THE WAY TO TOTAL AUTOMATION OF CALPROTECTIN MEASUREMENT IN FAECES WITH BÜHLMANN fCAL® TURBO

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Background

Calprotectin, an important marker to detect and monitor inflammatory processes in the gastrointestinal tract, has been well established during the last few years. As a consequence the amount of Calprotectin determinations in the laboratory has increased rapidly. Calprotectin has to be extracted from faecal specimens. Due to the nature and inherent inhomogeneity of this specimen the work load in the laboratory increased over time and reduction of hands-on-time is needed by simplification and automation.

In our laboratory we evaluated the user-friendly CALEX® Cap Extraction device to speed up and streamline the cumbersome and labour-intensive extraction procedure. As a second step we established and validated the new turbidimetric test fCAL turbo on our Roche Cobas® c501 analyzer. The fCAL turbo allows random access sample handling with a time to result of 12 min and with a measuring range of 20 – 8'000 µg/g. The time saving on hands-on-time was 70% and the total turn-around-time decreased to 20 minutes. The combination of the CALEX® Cap extraction device together with the new turbidimetric assay fCAL turbo (PETIA; particle enhanced turbidimetric immuno assay) is a paradigm-shift to total automation of Calprotectin quantification in faeces. Moreover the CALEX® Cap, with its unique stability of 3 days at room temperature, would allow extraction by the patient at home increasing efficacy even more.

Conclusions

The preparation of stool specimens for Calprotectin determination with CALEX® and the analysis with the new fCAL turbo on the Cobas® c501 module (Roche Diagnostics) is practically a fully automated testing procedure. As the consequence this setup can be handled as a routine- and random-access procedure. The hands-on-time reduces to a few minutes only and the time to result is close to 12 minutes. Calprotectin shows with 3 days at room temperature a fair stability in the buffer solution of the extraction device CALEX®. Therefore, faeces collection and transfer into the CALEX® might be easily done at home.

Material & Methods

Stool collection was done on a stool specimen collector. A little portion was gently homogenized, portioned and directly transferred into a stool collection tube of a faecal sample preparation kit (Roche Diagnostics), containing no additives and into a CALEX® Cap extraction device (BÜHLMANN) respectively for comparison reasons.

Both extraction procedures underwent a measuring protocol with the fCAL ELISA assay (BÜHLMANN) on the Euroimmun Analyzer 1 instrument.

Preparation steps with the faecal sample preparation kit: After a short extraction procedure using one volume of faeces and 49 volumes of extraction buffer, the test allows for the selective measurement of Calprotectin-antigen by sandwich ELISA. A monoclonal capture antibody highly specific to the Calprotectin heterodimeric and polymeric complexes (4-5), respectively, is coated onto the microtiter plate. Calibrators, controls and patients extracts are incubated at room temperature for 30 minutes. After a washing step a detection antibody conjugated to horseradish peroxidase detects the Calprotectin molecules bound to the monoclonal antibody coated onto the plate. After incubation and a further washing step, tetramethylbenzidine is added, followed by a stopping reaction (changing from blue to yellow colour). The absorption is measured at 450 nm.

Preparation steps with the CALEX® Cap device: The sampling pin of the CALEX® Cap device is dipped into the stool specimen and a few times removed in order to fill the grooves. Then reintroduced into the buffer solution and vortexed vigorously. The supernatant can be used for the Calprotectin measurement on the described systems above (Fig 1).

In a second step, we compared the extractions performed with the CALEX® Cap device by measuring them either with the fCAL ELISA on the Euroimmun Analyzer 1 (Euroimmun) or with the fCAL turbo (PETIA), a ready-to-use-kit on the Roche Cobas® c501 module (Fig 2).

Eventually, we determined the stability of Calprotectin in the CALEX® extraction buffer solution at room temperature to prove the 3 days stability for a possible outsourcing of sample collection at the patients' home. For this purpose, we tested two concentration levels of 200 µg/g and 50 µg/g Calprotectin (Fig 3).

