CALEX® CAP, A NOVEL STOOL COLLECTION AND EXTRACTION DEVICE: PERFORMANCE ANALYSIS AND STABILITY OF CALPROTECTIN IN STOOL EXTRACTS

J. Weber[1], M.-E. Ueberschlag[1], M. Prica[1], P. Spies[2], T. Jermann[1], C. Rothen[3], D. Gygax[2], J.-P. Rothen[3]

[1]BÜHLMANN Laboratories AG, Schönenbuch, Switzerland (correspondence: jw@buhlmannlabs.ch); [2]University of Applied Sciences, Muttenz, Switzerland; [3]ROTHEN Medizinische Laboratorien AG, Basel, Switzerland

University of Applied Sciences and Arts Northwestern Switzerland
School of Life Sciences

BACKGROUND & OBJECTIVES

Inflammatory Bowel Disease (IBD) is a chronic inflammation of the gut. IBD can be diagnosed and its disease course can be followed by biomarkers such as calprotectin which is measured in extracted stools. The first objective was to validate a new stool preparation tool, CALEX® Cap (**Fig. 1**), and to compare its performance for the extraction of calprotectin with "gold standard" weigh-in and manual extraction. The second objective was to determine the stability of calprotectin in the CALEX® Cap stool extracts and to compare them with extract stabilities in other commercial devices.

Fig. 1: CALEX® Cap device consisting of white sampling pin with grooves at its tip, transparent body with funnel (filled with extraction buffer to provide an extractive dilution of 1:500), and blue screw cap.

METHODS

All stools used for the different studies were anonymised leftover samples kindly provided by Labor ROTHEN (Basel, Switzerland). The reproducibility and recovery of i) stool sampling and ii) stool extraction using the CALEX® Cap device was determined. The calprotectin concentrations measured in 67 stool samples prepared by the CALEX® Cap device were compared with extracts of the same stools prepared by a manual weighing and extraction in a centrifuge tube by vigorous vortexing for 2 x 30 sec. 15 stool samples with calprotectin target values from 49 to 3147 µg/g were extracted with CALEX® Cap and three other commercial stool collection and extraction tools. The resulting extracts were stored for 0, 1, 2, 3, and 6 days at 23°C, then analyzed in the respective ELISA tests of the different manufacturers and compared to each other. All statistical analyses were carried out with Analyse-it for Microsoft Excel.

RESULTS

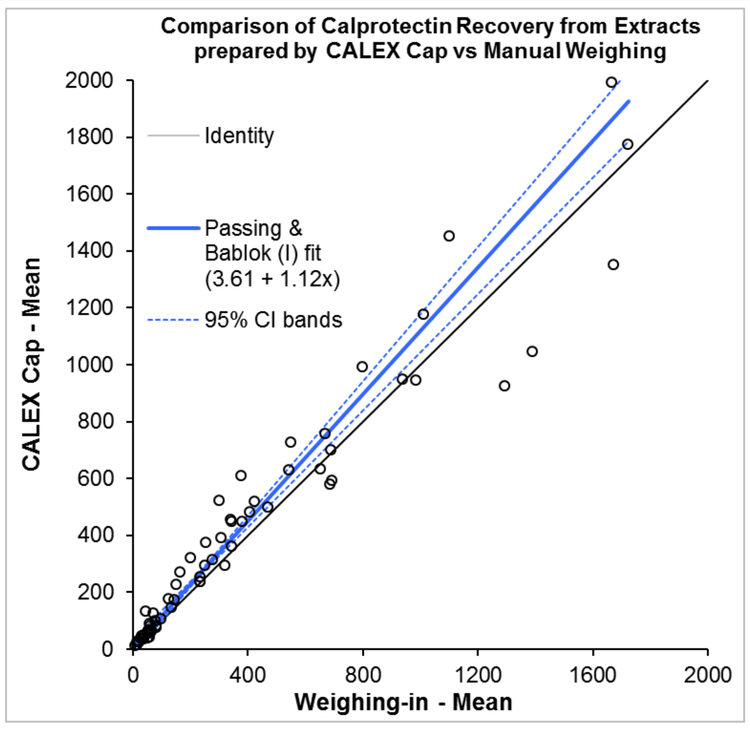

Fig. 2: Quantitative comparison of CALEX® Cap extracts to manually extracted stools measured in the BÜHLMANN fCAL® ELISA (n=67, mean of triplicates for both methods).

