Quantum Blue®
fCAL high range

Quantitative
Lateral Flow Assay

For research use only.
Not for use in diagnostic procedures.

LF-CHR25-U 25 tests

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ENGLISH

INTENDED USE

The BÜHLMANN Quantum Blue® fCAL high range is an *in vitro* test for the quantitative determination of calprotectin in human stool specimens.

For research use only. Not intended for use in diagnostic procedures.

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 and the control line are measured quantitatively by the Quantum Blue® Reader.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Comments
Test Cassette	25 pieces	B-CAL-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-CHR-CONSET	Ready to use
RFID Chip Card	1 piece	B-CHR-RCC	**Red** plastic card

Table 1

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

STORAGE AND SHELF LIFE OF REAGENTS

All kit components are stable at 2-8 °C until the expiration date printed on the labels.

REAGENTS & MATERIAL SUPPLIED SUPPLEMENTARY

Fecal extraction devices

Fecal extraction devices described below are not delivered with the kit and either of them has to be ordered with the kit.

Extraction Devices	Quantity	Code
CALEX® Cap Device	50, 200 or 500 tubes filled with 5 mL extraction buffer / ready to use	B-CALEX-C50 B-CALEX-C200 B-CALEX-C500
BÜHLMANN Smart-Prep	50 tubes consisting of spatulas and base caps	B-CAL-RD
ScheBo® Quick-Prep™	50 tubes consisting of tube, cone & dosing tip. Filled with 1.3 mL extraction buffer / ready to use	B-CAL-SO50

Table 2

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.
- 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.
- Unused solution should be disposed of according to local State and Federal regulations.

Technical precautions

Kit components

- All reagents and test samples must be equilibrated to room temperature (18-28 °C) before starting the test.
- Components must not be used after the expiration date printed on the labels.
- Do not mix different lots of reagents.
- Test cassettes cannot be re-used.

Test procedure

- Read carefully the instructions prior to carrying out the test. Test 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.
- The Quantum Blue® Reader must be switched on and programmed for the Quantum Blue® fCAL high range test (CHR_0 or CHR_900) before starting the test (see Quantum Blue® Reader Manual).
- Use the red RFID chip card in order to change lot-specific test parameters.
- 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 device.
- If not used directly, diluted samples should be stored at 2-8 °C and should be used within twelve hours.
- With BÜHLMANN Smart Prep and ScheBo® Quick-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. The extracts can be stored directly in the CALEX® Cap Device. For re-use/ re-measurement of the extracts see step 2 under the chapter assay procedure.

SPECIMEN COLLECTION AND STORAGE

If the extraction devices are used, less than 1 g of native stool specimen is needed for the extraction procedure.

Stool specimens should be collected into plain tubes.

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

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.

Extract storage

Calprotectin in extracts obtained by the CALEX® Cap Device is stable at room temperature (18-28 °C) for 3 days, at 2-8 °C for 6 days and at -20 °C for 18 months.
Calprotectin in extracts obtained by manual weighing method, by BÜHLMANN Smart-Prep or by ScheBo® Quick-Prep™ is stable at 2-8 °C for 6 days or at -20 °C for 18 months.

ASSAY PROCEDURE

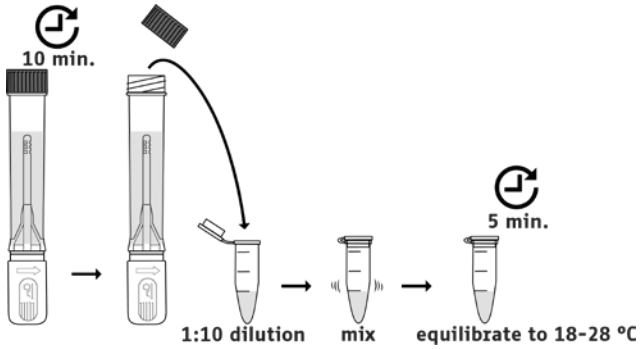
The assay procedure consists of three steps:

