Evaluation of BÜHLMANN CALEX® Cap Stool Extraction Devices for the extraction of faecal calprotectin

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Background

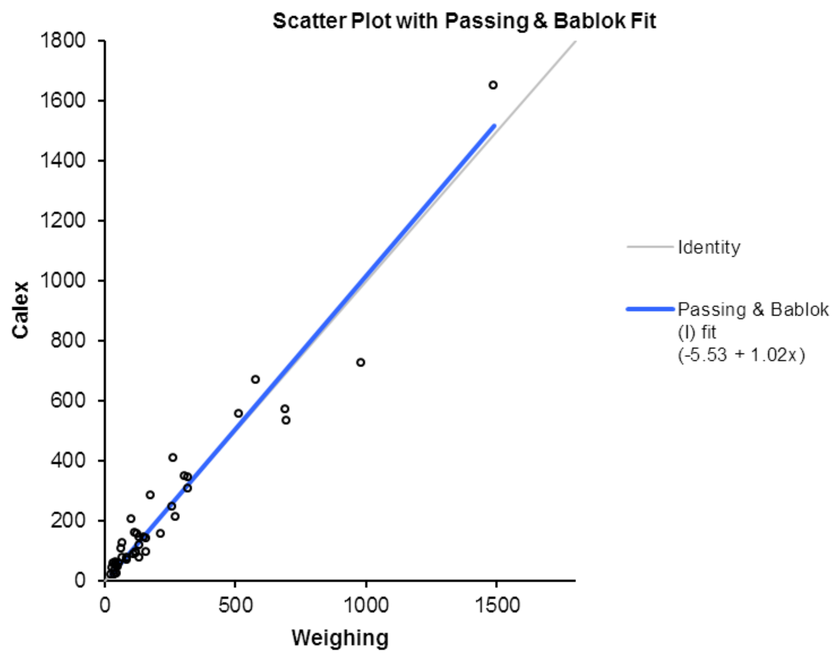
The gold standard method for faecal calprotectin extraction is the manual weighing method. This method however is time consuming and prone to human error. To improve the extraction phase and throughput of the faecal calprotectin assay, Royal Wolverhampton NHS Trust evaluated a commercial alternative to the manual weighing method known as BÜHLMANN Calex® Cap Stool Extraction Devices. The performance of the Calex® devices was assessed and compared to the gold standard manual weighing method.

Methods

1. A comparison was performed by extracting 67 homogenised stool samples (including 11 EQA specimens), ranging from faecal calprotectin <20 to >1932 µg/g, using both the Calex® devices and the manual weighing method.
2. Stool samples with low, medium and high concentrations of faecal calprotectin were homogenised, aliquoted and extracted using both extraction methods 10 times over different days to calculate inter-batch imprecision.
3. Stability was assessed by storing 3 samples extracted with Calex® devices at 4 ˚C for 14 days and faecal calprotectin was measured in the extracts on days 0, 5, 8 and 14 days post-extraction.

All extracts were analysed using BÜHLMANN fCAL® turbo reagent on an Abbott ARCHITECT c16000 platform.

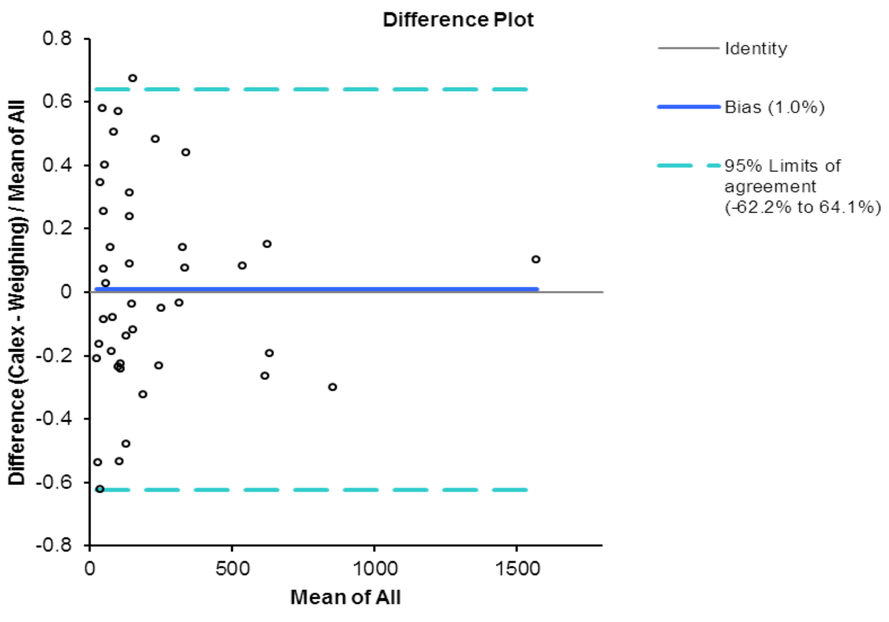
Results 1 - Comparison



n	42	(cases excluded: 14 due to missing values)
Range	26.00 to 1487.90	
	Replicates	
Weighing	1	
Calex	1	
	Bias	95% CI
Constant	-5.53	-18.33 to 14.42
Proportional	1.02	0.84 to 1.14

H_0: Constant bias = 0. H_1: Constant bias ≠ 0.