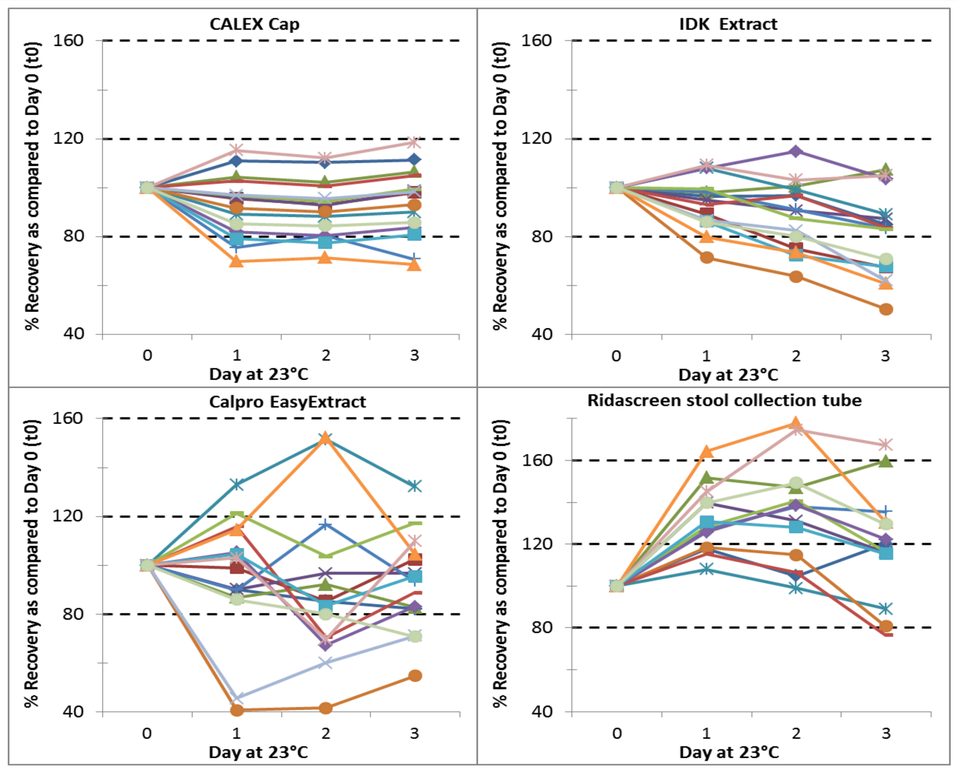

Fig. 3: Stability of calprotectin in 15 stool extracts prepared by four commercial devices and measured in the respective fCAL ELISAs of the different manufacturers.

