Quantum Blue®
fCAL extended

Quantitative
Lateral Flow Assay

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

LF-CALE25-U 25 tests

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ENGLISH

INTENDED USE

The BÜHLMANN Quantum Blue® fCAL extended is a 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 (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.

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 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
ScheBo® Quick-Prep™	50 tubes consisting of tube, cone & dosing tip, each 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.
- Reagents and chemicals have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

Technical precautions

Kit components

- All reagents and test samples must be equilibrated to room temperature (18-28 °C) before starting the assay.
- Mix well (vortex) the reagents before use.
- Components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.
- 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.
- 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.
- 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.

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 manual weighing method, by BÜHLMANN Smart-Prep or by ScheBo® Quick-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.

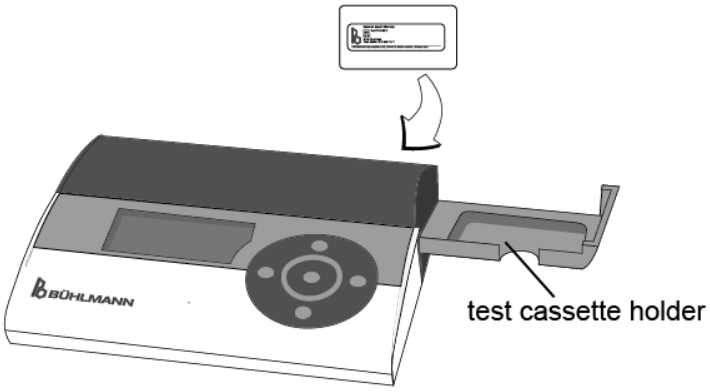
2. Sample processing

- Smart-Prep or ScheBo® Quick 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.

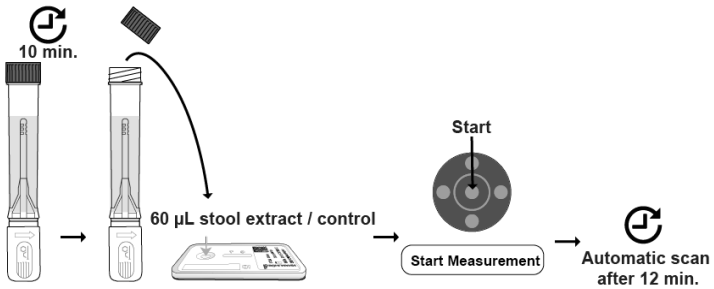
3.1. Method with internal timer

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. Add 60 µL of diluted 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.



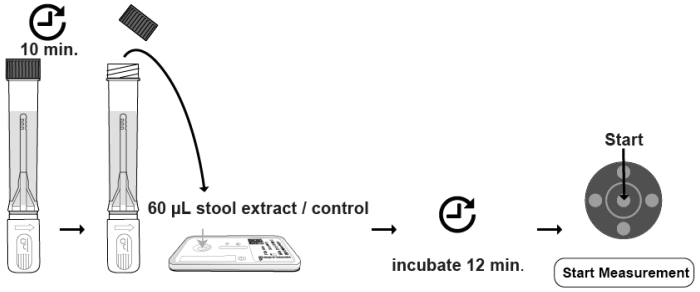
3.2. Method without internal timer

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. Add 60 µL of diluted 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

- The Quantum Blue® fCAL extended is standardized with the BÜHLMANN fCAL® ELISA (order code: EK-CAL).
- The Quantum Blue® Reader uses a lot-specific standard curve to calculate the calprotectin concentration. 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.
- In rare cases, when calprotectin levels are extremely high (above 4000 µg/g), the test system may be prone to a high dose hook effect, that can result in values below the expected 850 µg/g upper limit of the linear range.

PERFORMANCE CHARACTERISTICS

The following performance characteristics have been established with the Quantum Blue® Reader 2nd Generation and were verified on the Quantum Blue® Reader 3rd Generation.
Indicated performance characteristics apply for both Reader generations.

Method comparison

Bias at:

80 µg/g: 4.2% (95% CI: -2.0 - 19.3%)

100 µg/g: 2.1% (95% CI: -2.7-14%)

160 µg/g: -1.1% (95% CI: -4.1 - 6.6%)

300 µg/g: -3.6% (95% CI: -7.0 - 2.3%)

The method comparison study was performed according to the CLSI guideline EP09-A3. One hundred and eighty six (186) extracted stool samples were measured according to the instruction for use with the Quantum Blue® fCAL extended test and with the BÜHMANN fCAL® ELISA. Measurements were performed over three days using three Quantum Blue® fCAL extended test cassette lots (figure 2).

Recovery: 102%-121%

Six stool specimen extracts were spiked with 150 µg/g calprotectin in calibrator material of human serum origin. The baseline extract was spiked with the corresponding amount of extraction buffer. Baseline and spiked samples were measured in eight replicates. One test cassette lot was used. The results are summarized in table 3.

