BÜHLMANN Quantum Blue® high range test (order code: LF-CHR25).

LIMITATIONS

- Reagents delivered with the BÜHLMANN Quantum Blue® fCAL extended kit are intended for the determination of calprotectin levels in human stool samples only.
- 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. 19-20).
- In rare cases, when calprotectin levels are extremely high (above 5000 µg/g, e.g. in acute UC), the test system may be prone to a high dose hook effect, that can result in values below the expected 1000 µg/g assay range limit. It is advised to give particular attention to results above 300 µg/g when accompanied by strong symptoms.
- Patients who are taking NSAIDs regularly may have 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. 21-24).

INTERPRETATION OF RESULTS

I. Distinguishing organic disease from functional gastrointestinal disease

The determination of fecal calprotectin levels can be used as a reliable and simple aid in distinguishing organic from functional gastrointestinal diseases (ref. 1-7).
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.

Clinical thresholds

The following data were established with the BÜHLMANN fCAL® ELISA (order code: EK-CAL).

Results from 58 clinical samples from patients diagnosed with IBS and 131 clinical samples from patients diagnosed with IBD, from an international clinical study, were analyzed to obtain the values described in table 4.

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

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.

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 above 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 Quantum Blue® fCAL extended test to discriminate between patients with IBD and other non-inflammatory GI disorders, including IBS, was evaluated using clinical samples collected from 278 patients and extracted using the CALEX® Cap device. One hundred and twenty-four (124) patients had a final diagnosis of IBD (Crohn's disease, ulcerative colitis or indeterminate colitis), 92 patients suffered from IBS and 62 patients presented with abdominal pain and/or diarrhea, or other GI-related non-inflammatory conditions (refer to table 5). Final diagnosis was supported by endoscopic as well as other clinical findings.

A clinical sensitivity of 91.9 % at 80 µg/g and a clinical specificity of 78.6 % at 160 µg/g, can be reached in the differentiation between IBD and GI-related non-inflammatory conditions, including IBS. ROC curve analysis resulted in an AUC of 0.901 (refer to table 6).

A clinical sensitivity of 91.9 % at 80 µg/g and a clinical specificity of 80.4 % at 160 µg/g, can be reached in the differentiation between IBD and IBS. ROC curve analysis resulted in an AUC of 0.913 (refer to table 7).

The optimal cut-off combination for these patient pools could be defined by ROC analysis at 80 µg/g and 160 µg/g calprotectin, 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 of 200 µg/g** with slightly lower sensitivity (table 8 and 9).

II. IBD monitoring

Clinical thresholds

The determination of fecal calprotectin is also a reliable and simple way to assist the monitoring of IBD patients (ref. 7-18).

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. 7-18).

Calprotectin values between 100 and 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. 7-18).

Clinical evaluation

Correlation of calprotectin levels and the inflammatory status of patients' 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).

PERFORMANCE CHARACTERISTICS

The presented performance characteristics have been established on the Quantum Blue® Reader 3rd Generation, with the excepton of linearity presented for both reader generations.

Quantum Blue® fCAL extended was validated on both Quantum Blue® Reader 2nd and 3rd Generation instruments. The indicated performance characterstic specifications apply to both reader generations.

Method comparison

Bias at clinical decision points and mean bias: ≤15 %

The method comparison study was performed according to the CLSI guideline EP09-A3. One-hundred and eighty-three (183) stool samples extracted with the CALEX® Cap device were measured over 10 days with three Quantum Blue® fCAL extended reagent lots. Reference values, with a final calprotectin concentration interval of 30.5 to 925.8 µg/g were established in a clinical study with the BÜHMANN fCAL® ELISA using the manual weighing and extraction method. The results are summarized in tables 12 and 13.

Accuracy / Recovery: within 80 %-120 %

Eight stool specimen extracts were spiked with 60.2 µg/g and 120.4 µg/g calprotectin in calibrator material of stool extracts, at 5 % and 10 % of the specimen extract volume, respectively. “Baseline” samples were spiked with the corresponding volume of extraction buffer. “Baseline” and “baseline + spike” samples were measured in 13 replicates. The results are summarized in table 14.

Repeatability: **≤25 % CV**

Within-laboratory precision: ≤25 % 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. Six pooled stool specimen extracts with calprotectin concentrations ranging from 49.9 – 485.0 µg/g were tested. The results are summarized in table 15.

Between-lot precision: ≤25 % 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. Six pooled stool specimen extracts with calprotectin concentrations ranging from 55.3 - 552.5 µg/g were tested. The results are summarized in table 16.

