Analytical Performance of a Fecal CALPROTECIN (fCAL) PETIA Test

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BACKGROUND & OBJECTIVE

Calprotectin is a multifunctional protein that plays an important role in the diagnosis and follow-up of inflammatory bowel disease (IBD). High levels of calprotectin in stool samples are associated with inflammation of the intestinal tract. We evaluated the analytical performance of a new particle enhanced turbidimetric immunoassay (PETIA) on the clinical chemistry analyser BS-380 (MINDRAY) including linearity, security zone, precision and correlation to BÜHLMANN fCAL™ ELISA.

METHODS

The new latex based turbidimetric calprotectin assay (BÜHLMANN fCAL™ turbo) from BÜHLMANN Laboratories AG, Switzerland applies particles coated with anti-human calprotectin (MRP8/14) antibodies: the agglutination is proportional to the calprotectin concentration. Calprotectin levels are measured in extracts of human stool samples collected with the BÜHLMANN CALEX® Cap Device. Calprotectin levels are reported in µg/g stool sample considering a sample dilution of 1:500 in extraction buffer.
For linearity study serial dilutions were analysed and theoretical values were calculated from measured values of undiluted specimen. The intra-assay precision was performed with 5 different stool extracts containing different calprotectin concentrations in the range from 30 to 1300 µg/g. The inter-assay (total) precision was evaluated by measuring the same samples over a period of 20 days (2 runs per day in 2 replicates). Extracts of 60 fecal patient samples were analysed on the BS-380 and compared with the results generated with the BÜHLMANN fCAL™ ELISA.

RESULTS

Sensitivity estimation according CLSI EP17-A results in LoB of 2.5 µg/g, LoD of 6 µg/g and LoQ of 11 µg/g. **Linearity**: The assay has been tested to be linear in the range from 9 to 2059 µg/g calprotectin in stool. The obtained recovery values were between 99.7 and 112.5% (Fig.1) **Security zone**: Samples up to 10'000 µg/g results in concentrations above the upper assay limit of 2000 µg/g (Fig.2). **Precision:** The intra- and inter-assay precision (CV) were ≤ 4.5% (Fig.3). **Method comparison:** Passing - Bablok regression analysis revealed an intercept of -5.2 (-14 to 1.9) µg/g (95% CI), a slope of 1.04 (0.96 to 1.12) (95% CI), and a regression coefficient (r) of 0.95, suggesting that the new PETIA method showed a good correlation compared to matched ELISA assay. **Sample carry-over** assessed according CLSI EP10 was ≤ 0.5%. **Interference** With several drugs, vitamins, haemoglobin and lipid no interferences has been observed.