Repeatability: **15.3-19.1% CV**

Within-laboratory precision: 18.0-23.0% CV

Repeatability and within-laboratory precision were determined according to CLSI guideline EP05-A2. Four extracted stool samples were measured over ten days, in two independent runs each day, with two replicates per run. A single reagent lot was used (table 4).

Inter-lot precision: 16.5-20.6% CV

Inter-lot precision was determined according to CLSI guideline EP05-A2. Four extracted stool samples were measured using three different reagent lots. The measurements were performed over five days, in a single run each day, with two replicates per run (table 5).

Limit of Blank (LoB):

QB2: 6.7 µg/g calprotectin.

QB3G: 12.5 µg/g calprotectin.

The LoB was established according to CLSI guideline EP17-A using extraction buffer to obtain 60 blank values. The study was performed with two different test cassette lots.

Limit of Detection (LoD):

QB2: 18 µg/g calprotectin.

QB3G: 20.2 µg/g calprotectin.

The LoD was established according to CLSI guideline EP17-A. Two extracted stool samples with calprotectin concentrations of 58 and 62 µg/g were diluted in extraction buffer to obtain a total of six samples with concentrations ranging from 1 x LoB (6.3 µg/g) to 4 x LoB (25.1 µg/g). The samples were measured in ten replicates to obtain 60 values. The study was performed with two different test cassette lots.

Limit of Quantification (LoQ):

Lower LoQ: ≤30 (28.2) µg/g calprotectin.

Upper LoQ: ≥1000 (1002) µg/g calprotectin.

The LoQ was established according to CLSI guideline EP17-A. To determine the lower LoQ, four extracted stool samples with calprotectin concentrations ranging from 19.1

to 37.3 µg/g were measured in ten replicates to produce 40 values (table 6). To determine the upper LoQ, four extracted stool samples with calprotectin concentrations ranging from 628 to 1001.7 µg/g were measured in ten replicates to produce 40 values (table 7). The study was performed with two different test cassette lots. Reference calprotectin values of extracted stool samples were determined with the BÜHLMANN fCAL® ELISA. The relative total error was calculated using the RMS model from estimates of precision and bias to the reference values, for each sample. The LoQ was defined as the lowest and highest sample concentration, for the lower LoQ and upper LoQ, respectively, which met the acceptance criterion of 30% relative total error.

Linearity: 30-850 µg/g

The linear range of the Quantum Blue® fCAL extended assay was determined according to CLSI guideline EP06-A. Two extracted stool samples with low and high calprotectin concentrations were blended to obtain a total of 14 concentration levels covering and exceeding the expected measuring range. The blends were assayed in ten replicates on two test cassette lots. Mean calprotectin concentration values of each blend were plotted against the dilution factor used to obtain the blend. Linear as well as second and third order polynomial fitting was applied. Where coefficients of the second and third order polynomial fits were determined to be significant, the linear range was defined as the interval of calprotectin concentration in which deviation from the linear fit did not exceed 20% relative concentration value or 20 µg/g (figure 3).

High dose hook effect

No high dose hook effect was observed for calprotectin concentrations up to 1500 µg/g. Decrease in mean signal below the 850 µg/g upper linear range limit was estimated for calprotectin concentrations above 4000 µg/g. No value below 300 µg/g was observed for any of the single replicate results for all high samples tested. Seven to eight extracted stool samples with calprotectin concentrations ranging from 1361 µg/g to 13`817 µg/g were measured in five replicates on three different test cassette lots.

APPENDIX I

TABLES AND FIGURES

Test Results

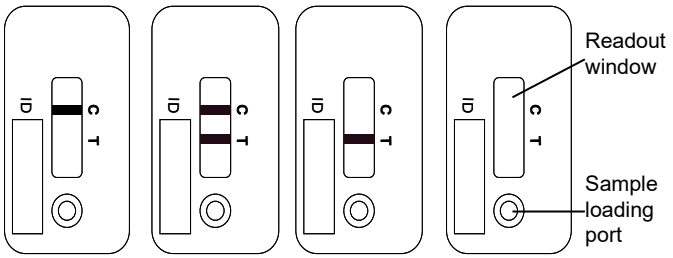


Figure 1A Figure 1B Figure 1C Figure 1D

Figure 1

Recovery

Sample	1	2	3	4	5	6
Base value [µg/g]	77	111	141	240	232	349
Spike value [µg/g]	150	150	150	150	150	150
Expected value (base + spike) [µg/g]	227	261	291	390	382	499
Observed value (base + spike) [µg/g]	260	267	345	409	441	605
% Recovery (observed/expected)	115	102	118	105	115	121

Table 3

Within-Laboratory Precision

Mean Conc. [µg/g]	Repeatability CV [%]	Between -run Precision CV [%]	Between -day Precision CV [%]	Within-lab Precision CV [%]
51.7	19.1	10.2	7.6	23.0
118.6	18.3	0.0	3.3	18.6
295.0	15.3	5.3	7.9	18.0
647.8	16.8	11.1	5.9	21.0

Table 4

Inter-Lot Precision

Mean Conc. [µg/g]	Inter-lot precision CV [%]
49.2	16.5
112.2	17.0
278.4	20.1
682.1	20.6

