Royal United Hospitals Bath
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Evaluation of the BÜHLMANN fCAL®TURBO Assay on the Beckman Coulter AU5822 Automated Chemistry Analyser

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Background

- Faecal Calprotectin analyses is used as a tool in the assessment of intestinal mucosal inflammation and an aid to IBD disease monitoring.
- Since 2021 Royal United Hospitals Bath (RUH) have employed the faecal calprotectin (fCAL) BÜHLMANN ELISA method on the Werfen DS2 analyser.
- With a desire to further automate the assay and improve efficiencies, we sought to evaluate the fCAL BÜHLMANN TURBO method on the Beckman Coulter AU5822.

Principle of Method

- Faecal samples were extracted using the CALEX® Cap extraction device (within 1-3 days of sample receipt).
- fCAL BÜHLMANN TURBO method is a particle enhanced turbidimetric immunoassay (PETIA):

➢ The extracted sample is incubated with reaction buffer and mixed with polystyrene nanoparticles which are coated with calprotectin-specific antibodies.

➢ If calprotectin is available in the sample, an immunoparticle agglutination occurs.

➢ The turbidity of the sample is measured by light absorbance; an increase with calprotectin-immunoparticle complex formation is proportional to calprotectin concentration which is determined from the established calibration curve.

Method

- fCAL BÜHLMANN TURBO was assessed for:

➢ **Inter-assay precision** (manufacturer IQC run >20x over the course of the verification)

➢ **Intra-assay precision** (individual patient extracts run ≥10x on the same day)

➢ **Accuracy** (UKNEQAS EQA sample extracts (x6) run and compared to ALTM and method means)

➢ **Bias** (patient extracts)

- Patient sample extracts (n=85) across the analytical range of the assay were run on both ELISA and TURBO methods within the same 24h period to minimise any effects of sample storage.

Results

- **Precision:** fCAL TURBO method inter-assay precision data was good and in keeping with manufacturer quoted precision. The intra-assay precision using patient samples was high in the low level patient sample but not clinically significant and likely to represent imprecision at the low end of the assay dynamic range:

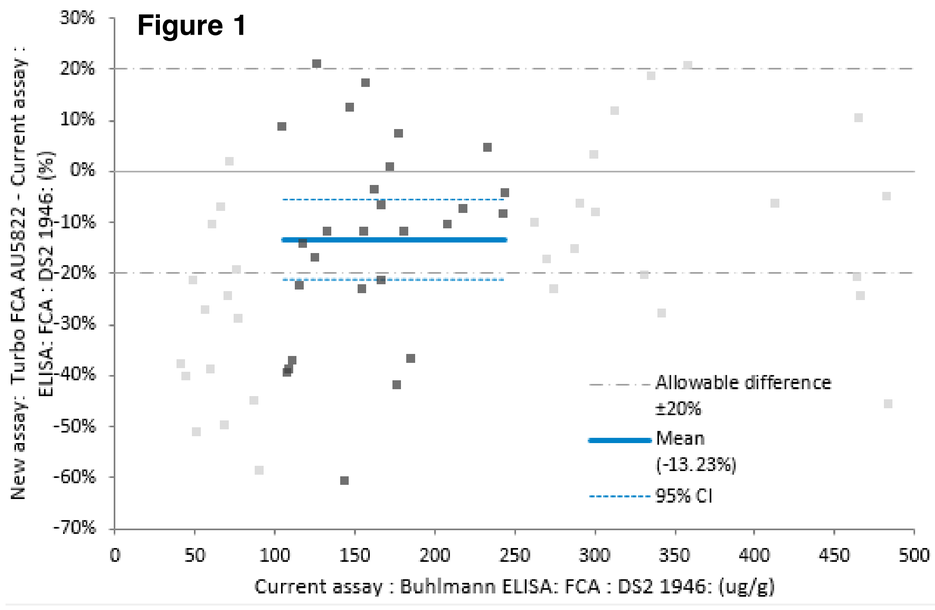
Inter-assay precision	QC1 lot 4719 (n=29)	QC2 lot 4719 (n=29)
Mean (µg/g)	87	280
SD (µg/g)	2.8	7.0
CV (%)	3.2	2.5

Intra-assay precision	Low patient (n=11)	Intermediate patient (n=11)	High patient (n=10)
Mean (µg/g)	37	168	1945
SD (µg/g)	8.0	7.8	36.4
CV (%)	21.3	4.7	1.9

- **Accuracy:** A limited range EQA samples were available, we obtained results as expected from UKNEQAS reports for all 6 samples analysed.
- **Bias:** An overall negative bias was demonstrated in patient samples run on the fCAL TURBO in comparison to results obtained on the fCAL ELISA (Figure 1).