1. Extraction of stool samples

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

2. Sample processing

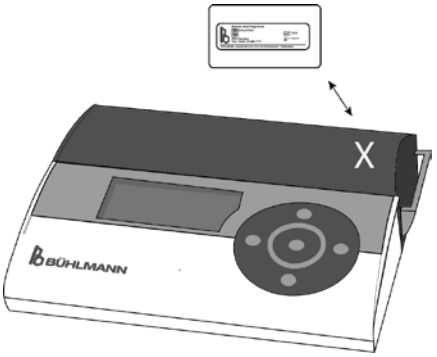
- Smart Prep or ScheBo® Quick Prep™: After extraction, let the stool extract settle for 10 minutes. Dilute the supernatant 1:100 with extraction buffer (e.g. 20 µL extract and 1980 µL extraction buffer) and mix well. Let the samples equilibrate for 5 minutes at 18-28 °C prior to proceeding to the next step (step. 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 and dilute the supernatant 1:10 with extraction buffer (e.g. 50 µL 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. 3).



3. Lateral flow assay procedure and readout

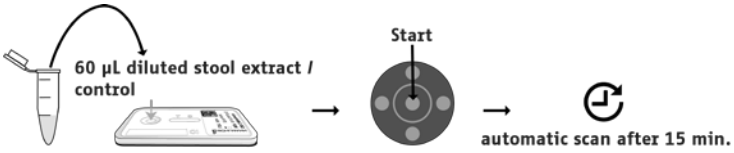
There are two alternative methods available on the Quantum Blue® Reader: <CHR_900> and <CHR_0>. Select one of these methods before starting the experiments.

Load the lot specific parameters from the red RFID chip card.



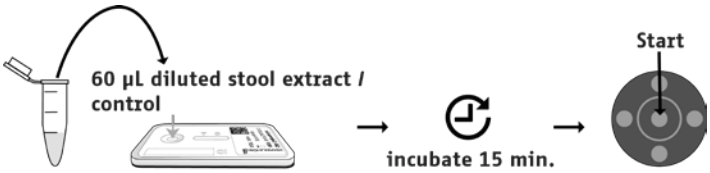
3.1 Method <CHR_900> with internal timer

- Load the test cassette onto the test cassette holder of the Quantum Blue® Reader.
- Add 60 µL of diluted stool extract onto the sample loading port of the test cassette.
- Close the cassette holder and start the measurement by pressing the start button.
- The scan starts automatically after 15 minutes (900 seconds).
- For low / high controls: Repeat step 3.1 using 60 µL of control instead of diluted stool extract.



3.2 Method <CHR_0> without internal timer

- Add 60 µL of diluted stool extract onto the sample loading port of the test cassette.
- Incubate for 15 minutes ± 1 minute (set a timer manually).
- Load the test cassette onto the test cassette holder of the Quantum Blue® Reader.
- Scan the cassette with the Quantum Blue® Reader by pressing the start button immediately.
- For low / high controls: Repeat step 3.2 using 60 µL of control instead of diluted stool extract.



Remark: Please refer to the Quantum Blue® Reader Manual to learn about the basic functions and how to initialize and operate the Quantum Blue® Reader, especially how to select test methods, and how to load lot specific parameters from the RFID chip card in order to get the samples measured.

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 and the timing ii) expiration dates of reagents and iii) storage and incubation conditions.
- Result of the self-test of the Quantum Blue® Reader performed at startup of the instrument 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 15 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 15 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 15 minutes of incubation time (figure 1C), the test result is invalid and the Quantum Blue® fCAL high range test has to be repeated using another test cassette.
- If neither the Control Line (C) nor the Test Line (T) are detectable after 15 minutes of incubation time (figure 1D), the test result is invalid and the Quantum Blue® fCAL high range test 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 15 minutes of incubation time, the test result is also invalid and the Quantum Blue® fCAL high range test has to be repeated using another test cassette.

