Evaluation of the BÜHLMANN fCAL™ Turbo Calprotectin Method on the Roche Cobas 6000 (c501)

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Background:

Faecal Calprotectin results are used to assist clinicians with the differential diagnosis of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Many laboratories use ELISA technology to analyse faecal samples for Calprotectin. We investigated the performance of the new BÜHLMANN fCAL™ turbo method which is CE marked for use on a number of mainline chemistry analysers.

Method:

The BÜHLMANN fCAL™ turbo particle enhanced turbidimetric immunoassay (PETIA) method on the Roche Cobas 6000 (c501) was compared to the BUHLMANN Calprotectin ELISA method on the Dynex DS2. Samples were extracted using the BÜHLMANN CALEX® extraction device prior to analysis on both methods.

- Regression analysis and Bland-Altman plots were used to compare results on 58 patient samples.
- Intra-assay precision was determined using 10 replicates of patient samples and inter-assay precision was calculated using 17 replicates of internal quality control material.
- NEQAS samples were analysed and bias relative to the all laboratory trimmed mean (ALTM) was assessed.

Results:

Comparison of patient results showed good correlation (R^2 = 0.97) consistent with previous studies[1,2]. Regression analysis produced the following equation: fCAL™ turbo = 1.14 DS2 result -23.42.

As shown in figure 1 the fCAL™ turbo method demonstrated a negative bias at concentrations <100µg/g and a positive bias at higher concentrations when compared with the ELISA method as observed in other studies[1]. Of note our ELISA method was running high compared to the ELISA mean on EQA during the evaluation.

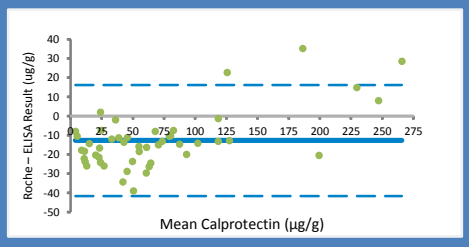

Figure 1
Bland Altman plot of patient comparisons (mean difference of - 12.8µg/g)

Intra-assay precision (%CV) was 3.1% and 1.3% at concentrations of 48µg/g and 247µg/g respectively (n=10). Inter-assay precision was 3.3% at 73 µg/g and 1.1% at 247 µg/g (n=17). This is consistent with De Sloovere *et al* who demonstrated %CV's of ~3% using the fCAL turbo method[2]. Since running the method routinely the IQC data shows a running CV of 4.5% at 75µg/g and 2.6% at 245µg/g (n=23)

To further investigate precision 10 separate extractions were performed on the same sample and analysis carried out on the Cobas platform. This showed a CV of 15.6% at a mean of 53.3µg/g.

Since the introduction of the method locally (November 2016) our EQA results have shown a positive bias compared to the ALTM (mean difference of +195µg/g). This is most marked at higher concentrations (Figure 2). This consistent with other fCAL™ turbo users registered with NEQAS (n=2).

A field safety notice (FSN) distributed in March 2017 stated that a positive bias of 15.6% was observed using the BÜHLMANN CALEX® extraction devices. This has subsequently been corrected with the CALEX® Cap "N" devices. We await further NEQAS results to determine the effects of this corrective action.

Local serial dilution studies show good recovery and an R^2 of 1.0 as shown in Figure 3.

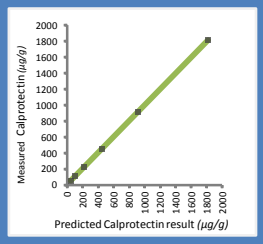

Figure 3
Serial dilution

Calprotectin (ug/g)	
fCAL turbo	EQA ALTM
<30	7.5
<30	13.3
<30	14
<30	16
<30	17.6
<30	24.3
<30	36
<30	40
93	41.9
72	54.5
84	54.8
136	57.8
228	136
304	140
1120	694
248	231
730	400
754	410
710	479
398	543
741	598
1672	708

Figure 2
Local EQA results compared to NEQAS All Laboratory Trimmed Mean (ALTM). Results <50µg/g shown in green.

Results are now reported locally with the following guidance:

- <50 µg/g – Probable IBS. Low probability of organic pathology
- 50-200 µg/g – Indeterminate range. Repeat after 4 weeks
- >200 µg/g – Moderate probability of organic pathology. Suggest referral
- >300 µg/g – High probability of organic pathology. Fast track referral.

Conclusion:

The BÜHLMANN fCAL™ turbo method demonstrates acceptable performance. The positive bias at higher concentrations is accounted for in local guidelines. A commutable reference material for calprotectin is required to define analytical accuracy in the future.

References:

(1) De Sloovere M *et al.* Analytical and diagnostic performance of two automated fecal calprotectin immunoassays for detection of IBD. *Clin Chem Lab Med* 2017 (Epub ahead of print)
(2) Nilsen T *et al.* A novel turbidometric immunoassay for fecal calprotectin optimized for routine chemistry analysers. *Journal of Clinical Laboratory Analysis* 2016; 1-6

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