Between-instrument reproducibility: ≤25 % CV

Between-instrument precision was established according to the CLSI guideline EP05-A3 using a 3 instruments x 5 days x 5 replicates study design. Six pooled stool specimen extracts with calprotectin concentrations ranging from 48.5 – 502.8 µg/g were tested. The results are summarized in table 17.

Limit of Detection (LoD): ≤30 µg/g

The LoD was established according to the CLSI guideline EP17-A2 using the classical approach, parametric analysis and a LoB <20 µg/g, determined using a non-parametric analysis.

Limit of Quantitation (LoQ): ≤30 µg/g

The LoQ was established according to the CLSI guideline EP17-A2, based on 90 determinations and a precision goal of 25 % CV.

Linearity: 25.2 to 908.9 µg/g

The linear range of the Quantum Blue® fCAL extended was determined according to CLSI guideline EP06-A. Measurements were performed in 10 replicates on a total of four reagent lots. A maximum deviation from linearity of 20 % or 15 µg/g, for samples below 75 µg/g, was allowed. The results are summarized in table 18.

High dose hook effect

High dose hook effect testing was performed on two reagent lots. Samples with calprotectin concentrations up to 5000 µg/g were correctly indicated as above 1000 µg/g for all replicates. For samples with higher calprotectin concentraiton values (6308.2 - 11214.4 µg/g) replicates with values below 1000 µg/g (643.4 µg/g lowest) were observed.

PREANALYTICS

CALEX® Cap extraction reproducibility ≤ 30 % 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. Eight clinical stool specimens with calprotectin concentrations ranging from 51.2 - 615.3 µg/g were assayed. The results are summarized in table 19.

INTERFERING SUBSTANCES

The susceptibility of the Quantum Blue® fCAL extended assay to oral pharmaceuticals, nutritional supplements, haemoglobin as well as entheropathological microorganisms was assessed according to the CLSI guideline EP07-A2. Bias in results exceeding 20 % was considered interference.

No interference was detected with listed substances, in Table 20, up to the indicated concentrations.
No interference was detected with entheropathological microorganisms, listed in Table 21, up to the indicated amounts of colony forming units (CFU) per mL of stool specimen extract.

TABLES AND FIGURES

Test Results

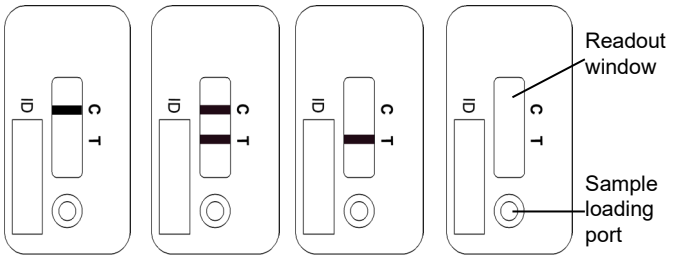


Figure 1A Figure 1B Figure 1C Figure 1D

Figure 1

Clinical Study – distinguishing organic disease from functional gastrointestinal disease

Final diagnosis	Distribution of patients' results in numbers (percent) within Quantum Blue® fCAL extended diagnostic ranges			
	< 80 µg/g	80 - 160 µg/g	> 160 µg/g	Total
IBD	10 (8.1%)	12 (9.7%)	102 (82.3%)	124
IBS	62 (67.4%)	12 (13.0%)	18 (19.6%)	92
Other GI	38 (61.3%)	9 (14.5%)	15 (24.2%)	62

Table 5

IBD vs. non-IBD	Clinical decision point	
	80 µg/g	160 µg/g
Sensitivity (95% CI)	91.9% (85.7%, 96.1%)	82.3% (74.4%, 88.5%)
Specificity (95% CI)	64.9% (56.8%, 72.4%)	78.6% (71.2%, 84.8%)
PPV (95% CI)	67.9% (60.2%, 74.8%)	75.6% (67.4%, 82.5%)
NPV (95% CI)	90.9% (83.9%, 95.6%)	84.6% (77.6%, 90.1%)
ROC AUC (95% CI)	0.901 (0.865, 0.938)	

Table 6

IBD vs. IBS	Clinical decision point	
	80 µg/g	160 µg/g
Sensitivity (95% CI)	91.9% (85.7%, 96.1%)	82.3% (74.4%, 88.5%)
Specificity (95% CI)	67.4% (56.8%, 76.8%)	80.4% (70.9%, 88.0%)
PPV (95% CI)	79.2% (71.6%, 85.5%)	85.0% (77.3%, 90.9%)
NPV (95% CI)	86.1% (75.9%, 93.1%)	77.1% (67.4%, 85.0%)
ROC AUC (95% CI)	0.913 (0.876, 0.950)	