STANDARDIZATION

- The Quantum Blue® fCAL high range is standardized against the BÜHLMANN fCAL® ELISA (order code: EK-CAL).
- The Quantum Blue® Reader uses a lot-specific standard curve to calculate the calprotectin concentration. This lot-specific standard curve is generated with the mean values (n ≥ 20 measurements each) from 13 calibration points obtained from different stool samples with known calprotectin concentrations. The test range is between 100 and 1800 µg/g.
- For quantitative measurements, unknown samples reading above 1800 µg/g can be further diluted 1:10 with extraction buffer and assayed again according to the procedure. The resulting dilution factor must be multiplied by the measured concentration to obtain the final results.
- For quantitative measurements, unknown samples reading below 100 µg/g can be re-tested in the Quantum Blue® fCAL (order codes: LF-CAL25).

LIMITATIONS

- Reagents delivered with the Quantum Blue® fCAL high range kit are intended for the determination of calprotectin levels in human stool samples only.

PERFORMANCE CHARACTERISTICS

Method comparison

Bias at 200 µg/g: -1.6 % (95 % confidence interval: -12.0 to 15.2)

The method comparison study was performed according to the CLSI guideline EP09-A3. 103 samples were measured according to the instruction for use with the Quantum Blue® fCAL high range test and with the BÜHLMANN fCAL® ELISA. Measurements were performed over three days using two Quantum Blue® fCAL high range test cassette lots. The correlation data is illustrated in figure 2.

Repeatability: 20.0-22.7 % CV

Within-laboratory precision: 22.4-28.1 % CV

Repeatability and within-laboratory precision were established according to CLSI guideline EP05-A3. Five extracted stool samples with calprotectin concentrations ranging between 180 and 1224 µg/g, were evaluated by one operator over 20 days, in two independent runs per day with two replicates per run. The test results are summarized in table 3.

Reproducibility: 19.2-28.9 % CV

Reproducibility was established according to CLSI guideline EP05-A3 by performing measurements on three different Quantum Blue® Reader instruments with three different test cassette lots. Five extracted stool samples with calprotectin concentrations ranging between 202 and 1328 µg/g, were tested over 5 days, in one run with five replicates per run. Each Quantum Blue® Reader instrument was operated by a different operator on three different sites. The results are summarized in table 4.

Limit of Blank (LoB): 60 µg/g calprotectin

The LoB was established according to the CLSI guideline EP17-A2 with four stool samples diluted to a target concentration below 10 µg/g calprotectin. The samples were measured over three days in five replicates each day to produce 60 blank values. The study was performed on two different test cassette lots. The LoB was evaluated by using non-parametric analysis.

Limit of Detection (LoD): 100 µg/g calprotectin

The LoD was established according to the CLSI guideline EP17-A2 with five samples with concentrations of 59, 70, 82, 117 and 148 µg/g calprotectin. The samples were measured over three days in five replicates each day to produce 75 values. The study was performed with two different test cassette lots. The LoD was calculated using parametric analysis.

Limit of Quantification (LoQ): 100 µg/g calprotectin

The LoQ was established according to the CLSI guideline EP17-A2 with six samples with reference concentrations of 59, 70, 82, 117, 148 and 166 µg/g calprotectin established with the BÜHLMANN fCAL® ELISA. The samples were measured over three days in five replicates each day to produce 90 values. The study was performed with two different test cassette lots. The relative total error was calculated using the RMS model from precision and bias estimates for each sample. The relative total error values were log transformed and plotted against the reference calprotectin concentration of the samples. The LoQ was defined as the intersection of the linear regression model obtained for the plot and the acceptance criterion of 30 % relative total error. The results are summarized in table 5.

Linear range: 67-2153 µg/g calprotectin

The linear range of the Quantum Blue® fCAL high range test was determined according to the CLSI guideline EP06-A. Two sample pools, low and high, were blended to obtain 17 concentration levels covering and exceeding the expected measuring range. The blends were assayed in 10 replicates on two test cassette lots. The linear range was defined as the interval of concentration levels in which coefficients of the second and third order fits were determined as not significant. Results for one test cassette lot are shown in figure 3.