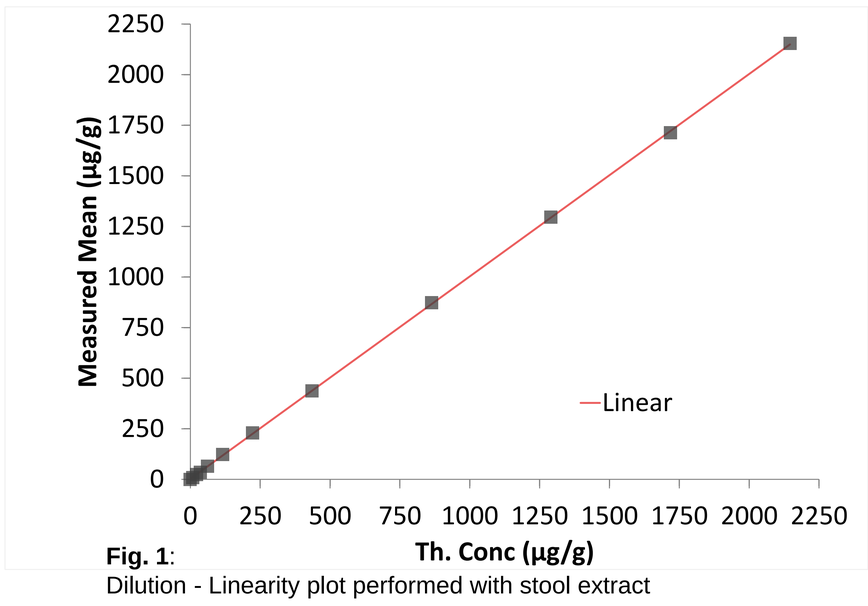

Fig. 1:
Dilution - Linearity plot performed with stool extract

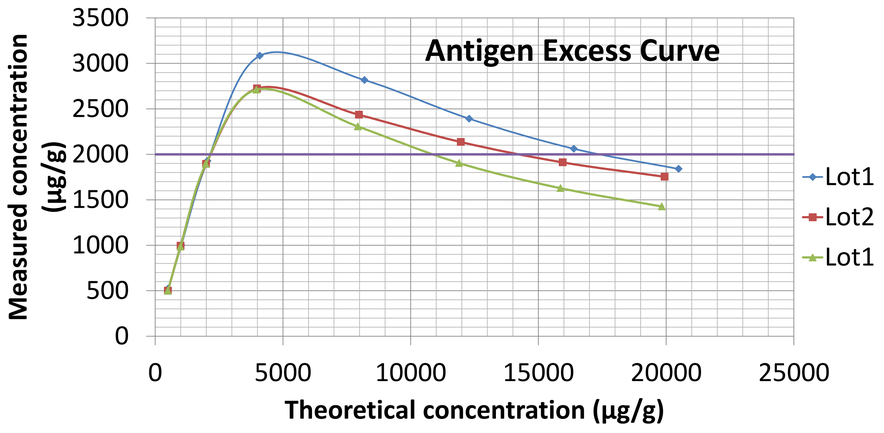

Fig. 2:
Antigen Excess curve of independent particle lots. Highest calibrator at 2000 µg/g.

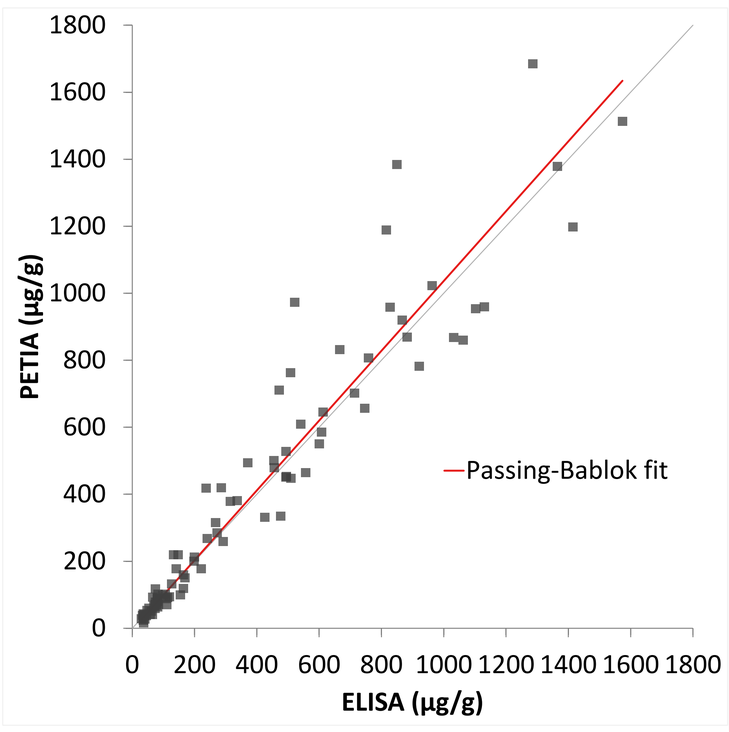

Fig. 4:
Passing-Bablok regression analysis of fCAL turbo assay to fCAL ELISA with 91 extracts. Intercept: -5.2, slope: 1.04, regression coefficient r: 0.954.

