Fecal Calprotectin Quantitation Demands for Enhanced Automation

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BACKGROUND

Fecal calprotectin (fCAL) is well recognized as an important screening marker in the diagnosis of inflammatory bowel diseases (IBD) as well as in the monitoring of IBD patients suffering from this lifelong chronic disease. The increasing amount of fecal calprotectin analysis in the specialized immunological or microbiological laboratories increased the demands for simplifying the pre-analytical work for fecal extraction.

The manual weighing method with a minimal amount of 50-100 µg fecal sample extracted in 50 volumes of extraction buffer was established in the early 2000 and guarantees the best possible quantification of fCAL. The method is regarded as the reference method for fCAL quantification. New devices simplifying the time consuming reference method have been introduced by many different immunoassay manufacturers.

The new CALEX® Cap extraction device from BÜHLMANN was validated in this study.

CALEX® Cap

METHODS

Leftover samples were used for the validation of the CALEX® Cap extraction device. Method comparison between reference method and extraction device, reproducibility and extract stability studies were done applying the BÜHLMANN fCAL® turbo assay.

Repeatability of extraction: 6 stool samples with defined fCAL levels were repeatedly extracted with the CALEX® extraction device over a period of 3 days by two operators. A total of 12 extractions were performed for each sample. The extracts were stored at -20°C and measured within one single run of fCAL turbo.

Extract stability: 31 left-over stool specimens within the BÜHLMANN fCAL® ELISA measuring range (10–1800 µg/g), extracted in a 1:500 dilution (CALEX® extraction), and aliquoted. The aliquots were stored at 28°C, and sampled for calprotectin level determination at the time points day 0 to day 6. Calprotectin value determination was performed the next day after stool specimen extraction, denoting t = 0.

CALEX® Cap extraction, designed for the application on Total Laboratory Automation (TLA) solutions, was tested for usability in a fully automated routine laboratory.

RESULTS

Method Comparisons

The redesigned CALEX® Cap tube shows highest comparability (r^2=0.96) to the existing, and well established extraction device (see figure 2). The CALEX® extraction device is directly comparable to the manual weighing, reference method (y=1.10x-7.9; r^2=0.89) with a minimal bias of <5%. The higher extraction ratio (extraction dilution 1:500) results in a more efficient extraction in samples with elevated levels, >500 µg/g (figure 1).

Extraction Reproducibility

The extraction reproducibility observed with the CALEX® Cap (9.8-19.1%CV, see table 1) is highly comparable to the reference method shown in former studies (7.9-16,9%CV, data not shown). Reproducibility depends strongly on sample consistency and homogeneity of the stool sample.

Method Comparison: Manual weighing vs. CALEX®

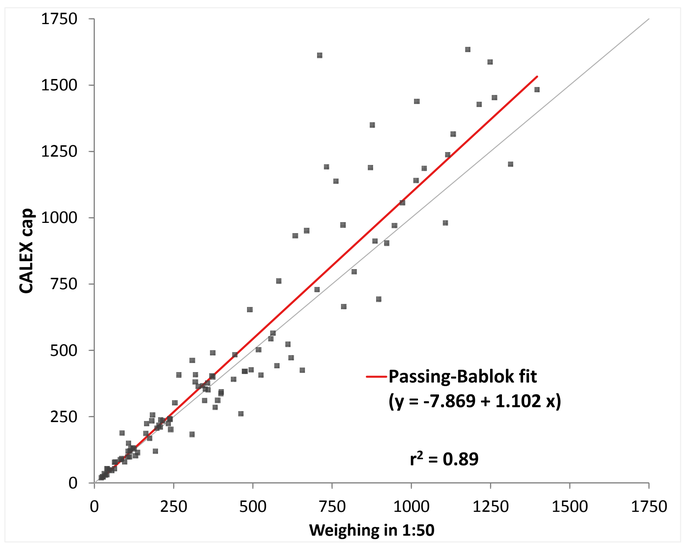

Figure 1: Method Comparison Reference (manual; 1:50) vs. CALEX® Cap (1:500) extraction. Passing-Bablok fit.

Method Comparison: CALEX® existing vs. new version

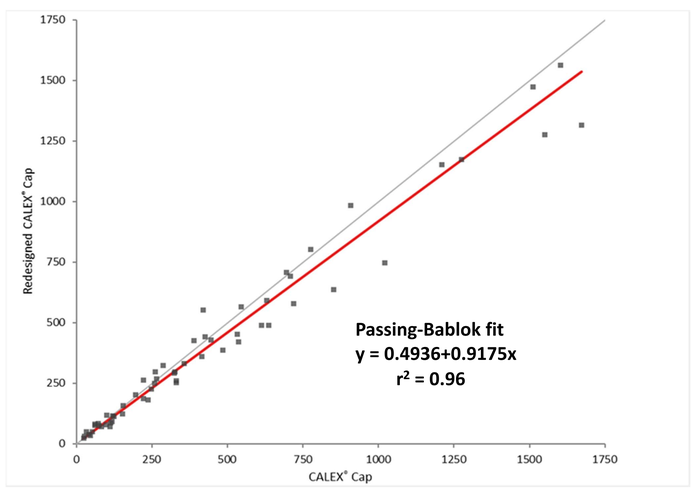

Figure 2: Method comparison of existing CALEX® Cap vs. redesigned CALEX® Cap version. Passing Bablok fit, n=59 ranging from 25 to 1673 µg/g. Altman bland mean difference -6.17% (95% CI -10.46-1.88).