Table 5

Limit of Quantification – Lower LoQ

Lower LoQ	Reference value (R) [µg/g]	Mean value obtained (O) [µg/g]	Bias [µg/g] (R-O)	Precision [% CV]	Total Error [%]
Lot M0527	37.3	29.2	8.1	17.5	25.7
	28.2	21.3	6.9	16.7	27.8
	23.6	17.6	6.0	25.6	31.9
	19.1	13.6	5.5	20.6	32.2
Lot M2128	37.3	37.4	-0.2	24.1	24.2
	28.2	25.1	3.1	15.8	17.8
	23.6	21.0	2.6	29.1	28.2
	19.1	18.5	0.6	20.7	20.3

Table 6

Limit of Quantification – Upper LoQ

Upper LoQ	Reference value (R) [µg/g]	Mean value obtained (O) [µg/g]	Bias [µg/g] (R-O)	Precision [% CV]	Total Error [%]
Lot M0527	1001.7	752.6	249.1	18.4	28.4
	746.0	706.9	39.1	16.2	16.2
	678.6	704.2	-25.6	14.0	15.1
	628.0	668.4	-40.4	21.3	23.5
Lot M2128	1001.7	783.0	218.7	22.6	28.1
	746.0	670.4	75.6	13.9	16.1
	678.6	629.9	48.7	17.2	17.5
	628.0	636.8	-8.8	20.9	21.1

Table 7

APPENDIX I

TABLES AND FIGURES

Method Comparison

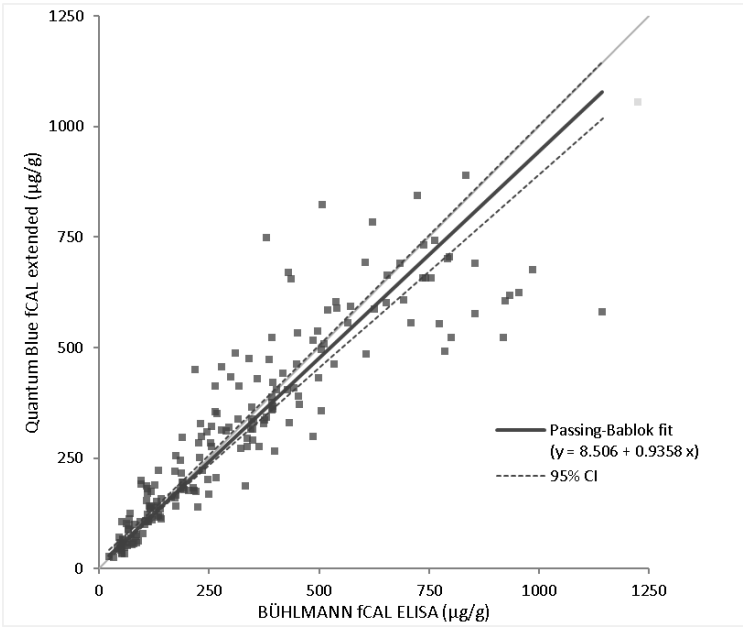


Figure 2

Linearity

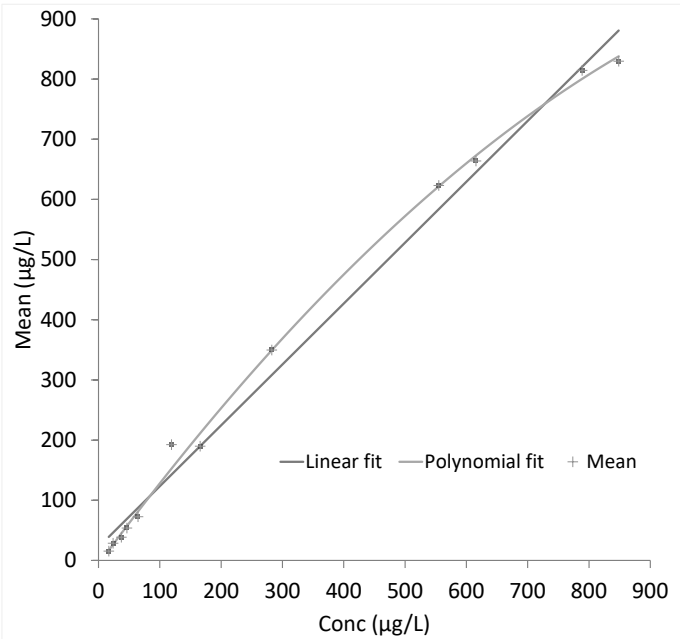


Figure 3

APPENDIX II

SYMBOLS

Symbol	Explanation
	Use by
REF	Catalogue number
LOT	Batch code
Σ	Content sufficient for <n> tests
	Consult instructions for use
	Temperature limitation

Symbol	Explanation
TC	Test cassette
BUF EX	Extraction buffer
CONTROL L	Control low
CONTROL H	Control high
RCC	RFID chip card
BCC	Barcode card