A Commitment to Diagnostics

Calprotectin ELISA

Simplified and Efficient Stool Extraction using a *Faecal Sample Preparation Kit*

To optimize and simplify the stool extraction procedure the *Faecal Sample Preparation Kit* provided by Roche Diagnostics GmbH (Cat. No. 10745 804) was evaluated.

Correlation

49 faecal samples with low to high amount of Calprotectin were extracted using both methods: Exact weighing of the samples by a precision balance (see Assay Procedure) and extraction with the "Faecal Sample Preparation Kit". The Sample chamber carries about 85 mg stool sample. A standard volume of 4 ml Extraction Buffer (B-CAL-EX) was added to the tube and homogenized on a vortex mixer for multiple test tubes. The extracts were tested in the Calprotectin ELISA (EK-CAL) according to the assay procedure.

The correlation of the two different extraction procedures was excellent: Coefficient of correlation: R^2 = 0.96 with a slope of 1.05 and a intercept of 0.62 µg/g (Figure 1).

Reproducibility of Net Weight

10 aliquots from 3 different faecal samples each (n=30), were collected with the *Faecal Sample Preparation Kit*. Net weights of the aliquots were estimated by a precision balance. An average of 86.9 mg (95% CI: 83.9-89.8; SD 7.9) stool sample was drawn with the device corresponding to a coefficient of variation (CV) of 9%.

Reproducibility of the Extraction

Seven stool samples with different concentrations of calprotectin were extracted and measured 10 times. The mean coefficient of variation is 18.7%.

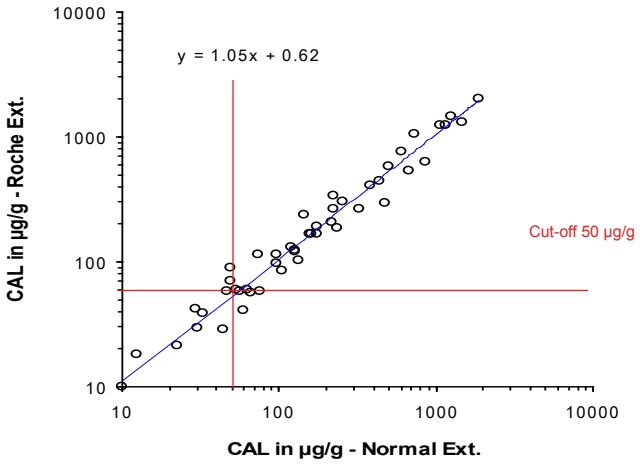


Figure 1

Conclusion

The reproducibility results on net weight and the extraction itself clearly indicate that, due to the consistency, homogenicity, content of plant fibres of the stool etc., the extraction of the sample has a higher impact on the outcome than the extraction methods described above.

Thus the Roche Faecal Sample Preparation Kit is recommended in order to simplify the extraction procedure.

Faecal Sample Preparation Kit – Summary extractions steps

Draw stool sample

Add 4ml Extraction Buffer

Vortex for 1 minute

Centrifuge for 5 min at 3000 x g

Transfer and dilute supernatant

BÜHLMANN

Faecal Sample Preparation Kit

Roche Diagnostics GmbH (Cat. No. 10745 804)

The Faecal Sample Preparation Kit from Roche Diagnostics is designed for the fast and reproducible preparation of stool extracts e.g. for the Calprotectin determination using Calprotectin ELISA (EK-CAL) from Bühlmann Laboratories AG.

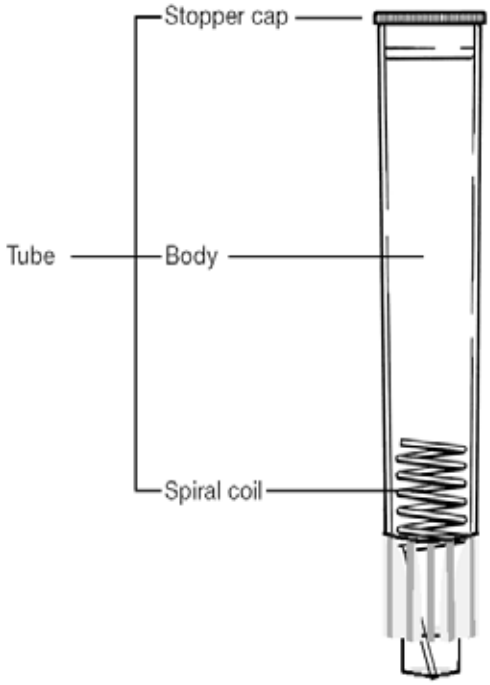
The Extraction Buffer (B-CAL-EX) provided with the kit must be used.

The usage of the Faecal Sample Preparation Kit guaranties a high consistency in the net weight of the samples so that a **standardized amount of 4 ml Extraction Buffer** can be added to the tube.

Bühlmann recommends vortexing each single sample for **for 1 minute on a (Multitube) vortex mixer** to guarantee a complete extraction of samples.

After the homogenization, ~1 ml of the extract is transferred into a fresh, labeled Eppendorf tube and **centrifuged for 5 minutes** at 3'000 x g in a microcentrifuge.

The supernatant is to be decanted into a fresh labeled tube and measured in the ELISA directly or stored at -20°C. The frozen extracts are stable for at least 4 months.



Preparation of sample

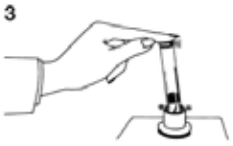
1

Carefully press the sample into the hollow cavity in the base cap and level off the surface

2

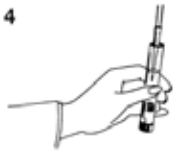
Firmly press the tube onto the base cap, detach the tag, and add 4ml of Extraction Buffer (B-CAL-EX)

3



Carefully re-cap the tube and homogenize the sample for about 1 minute using a vibration mixer (e.g. Vortex mixer). Centrifuge for 5 minutes at 3'000 x g (for centrifugation transfer into an Eppendorf tube)

4



Collect the supernatant and use an appropriate dilution for the quantification of Calprotectin.

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Ordering code Calprotectin:

EK-CAL	96 wells
LF-CAL20-RD	20 cassettes