Extract Stability

The results of the extract stability study are presented in table 2. The amount of samples with stable calprotectin levels was 90% after 3-days storage at elevated temperature. Three samples out of 31 samples show a confirmed decrease of measured levels. One sample (#27) shows a decrease from 60 µg/g to 30 µg/g after two days and two samples (#3,4) a decrease after 3 days (362 and 167 µg/g). Four samples on day six showed calprotectin values exceeding 120%. Analyte stability at elevated room temperature (28°C) within the BÜHLMANN extraction buffer indicated a sample dependency with unknown reason. High extract stability supports simplification of fecal calprotectin analysis.

Extraction Reproducibility

Sample Nr	fCAL® turbo	
	Mean calprotectin concentration [µg/g]	CV [%]
1	53	16.2
2	124	19.1
3*	138	[32.4]
4	404	11.2
5	1367	9.8
6	639	16.8
Mean Total		**14.6**

Table 1: Summary of extraction reproducibility study results. Samples were extracted independently twelve times. Extracts were measured with BÜHLMANN fCAL® turbo assay. Sample no. 3 was excluded from data evaluation due to liquid/mucous consistency .

Extract Stability

Samples	Calprotectin conc in [µg/g] - Extracts 1:500				
	Stored at 28°C				
	Day0	Day1	Day2	Day3	Day6
1	59	85.7	84.4	87	82
2	63.8	87	92	92	94
3	362.6	498	499	257	273
4	167.2	215	233	115	119
5	230.3	258	246	253	268
6	38	50.2	52	48	48
7	109	125	120	119	119
8	78	124.9	106	103	103
9	25.4	23.4	22	24	20
10	237	276	289	285	313
11	196	241	220	242	269
12	47	56	52	54	52
13	36	45	44	43	41
14	70.4	96	93	94	91
15	38.1	46	43	28	38
16	29.6	33	31	29	27
17	853	972	912	1016	1007
18	457	483	395	421	416
20	351	483	356	380	322
21	69	81	72	74	75.5
22	249	320	295	298	330
23	790	983	978	999	910
24	123	146	159	153	181
25	78	98	94	98.5	95
26	781	951	1019	1127	1206
27	60	58	30	30	25
28	411	434	516	521	563
30	623	763	817	828	792
31	77	86	95.3	99	99
32	1125	1274	1404	1441	>1800
39	756	962	1588	1475	1643

Table 2: Analyte stability in BÜHLMANN extraction buffer (1:500). N=31.

Total Laboratory Automation Solution

The new design of the CALEX® Cap extraction device allows the direct application on TLA solutions including integrated centrifugation. A first successful installation has been realized in a Swiss private lab on an Inpeco based system (figure 3 A-D). The tubes were successfully tested on all the different modules of a TLA from Rack/Bulk Input module to Sealer and Rack Output module.

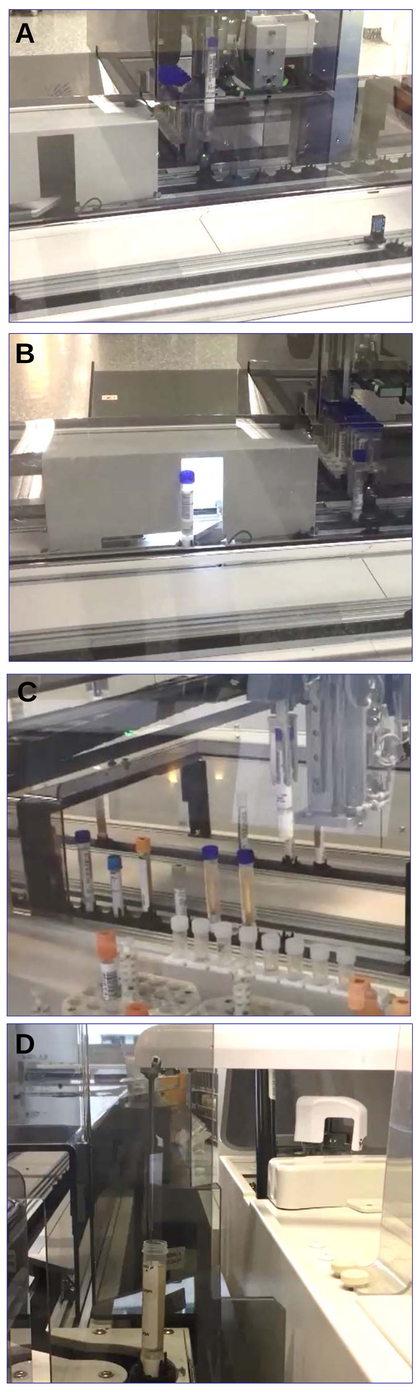

Figure 3: CALEX® Cap on Total Laboratory Automation solution (Inpeco); A: Rack Input Module (RIM); B: Tube Identification Module (TIM); C: Centrifuge Module (CM); and D: Sampling Connection to Clinical Chemistry Analyzer (Advia; Siemens)

CONCLUSIONS

The redesigned CALEX® Cap extraction device is suitable for automated quantitation of fCAL. The high quality of the device guarantees highly comparable and reproducible results as compared to the laborious manual weighing reference method. The analyte stability in the extraction buffer and design of the tube would even allow sample collection at the patients' home. Last but not least the properties and qualities of the CALEX® Cap allow the fully automated and simplified analysis of fCAL in high-throughput laboratories. The combination of CALEX® Cap with the automated, turbidimetric BÜHLMANN fCAL® turbo assay simplifies the laborious and cumbersome fecal calprotectin quantitation from sample collection to result reporting.

XXXVI Nordic Congress in Clinical Chemistry, 12-15 June, 2018, Helsinki