Technical Bulletin

Verification of Calprotectin ELISA Data

Transferring from Quantum Blue to the higher throughput Calprotectin ELISA (EK-CAL) is the natural step when demands for this innovative assay increase.
The ELISA is easy to use and gives you trusted results with excellent precision and correlation.

Calprotectin ELISA Correlation with Clinical Data

Data from the 2012 Manz paper[1] confirms the correlation of patient calprotectin ELISA test results with their clinical assessments.
575 consecutive patients with abdominal discomfort referred for endoscopy to the Department of Gastroenterology & Hepatology at the University Hospital Basel, Switzerland, were enrolled in the study.
Calprotectin was measured in stool samples, collected within 24 hours before their investigations, using the enzyme-linked immunosorbent assay. The presence of a clinically significant finding in the gastrointestinal tract was the primary endpoint of the study.
Final diagnosis was determined by two different gastroenterologists, who had no knowledge of the patients' calprotectin results.

Clinically significant finding	AUC (95%CI)	Cut-off (µg/g)	Sens (%)	Spec (%)	LR+	LR-	NPV (%)	PPV (%)	Accuracy (%)
Overall	0.877 (0.85 - 0.90)	50	73	93	10.8	0.29	88	84	85
Upper gastrointestinal tract	0.730 (0.66 - 0.79)	48	59	82	3.2	0.50	75	68	71
Lower gastrointestinal tract	0.912 (0.88 - 0.94)	50	84	92	10.6	0.17	82	93	89

Table 1: *Area under the receiver operating characteristics curve (AUC) with corresponding sensitivity (Sens), specificity (Spec), positive and negative likelihood ratio (LR+, LR-), and negative and positive predictive values (NPV, PPV) for faecal calprotectin to identify a clinically significant finding in the gastrointestinal tract.*
Overall accuracy was calculated using the following formula: (true positive test results + true negative test results)/total population.

Another study by Lehmann et al., at the University Hospital Basel, Switzerland, proves calprotectin concentrations measured with the BÜHLMANN Calprotectin ELISA correlate highly with clinical results and the current gold standard of colonoscopy.
405 symptomatic patients were included.
With a sensitivity of 84% and a specificity of 95% the BUHLMANN calprotectin ELISA confirmed the nature of the pathology behind the symptoms, and showed an excellent negative predictive value of 93%. In this study, the performance of the BUHLMANN monoclonal ELISA was superior to polyclonal calprotectin determinations and to lactoferrin measurements (not shown).
The cut off used was 50 µg/g calprotectin in faeces.

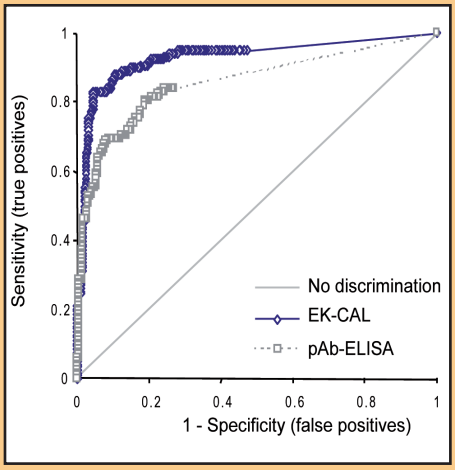
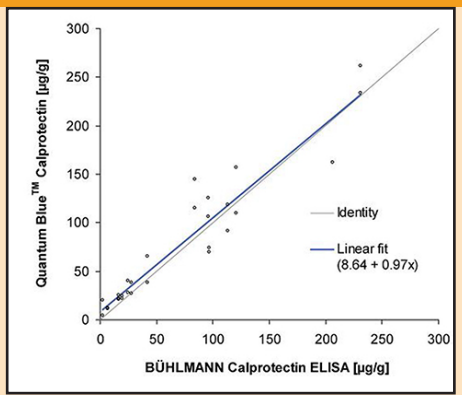


Figure 1:
ROC analysis of the ability of BÜHLMANN Calprotectin ELISA and of a polyclonal ELISA to discriminate between patients with CD and IBS. From Lehmann et al. (in prep).

Calprotectin ELISA Correlation with the Quantum Blue Test

Patients who have already had a calprotectin result obtained using the Quantum Blue test can be followed up using the Calprotectin ELISA EK-CAL kit with complete confidence.

Figure 2:
There is no discrepancy between results obtained with the two kits.



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The 50µg/g cut-off is common to all the Buhlmann Calprotectin tests

This is confirmed by an observational study of patients visiting a Gastroenterology clinic[1].