Table 7

IBD vs. non-IBD	Clinical decision point	
	50 µg/g	200 µg/g
Sensitivity (95% CI)	96.0% (90.8%, 98.7%)	79.0% (70.8%, 85.8%)
Specificity (95% CI)	50.6% (42.5%, 58.8%)	83.8% (77.0%, 89.2%)
PPV (95% CI)	61.0% (53.8%, 67.9%)	79.7% (71.5%, 86.4%)
NPV (95% CI)	94.0% (86.5%, 98.0%)	83.2% (76.4%, 88.7%)

Table 8

IBD vs. IBS	Clinical decision point	
	50 µg/g	200 µg/g
Sensitivity (95% CI)	96.0% (90.8%, 98.7%)	79.0% (70.8%, 85.8%)
Specificity (95% CI)	52.2% (41.5% ,62.7%)	83.7% (74.5%, 90.6%)
PPV (95% CI)	73.0% (65.5%, 79.7%)	86.7% (79.1%, 92.4%)
NPV (95% CI)	90.6% (79.3%, 96.9%)	74.8% (65.2%, 82.8%)

Table 9

non-IBD - IBS + other GI
CI – confidence interval
PPV – positive predictive value
NPV – negative predictive value
ROC AUC – area under receiver operating characteristic curve

Clinical Studies – IBD monitoring

Calprotectin[1] vs IBD activity determined by endoscopic findings	Study 1 Spain (ref. 9)	Study 2 Spain (ref. 10)	Study 3 Australia, New Zealand (ref.11)
Patient number and demographics	89 (CD[2]) Ages: 32-58 44% male	123 (UC[3]) Ages: 18-85 66.4% male	99 (CD[2] after resection) Ages: 29-47 46.5% male
Cut-off	272 µg/g	280 µg/g	100 µg/g
NPV	98%	86%	91%
PPV	76%	80.3%	53%

Table 10

[1] Study 1 & 2 – Quantum Blue® fCAL and Quantum Blue® fCAL high range
Study 3 – BÜHLMANN fCAL® ELISA
[2] CD = Crohn`s disease patients
[3] UC = Ulcerative Colitis patients

Clinical Studies – IBD monitoring

Calprotectin[1] vs future clinical remission or relapse	Study 4 UK (ref. 12)	Study 5 Spain (ref. 13)	Study 6 Spain (ref. 14)
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

[1] Study 4 – BÜHLMANN fCAL® ELISA
Study 5 & 6 – Quantum Blue® fCAL and Quantum Blue® fCAL high range
[2] CD = Crohn`s disease patients
[3] UC = Ulcerative Colitis patients

TABLES AND FIGURES

Method Comparision

Passing-Bablok Regression Analysis						
Slope (95% CI)	Intercept [µg/g] (95% CI)	Bias at 80 µg/g (95% CI)	Bias at 100 µg/g (95% CI)	Bias at 160 µg/g (95% CI)	Bias at 300 µg/g (95% CI)	r
1.123 (1.045, 1.221)	-2.7 (-11.3, 3.6)	8.9% (4.2%, 15.3%)	9.6% (4.6%, 16.8%)	10.6% (4.3%, 19.2%)	11.4% (3.8%, 21.1%)	0.900

Table 12

Bland-Altman Analysis		
Mean bias (95 % CI)	Lower LoA (95 % CI)	Upper LoA (95 % CI)
9.7% (4.9%, 14.5%)	-54.6% (-62.8%, -46.4%)	74.0% (65.8%, 82.2%)

Table 13

Recovery

ID	Spike value [µg/g]	Mean baseline [µg/g]	Expected baseline + spike [µg/g]	Observed baseline + spike [µg/g]	Recovery rate [%]
#1	60.2	52	112	110	99
#2	60.2	63	123	127	103
#3	60.2	63	123	131	107
#4	60.2	78	138	137	99
#5	60.2	115	175	179	102
#6	120.4	149	270	272	101
#7	120.4	221	341	341	100
#8	120.4	469	589	559	95

Table 14

Within-Laboratory Precision

ID	Mean [µg/g]	n	Within-run (Repeatability) %CV	Between-run %CV	Between-day %CV	Total precision %CV
S1	49.9	80	18.2	0.0	5.3	18.9
S2	87.1	80	17.0	0.0	2.9	17.2
S3	135.7	80	11.7	8.9	0.0	14.7
S4	213.2	80	14.5	6.5	1.8	16.0
S5	337.4	80	14.8	3.2	5.0	15.9
S6	485.0	80	21.4	0.0	0.0	21.4