Dilution linearity: 1:10-1:100

A sample with an estimated concentration of 18`000 µg/g calprotectin was diluted in extraction buffer to obtain target concentrations of 12`500, 10`000, 7500, 5000, 2500 and 1700 µg/g calprotectin. The samples were further diluted 1:10 to obtain calprotectin concentrations covering the measuring range of the assay. The dilutions were measured in five replicates in the Quantum Blue® fCAL high range test on two test cassette lots. The linearity of the obtained dilutions was assessed according to CLSI guideline EP6-A. Where coefficients of second or third order fits were determined to be significant, a maximum deviation from linearity of 20 % was allowed, with no deviation above 10 % observed. Results for one test cassette lot are shown in figure 4.

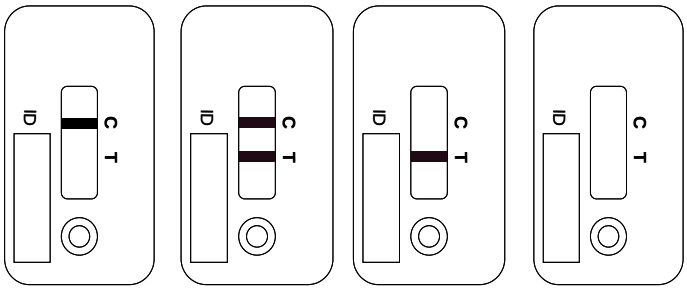
High Dose Hook Effect

A high dose hook effect was not observed for samples with calprotectin concentrations of up to 18`931 µg/g.

APPENDIX I

TABLES AND FIGURES

Test cassette results



A B C D

Figure 1

Method comparison

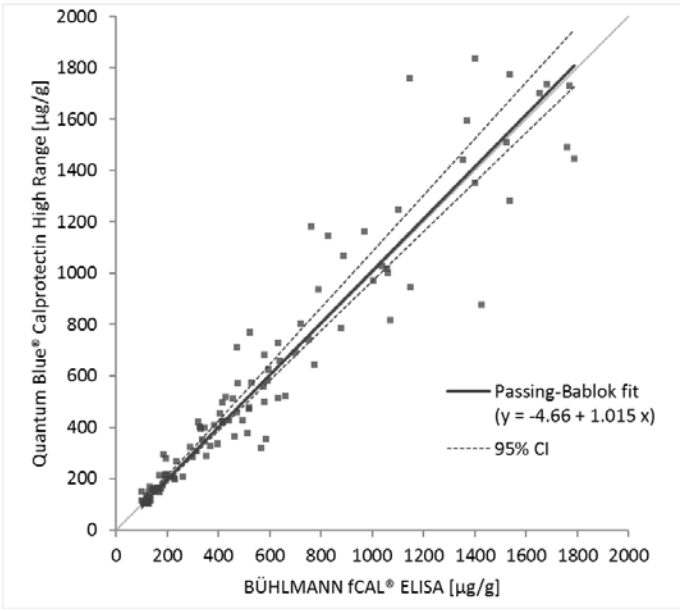


Figure 2

Within-laboratory precision

Mean Conc. [µg/g]	Repeatability CV [%]	Between -run Precision CV [%]	Between -day Precision CV [%]	Within-lab Precision CV [%]
180	21.0	0.0	12.6	24.5
219	21.5	5.1	17.5	28.1
330	20.6	15.5	0	25.8
582	22.7	6.4	13.1	27.0
1224	20.0	6.4	6.0	22.4

Table 3

Reproducibility

Mean Conc. [µg/g]	Repeatability CV [%]	Between -day Precision CV [%]	Between-Lot/Op/In[1] Precision CV [%]	Reproduc-ibility CV [%]
202	21.5	2.8	8.7	23.3
243	23.3	11.3	12.7	28.9
347	19.7	3.2	0	19.9
629	20.8	10.0	4.0	23.4
1328	16.9	9.0	0	19.2

