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Validation of the newly FDA-approved Buhlmann fCal Turbo assay for measurement of fecal calprotectin in a pediatric population

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ABSTRACT

Objectives: Inflammatory bowel disease (IBD) is an increasingly prevalent disorder marked by chronic intestinal inflammation. Fecal calprotectin has emerged as a useful biomarker for differential diagnostics and monitoring IBD activity. We validated the newly FDA-approved fCal Turbo fecal calprotectin assay in our pediatric hospital.

Design and methods: The performance of the fCal Turbo assay was assessed on the Vitros 5600 analyzer (Ortho Clinical Diagnostics, USA), including limit of quantitation, linearity, precision, and interference studies. Method comparison was performed with 20 fecal samples with the Buhlmann fCal ELISA, and reference range verification was performed with 33 fecal samples.

Results: The fCal Turbo assay on the Vitros 5600 was linear between 33.1 and 14,182.5 μg/g, with dilution studies extending the range to 33.1–22,000 μg/g, Reproducibility of the assay met acceptability criteria, with intra-assay CV of 0.3–3.2% and inter-assay CV of 5.2–8.9%. Interference studies identified acceptable thresholds for protein, bilirubin, and lipids. We verified a reference range of 33.1–60 μg/g in our patient population. Deming regression identified acceptable correlation with minor positive bias (2.7%) between the fCal Turbo and fCal ELISA methods.

Conclusions: The fCal Turbo assay performs well on the Vitros 5600 analyzer in our patient population, with the assay being easy to use in our routine chemistry workflow. We anticipate that the fCal Turbo assay will be useful as a rapid screening method for differential diagnostics and disease monitoring of IBD in our patient population.

1. Introduction

Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are increasingly prevalent disorders with overlapping clinical presentations. Distinguishing between these two classes of disease is important, as while IBS is an idiopathic functional disorder, IBD is characterized by chronic inflammation and tissue destruction [1]. IBD can have serious sequelae, such as the need for surgical bowel resection or colorectal cancer. Particularly in children, IBD can cause malnutrition, growth failure, and micronutrient deficiencies [2]. Timely identification and treatment of IBD can reduce these deleterious consequences.

The gold standard diagnostic method for differentiation between IBS and IBD is endoscopy with tissue biopsy [3]. The need for a skilled operator and the invasive and uncomfortable nature of the test make endoscopy undesirable for patients, and an unsuitable test

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for repeated monitoring. Yet, tracking disease activity over the course of time is desirable to monitor disease flares.

Fecal calprotectin (FC) is a biomarker that has shown promise for identification and monitoring of IBD. Calprotectin is a calcium- and zinc-binding protein that is abundant in polynuclear neutrophils, monocytes, and macrophages. Its concentration in stool is well correlated with disease activity of IBD, especially in respect to the colonic mucosa, as measured by histopathological examination [4]. Testing for FC is becoming more widely adopted, and compliance with FC testing for disease monitoring in the pediatric IBD population at our institution is good [5]. The most widely used method for measurement of fecal calprotectin is ELISA. A cutoff value of 50 μg/g FC has been widely studied for differentiation between IBD and non-IBD, with high sensitivity (83–100%) and specificity ranging from 51 to 100% [6]. Recently, manufacturers have developed automated immunoassays that are more compatible with most laboratory workflows for the measurement of FC [7].

Our institution has previously sent specimens to reference laboratories to perform fecal calprotectin testing, and these laboratories primarily use ELISA for screening of suspected IBD in symptomatic patients. However, the primary pitfall of ELISA is the long turnaround time for results (quoted as 2 h to result for the fCal ELISA [8], compared to 10 min for the fCal Turbo [9]), and submission of specimens for reference laboratory testing further prolongs this turnaround time. In order to reduce FC turnaround, our pediatric hospital has validated the recently FDA-approved Buhlmann fCal Turbo turbidimetric immunoassay on the Vitros 5600 automated chemistry analyzer.

2. Materials and methods

The Buhlmann fCal Turbo turbidimetric immunoassay (Buhlmann Diagnostics, Amherst, NH) was evaluated for use on the Vitros 5600 automated chemistry analyzer (Ortho Clinical Diagnostics, Raritan, NJ). The fCal Turbo assay yields measurements in μg/g. Samples used for validation were extracted from stool using the Calex cap extraction device according to the manufacturer's instructions (Buhlmann Diagnostics, Amherst, NH).