H_0: Proportional bias = 1. H_1: Proportional bias ≠ 1.



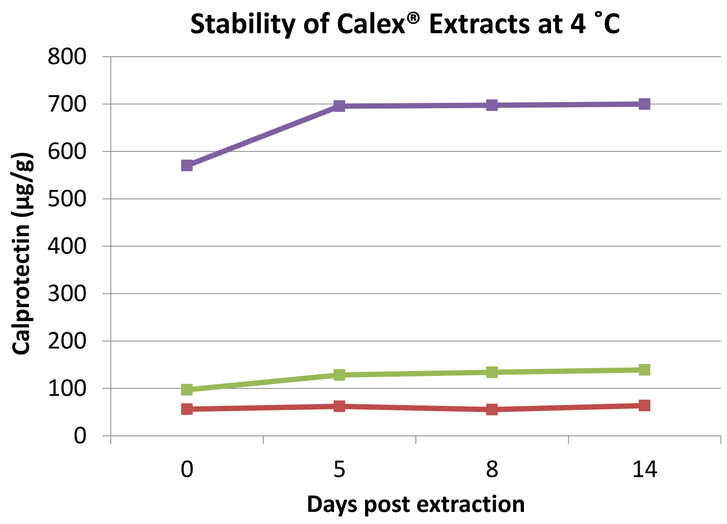
n	42	(cases excluded: 14 due to missing values)
Correlation - absolute difference v average	-0.21	
Bias	1.0%	
95% CI	-9.1%	to 11.0%
SE	4.97%	
t statistic	0.19	
DF	41	
p	0.8493	
SD of differences	32.2%	between single measurements
	95% Limits of agreement	95% CI
Lower	-62.2%	-79.5% to -44.9%
Upper	64.1%	46.8% to 81.4%

Results 2 – Intra batch imprecision

Calprotectin extracted using Calex® (µg/g)			Calprotectin extracted using weighing (µg/g)		
L	M	H	L	M	H
31	45.3	138.7	34.6	44.8	125.5
20.6	22.3	120.4	<20	37.1	153.7
<20	29.7	99.5	<20	35.9	112.3
<20	<20	111.7	<20	24.6	115.8
<20	24.6	106.5	<20	26.8	133.1
21.2	<20	110	<20	21.2	131.4
<20	22.3	119.8	<20	34.2	105.9
<20	23.4	118.7	<20	30.8	152
20	<20	124.5	<20	22.9	122.2
22.9	25.1	112.9	<20	21.2	137.7
Mean	23.1	27.5	116.3		30.0	129.0
SD	4.5	8.2	10.8		7.9	15.9
CV (%)		29.9	9.3		26.5	12.3

Results 3 – Calex® extract stability

Days post extraction	Calprotectin (µg/g)		
	Sample 1	Sample 2	Sample 3
0	56.3	97.3	570.2
5	62.3	128.5	695.5
8	55.4	134.1	697.5
14	63.9	139.2	699.9



	Calprotectin (µg/g)		
	Sample 1	Sample 2	Sample 3
Actual diff between 0 and 5 days	6.0	31.2	125.3
% diff between 0 and 5 days	10.7	32.1	22.0

	% difference compared to day 0		
	Sample 1	Sample 2	Sample 3
Day 5	11	32	22
Day 8	-2	38	22
Day 14	13	43	23

Discussion

1. Considering the non-homogenous nature of stool samples, the Calex® extraction devices showed good agreement with manual extraction. Results for Calex®-extracted EQA samples also compared better to the MLTM than those extracted using the manual weighing method. Result interpretation was altered for 12/56 samples (21%), however this can be explained by the imprecision of the assay and variation in calprotectin due to sampling location. The regression analysis contained 1 and 0 for 95% CI for slope and intercept, respectively.
2. Imprecision using the Calex® extraction devices is not significantly different to the weighing extraction method and is actually lower at higher concentrations. The imprecision at low concentrations is difficult to quantify due to many values being <LOQ.
3. Stability studies showed that concentration was not significantly affected by storage and therefore samples extracted by Calex® can be stored at 4 ˚C and analysed weekly. Although the concentration did change to a degree greater than that accounted for by imprecision in some cases this could be accounted for by differences in sampling site, which even homogenisation of the sample cannot overcome. The concentration did not follow an overall trend with continued storage. The percentage difference seen between 0 and 5 days is also very similar to the % CV in earlier studies and therefore storage of up to 5 days is acceptable.

Conclusion

Calex® extraction devices compare well to extraction via manual weighing. Calex® also demonstrated similar imprecision and accuracy to manual extraction. The main benefits of Calex®, speed and ease of use, will enable higher throughput and will allow the service to cope with future increases in demand. The devices are also more hygienic and the significant reduction in staff time and other reagents/consumables will no doubt result in an overall cost saving.