Table 4

Limit of quantification

Sample	1	2	3	4	5	6
Reference value [µg/g]	99.72	132.75	148.49	165.47	82.65	80.45
Bias [µg/g] (Mean value obtained – reference value)	17.29	16.07	0.84	-0.08	12.50	21.26
Precision [% CV]	28	23	19	18	27	19
% Total Error	33	26	19	18	31	32
Intersection of linear regression with acceptance criterion of 30 % relative total error[2] [µg/g]						**97.2**

Table 5

Linearity

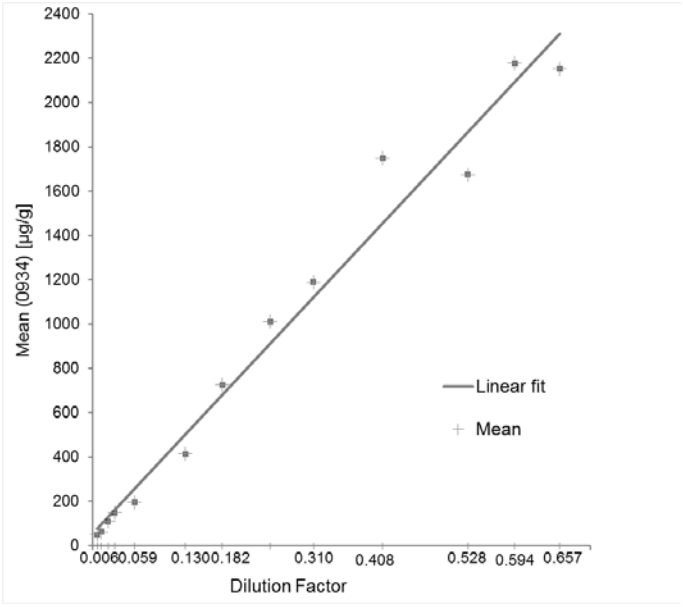


Figure 3

[1] Lot/Op/Inst. = Lot, Operator and Instrument
[2] The relative total error and the acceptance criterion were log transformed

APPENDIX I

TABLES AND FIGURES

Dilution linearity

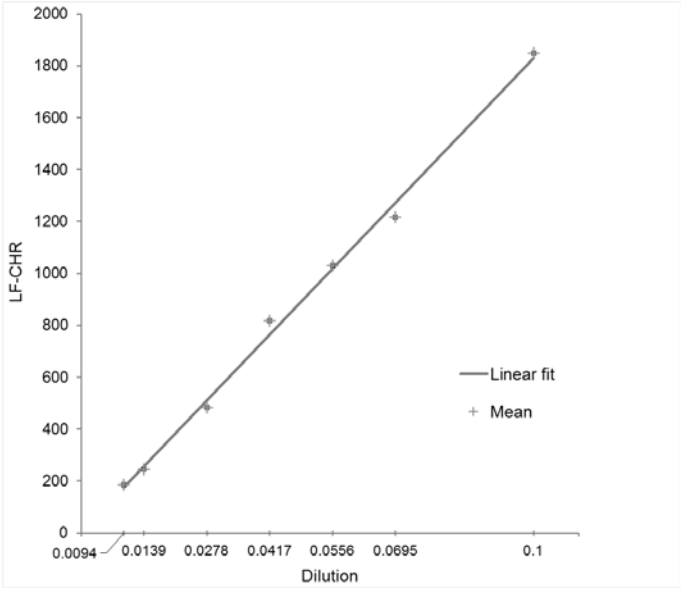


Figure 4

APPENDIX III

SYMBOLS

Symbol	Explanation
	Use By
REF	Catalogue number
LOT	Batch code
	Contains sufficient for <n> tests
	Consult Instructions for Use-

Symbol	Explanation
	Temperature limitation
TC	Test Cassette
BUF EX	Extraction Buffer
CONTROL L	Control Low
CONTROL H	Control High
RCC	RFID Chip Card