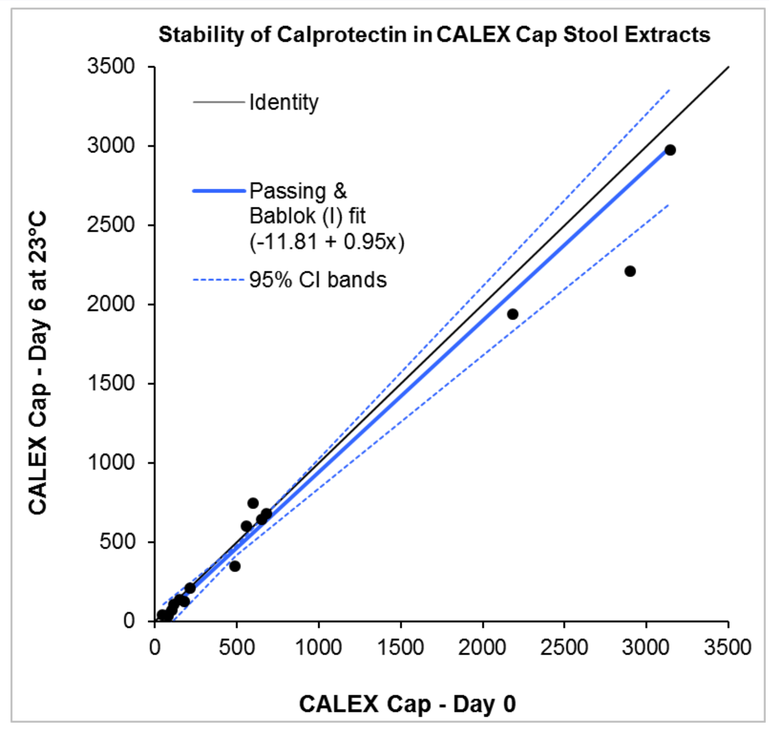

Fig. 4: Recovery of calprotectin from 15 stool extracts prepared by CALEX® Cap and stored for 6 days at 23°C as compared to fresh extracts (Day 0). Samples were measured in the BÜHLMANN ELISA.

Reproducibility of Stool Collection with CALEX® Sampling Pin and Stool Extraction with CALEX® Cap Device

50 stool samples of various consistency were collected in triplicates with the sampling pin and introduced in the CALEX® body through the funnel which stripped off excess amount of stool. The mean amount collected was 10.8±1.2 mg (CV, coefficient of variation, of 11.0% for singles, 8.1% for triplicates). Stool extraction of 14 samples (34 – 3250 µg/g as measured by the BÜHLMANN fCAL® ELISA) was repeated up to 20-times yielding a mean CV of 13.0% with CALEX® Cap, whereas manual extraction with weighing resulted in 13.4% CV.

Method Comparison of CALEX® Cap vs. Manual Weighing of Stools and Extraction

Passing and Bablok regression analysis revealed an intercept (bias) of 3.6 (-2.3 to 10.1) µg/g (95% CI, confidence interval), a slope (recovery) of 1.12 (1.02 to 1.18) (95% CI), and a regression coefficient (r) of 0.97 (**Fig. 2**).

Stability of Calprotectin in Stool Extracts prepared by and stored in four different Stool Collection and Extraction Devices

The stability of calprotectin (criterion: ≤ ±20% deviation for each single sample as compared to t_0; **Fig. 3**) for 3 days at 23°C was given for 87% of extracts stored in CALEX® Cap (BÜHLMANN, Switzerland), 60% in IDK Extract® (Immundiagnostik, Germany), 73% in EasyExtract™ (Calprolabs, Norway), and 33% in RIDASCREEN® stool collection tube (r-biopharm, Germany). The average calprotectin recovery (95% CI) after 6 days at 23°C determined by Passing and Bablok was 95% (84 to 108) for CALEX Cap® (**Fig. 4**), 86% (63 to 93) for IDK Extract®, 63% (56 to 81) for EasyExtract™, and 134% (104 to 172) for RIDASCREEN® stool collection tube, respectively.

CONCLUSIONS

- The stool sampling using CALEX® Cap is very reproducible, easier and more hygienic then manual weighing. The extraction of calprotectin out of stools is as reliable as with conventional methods. CALEX® Cap represents a fully quantitative "all-in-one" device.
- The stability of calprotectin in stool extracts is given for at least 3 days at ambient temperature when kept in the CALEX® Cap, and clearly more stable than in any other commercial device tested. Hence, CALEX® Cap devices containing sampled stool in a preserving buffer solution can be sent from the collection site (ie. patients' homes or GP's offices) to the testing lab via normal postal mail.

Euromedlab, June 21-25, 2015, Paris, France / Poster No. W115