Table 15

Between-Lot Precision

ID	Mean [µg/g]	n	Within-run (Repeatability) %CV	Between-day %CV	Between-lot %CV	Total precision %CV
S1	55.3	75	16.6	10.0	0.0	19.4
S2	94.4	75	16.4	8.7	0.0	18.5
S3	155.2	75	20.1	2.6	2.1	20.4
S4	227.0	75	17.3	2.8	0.0	17.5
S5	361.5	75	16.9	2.5	4.8	17.7
S6	552.5	75	17.3	6.8	4.6	19.1

Table 16

Between-Instrument Precision

ID	Mean [µg/g]	n	Within-run (Repeatability) %CV	Between-day %CV	Between-instrument %CV	Total precision %CV
L1	48.5	75	16.9	2.4	4.3	17.6
L2	86.9	75	12.4	5.6	0.0	13.6
L3	151.6	75	19.4	3.2	0.0	19.7
L4	224.1	75	17.5	4.2	3.5	18.3
L5	355.0	75	17.0	4.9	0.0	17.7
L6	502.8	75	19.8	7.3	4.5	21.6

Table 17

Linearity

Dilution Series	Lot	Measuring Interval [µg/g]	R2	p-value for non-linear coefficient	Linear range [µg/g]
1	M0527	15.5 to 939.1	0.911	<0.0001*	15.5 to 939.1
2	M2128	16.1 to 908.9	0.927	<0.0001*	25.2 to 908.9
3	M3048	11.7 to 972.9	0.856	0.018*	11.7 to 972.9
4	M4851	24.3 to 1004.2	0.939	<0.0001*	24.3 to 1004.2

Table 18: *significant

Pre-analytics extraction reproducibility

ID	Mean [µg/g]	n	Within-run %CV	Between-				Total %CV
				extraction %CV	day %CV	lot %CV	operator %CV	
S1	51.2	72	11.7	6.1	10.2	0.0	0.0	16.7
S2	63.5	72	19.0	9.9	4.3	0.0	0.0	21.9
S3	87.4	72	13.2	12.4	1.8	4.6	1.2	18.8
S4	159.5	72	16.6	0.0	5.0	0.0	2.1	17.5
S5	181.4	72	11.6	11.0	0.0	3.5	11.0	19.7
S6	270.5	72	15.1	12.5	6.6	9.6	6.4	23.7
S7	570.8	72	16.9	8.1	5.7	2.0	0.0	19.6
S8	615.3	72	17.0	8.9	9.3	0.0	0.0	21.3

Table 19

TABLES AND FIGURES

Interfering substances

Trade Name	Active Component	Concentration mg/50 mg stool
Duofer Fol	Iron (II) sulfate (contains 0.4 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
Bactrim	Sulfamethoxazole + Trimethoprim	1.7 + 0.35
Ciproxine	Ciprofloxacin	1.25
Vitamin E	DL-α-Tocopherol Acetate	0.30
Berocca	*B1 (1.4 mg), B2 (1.6 mg), B6 (2 mg), B12 (1 µg), C (60 mg), folic acid (200 mg), nicotinamid (18 mg), pantothensäure (6 mg), biotin (0.15 mg), calcium (120 mg), magnesium (120 mg), zink (9.5 mg)*	1.06
Hemoglobin	Hemoglobin	1.25

Table 20

Name	Final Concentration (CFU/mL)
Escherichia coli	2.9×10^6
Salmonella enterica subsp. enterica	8.2×10^6
Klebsiella pneumoniae subsp. pneumonia	4.5×10^6
Citrobacter freundii	5.5×10^6
Shigella flexneri	5.0×10^6
Yersinia enterocolitica subsp. enterocolitica	5.3×10^6

Table 21

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CHANGELOG

Date	Version	Change
2022-07-13	A4	Update to chapter *Storage and shelf life of reagents* Update to chapter *Reagents and materials supplied supplementary* Update to chapter *Precautions* Update to chapter *Standardization* Update to *Limitation* regarding high dose hook effect Update to Clinical performance data in chapter *Distinguishing organic disease from functional gastrointestinal disease, Clinical evaluation* Update to performance data in chapter *Performance characteristics* Introduction of chapter *Interfering substances* Revision of chapter *Symbols* Inclusion of notified body number to CE-mark – conformity assessment procedure according to IVDR 2017/746

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.

NOTES

SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1. In addition the following symbols and signs are used:

Symbol	Explanation
TC	Test Cassette
BUF|EX	Extraction buffer
CONTROL|L	Control Low
CONTROL|H	Control High
RCC	RFID Chip Card
BCC	Barcode Card
	**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: **www.buhlmannlabs.ch/support/downloads/**

Parts of the kit are patent protected by EP2947459(B1); US10620216(B2); AU2015261919(B2); JP6467436(B2)

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