Results

Method Comparison Extraction – CALEX® Cap device

Measuring of the two extracted solutions with the Roche faecal sample preparation kit cup and CALEX® on the Euroimmun Analyzer 1 system. The correlation was y = 0,80 x + 0,00; r = 0,88396. However, the discrimination at the cut-off of 50 µg/g showed no difference between the two methods (Fig 1).

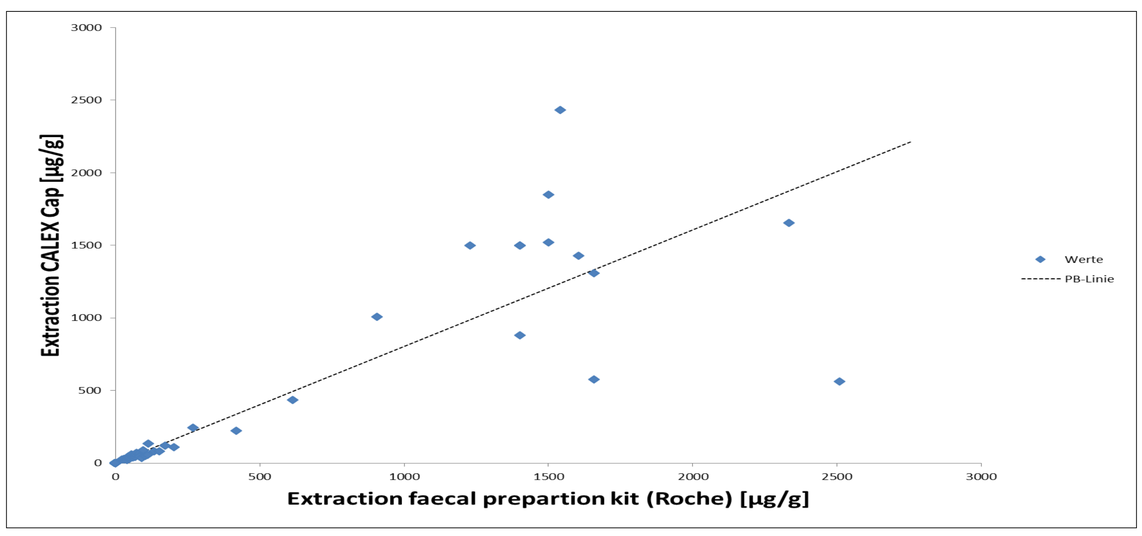

Fig. 1: Passing Bablok regression of the extraction procedures for Calprotectin either with faecal sample preparation kit and CALEX®, measured on the Euroimmun Analyzer 1 with the fCAL ELISA for Calprotectin.

Method Comparison fCAL ELISA vs. fCAL turbo

The faeces specimens got extracted with the CALEX® device. The Calprotectin in the buffer solution was measured on both systems Euroimmun Analyzer 1 with the fCAL ELISA reagent and on the Cobas® c501 module with the fCAL turbo (PETIA) reagent.
The correlation was y = x – 0,004; r = 0,9708. For the fCAL turbo reagent measured on the c501 we calculated for the 50 µg/g specimen a 1s of 2,78 µg/g and a coefficient of variation of 5,4% and for the 200 µg/g specimen a 1s of 14,03 µg/g and a coefficient of variation of 6,3% (Fig 2).