This bias was unsurprising as for unknown reasons the RUH fCAL ELISA method had a long demonstrated positive bias on the UKNEQAS EQA scheme when compared to the ALTM and method mean.

Figure 1

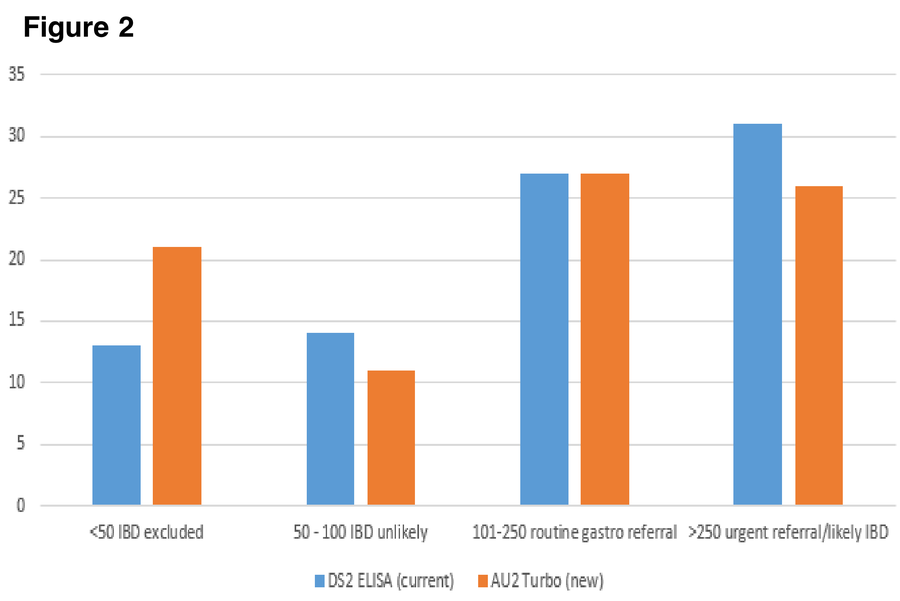


The difference in fCAL results was most significant at lower concentrations (-30% at <100 µg/g, -13% at 100-250 µg/g and -9% at >250 µg/g) (Figure 1).

An allowable difference of ±20% in included on the difference plots below to provide allowance for inherent analytical imprecision (2 x ELISA target %CV of 10%). A mean difference of -30% at concentrations <100 µg/g was deemed clinically insignificant.

- **Clinical impact:** given our intention to continue use of clinical thresholds based on the York Faecal Calprotectin Care Pathway), the verification samples results from both ELISA and TURBO fCAL methods were categorised as shown in Figure 2 (thresholds currently employed where patients have repeated fCAL tests).
- This analysis highlighted that we would likely see more patients classified as IBD excluded and a reduction in patients classified as IBD unlikely and those requiring urgent referral.

Figure 2



Conclusion

- The fCAL TURBO evaluation was successful and employed into routine use in February 2024, without amendment to interpretation thresholds. This is a more robust assay, with an increased measuring range (20 to 2000 ug/g on TURBO vs 10 – 600 ug/g on ELISA), improved accuracy (based on EQA results) and precision. The higher upper reportable limit on the fCAL TURBO assay (without the need for sample dilution) was in particular recognised as a significant improvement by the Gastroenterology team for monitoring and tailoring treatment of patients with known IBD.

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References:
Turvill J. *et al.* Evaluation of a faecal calprotectin care pathway for use in primary care. *Primary Health Care Research & Development*. 2016;17(5):428-436
BÜHLMANN fCAL® turbo Calprotectin turbidimetric assay Reagent kit package insert