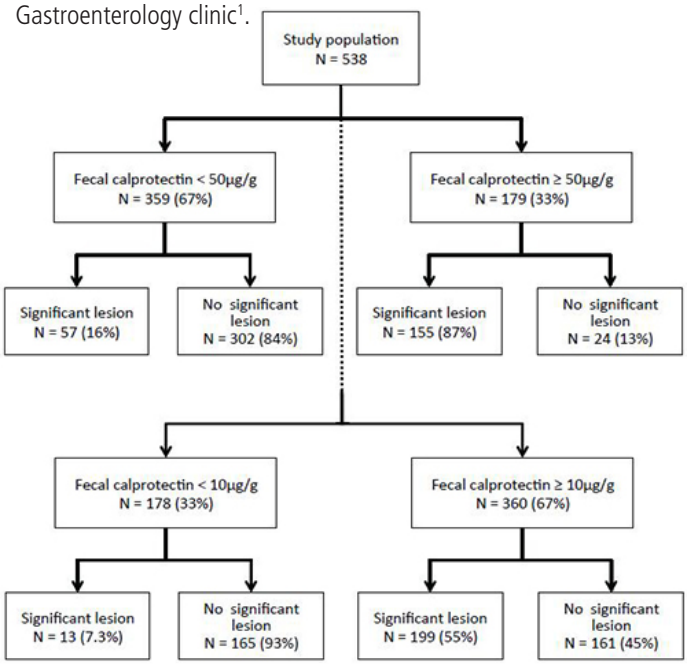
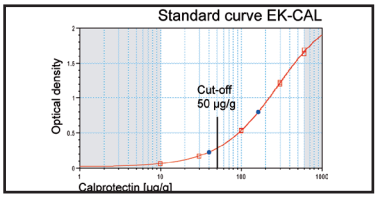
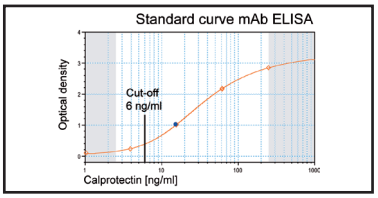


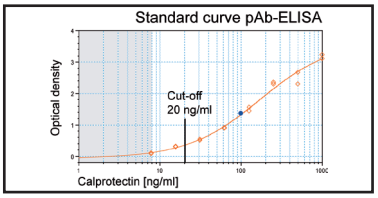
Figure 3 *Diagnostic accuracy of faecal calprotectin.*
The overall test accuracy of faecal calprotectin was 85% when 50 µg/g was used as cut off value (upper panel) and was 68% when 10 µg/g was used (lower panel).

Standard Curves and cut-offs obtained with EK-CAL (mAb ELISA), pAb ELISA and another mAb ELISA

The standard curves below show the cut-off ranges for the EK-CAL ELISA, a Calprotectin Polyclonal ELISA and another Monoclonal ELISA. The curves show that only EK-CAL is using the appropriate cut-off for the kit.



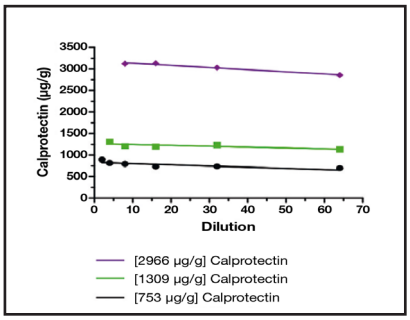




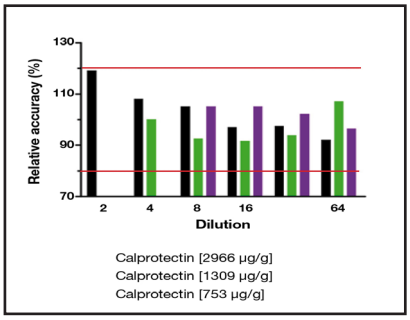
EK-CAL shows excellent linearity up to 4000µg/g [2]

This is important when studying mucosal healing or looking to predict potential flare-up of IBD symptoms.
In general, Calprotectin rises 3 months prior to a flare up.
Accurate results in the high range are essential for reliable patient monitoring.

Figure 4 *Assessment of assay parallelism for Calprotectin*



(A) Stool extracts with Calprotectin concentrations above the upper limit of quantification (ULOQ) were diluted into the working range of the assay.



(B) Parallelism was demonstrated by acceptable (relative) accuracy of tested stool extracts. Red lines indicate 20% upper and lower limits.

Use of EK-CAL is well established and supported

- EK-CAL is routinely used by Dr Tibble's group (Royal Sussex County Hospital, Brighton) and the Calprotectin UK reference centre (Kings College Hospital, London).
- CE marked protocols for EK-CAL on both the Dynex DS2 and DSx have been developed by Buhlmann.

Protocols for other ELISA processors are also available.

- The UK NEQAS scheme's preferred units for calprotectin measurement are µg/g. The National Laboratory Medicines Catalogue [NLMC] and the Pathology Harmony Initiative also recommended calprotectin measurement in µg/g.

EK-CAL is the only kit available that reports directly in µg/g, without requiring a conversion factor.

References

Cut-off/ Comparison with clinical findings

1. Manz M. et al. Value of faecal calprotectin in the evaluation of patients with abdominal discomfort: an observational study. BMC Gastroenterology 2012, 12:5

Dilution Linearity of EK-CAL

2. Vermunt R. et al. Calprotectin in biomarker-mediated drug development: Method validation for the quantification of faecal Calprotectin. Eurofins Poster

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