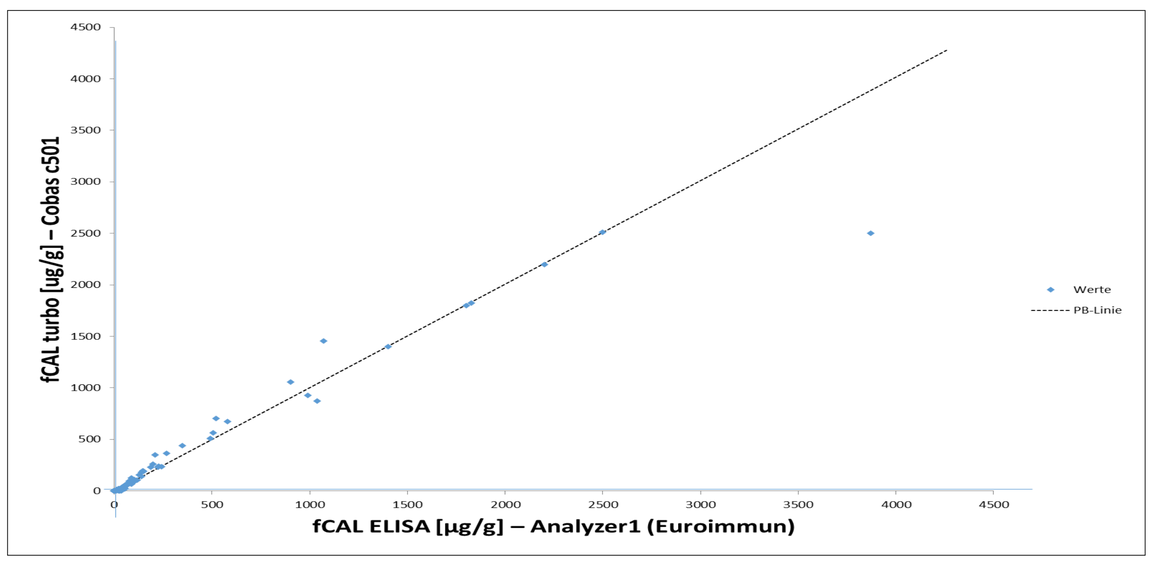

Fig. 2: Passing Bablok regression of the Calprotectin measurements with fCAL ELISA assay on Euroimmun Analyzer 1 and fCAL turbo on Cobas® c501.

Extract stability

Stored at room temperature, the CALEX® buffer solution shows over a period of 3 days a good stability for the 2 Calprotectin levels at 50 µg/g and 200 µg/g, respectively. The measurement was performed with fCAL turbo on the Cobas® c501 (Fig 3).

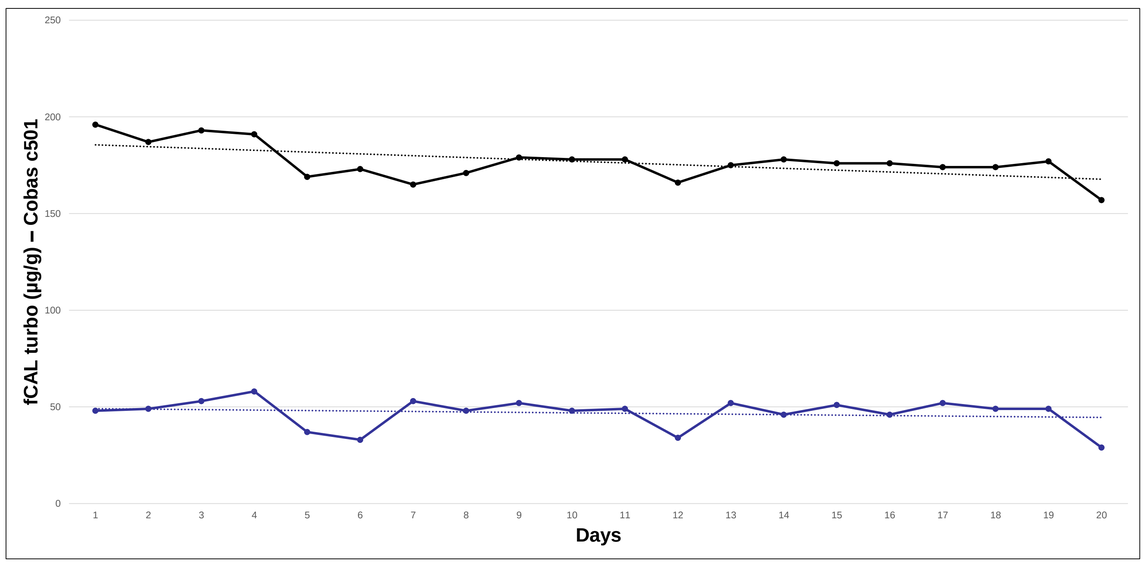

Fig. 3: Stability series at room temperature in CALEX® buffer solution at the level of 50 µg/g and 200 µg/g Calprotectin, measured with the fCAL turbo reagent on the Cobas® c501.

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