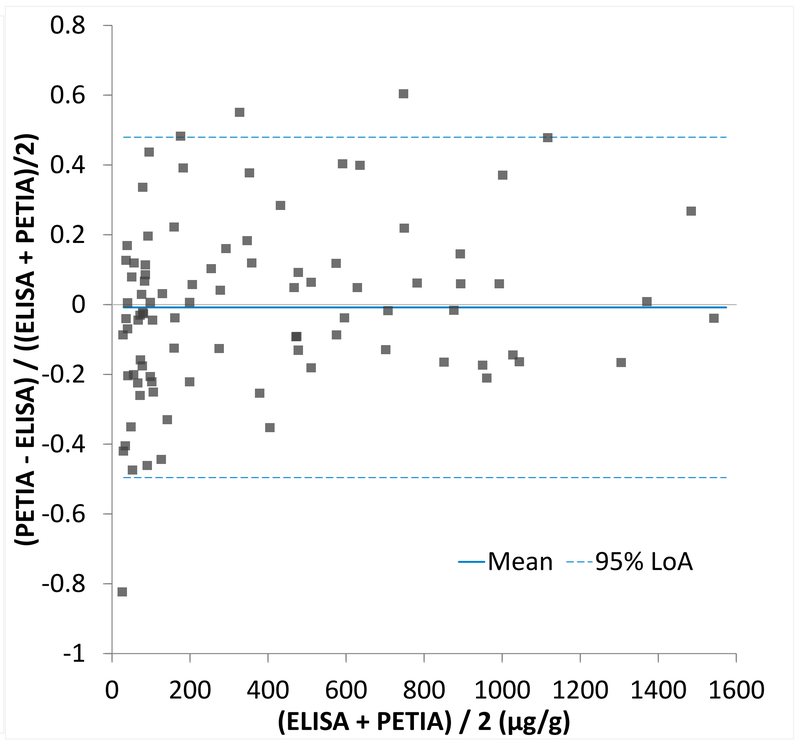

Fig. 5:
Altman Bland Difference Plot analysis of fCAL turbo assay to fCAL ELISA with 91 extracts. Bias: - 0.8%

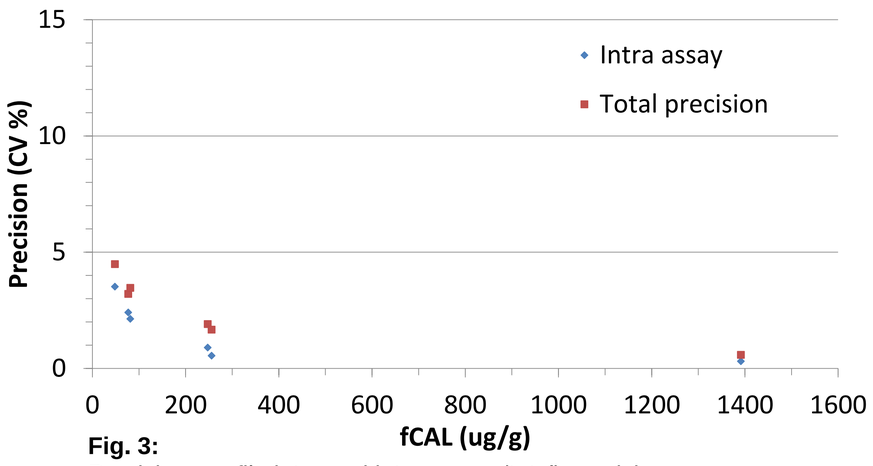

Fig. 3:
Precision profile intra and inter-assay (total) precision.

CONCLUSIONS

The new latex turbidimetric procedure for determining calprotectin is an attractive alternative to ELISA allowing random access and full automation of fecal calprotectin quantitation. Moreover, it represents an accurate and precise method to determine calprotectin levels in fecal extracts in a measuring range from 15 to 10'000 µg/g.

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