2.1. Precision studies

Intra- and inter-assay precision studies were performed in accordance with CLSI guidelines by measuring six concentrations (mean values 57.8, 88.8, 175.9, 670.4, 1539.5, and 6446.2 μg/g) of fCal Turbo calibrator material. Within-run precision was assessed by measurement of 4 replicates at each concentration within one run, and between-run precision was assessed by measurement of 4 replicates of each concentration twice a day for five days. CV was calculated for intra- and inter-run studies by standard deviation/mean X 100, and results were considered acceptable when CV $< 10\%$.

2.2. Analytical sensitivity

Analytical sensitivity was assessed by measuring 5 replicates of 4 aliquots of normal saline or dilution of low-concentration calibrator. Limit of detection was calculated as the limit of blank $+1.65$ X standard deviation. Limit of quantification was determined as the lowest concentration of analyte that was higher than the calculated limit of detection and measurable with less than 20% total error. Analytical specificity was assessed by spiking sample extracts with known concentrations of protein, triglyceride-rich lipid, or conjugated bilirubin (Sun Diagnostics, New Gloucester, ME). Interference from hemolysate was not evaluated, as bloody stool is a manufacturer-dictated criterion for sample rejection. Spiked and neat samples were run for each extract, and results considered acceptable if measured values differed by less than 20%.

2.3. Linearity

Linearity studies were carried out by measuring a set of 11 manufacturer-provided samples with assigned values ranging from 37.6 to 14,182.5 μg/g calprotectin for 3 replicates each, with allowable systematic error set at 10%. Dilution verification was performed by measurement of 4 high-concentration samples neat or diluted 10X, and results considered acceptable if measured values differed by less than 20%.

2.4. Accuracy

Accuracy of the fCal Turbo assay on the Vitros 5600 was determined by comparison with the FDA-cleared fCal ELISA (Buhlmann Diagnostics, Amherst, NH). Thirty three (33) random stool specimens were collected from previously tested samples at our institution, extracted using the Calex cap device using the same protocol as for other tested samples, and the extracts frozen at -80 °C prior to analysis. One aliquot of frozen extract was shipped to Buhlmann laboratories for analysis by ELISA, and all aliquots were thawed just prior to analysis.

2.5. Reference range verification

The reference range given by the manufacturer (<80 μg/g) [9] was verified using extracts from 22 stool specimens previously tested at our institution. We collected samples from refrigerated stool specimens from the previous week, as calprotectin has been demonstrated to be stable at 4 °C in stool for 6 days [4]. Patients were between 2 and 17 years of age. Specimens that were too firm to

homogenize, bloody or mucoid were excluded, as these are manufacturer-dictated criteria for sample rejection. Chart review was conducted to exclude samples from patients with a diagnosis of IBD or other GI disease, or those using NSAIDs or proton pump inhibitors, as these are demonstrated to increase calprotectin concentrations [10].

2.6. Statistical analysis

Statistical analysis for accuracy, precision, linearity, and reference range studies was carried out using EP evaluator. Method comparison studies were evaluated using Deming regression, and a Bland-Altman plot was generated to evaluate proportional bias.

3. Results

3.1. Precision

The fCal Turbo assay exhibited acceptable precision both within and between runs, yielding within-run precision of 0.3–3.2%, and between-day precision of 5.2–8.9% (Table 1).

3.2. Linearity and reportable range

The fCal Turbo assay was determined to be linear between 37 and 14157 μg/g, with total observed error of 3.0%. Dilution studies performed with high-concentration samples verified linearity of response up to a 10X dilution. Limit of blank was measured as 19.0 μg/g, with limit of detection 27.7 μg/g, and limit of quantitation 33.1 μg/g. The clinical reportable range for the fCal Turbo assay was verified to be 33.1–14157 μg/g (Fig. 1).

3.3. Method comparison

Method comparison studies of the fCal Turbo assay on the Vitros 5600 instruments with the manufacturer's ELISA indicated excellent agreement between the two methods. Comparison to ELISA yielded fCal Turbo = 1.030 X ELISA – 3.245, with R = 0.9977 and proportional bias of 2.66% (Fig. 2).

3.4. Reference range

Reference range verification studies were carried out using a total of 22 samples identified as normal based on our exclusion criteria. Sixteen (16) samples were measured at concentrations below 33.1 μg/g, which is below our reportable range. Based on this, our reference interval for patients without IBD was verified to be 33.1–60 μg/g.

3.5. Analytical interferences

Interference studies were carried out to evaluate the effects of protein, bilirubin, and lipids on measurement of fecal calprotectin concentrations by the fCal Turbo assay. All spiked compounds yielded some degree of interference with the assay, but no significant interference was identified below 66 mg/dL protein, 50 μg/dL conjugated bilirubin, or 340 μg/dL triglyceride-rich lipid.

4. Discussion

This report describes the validation of the fCal Turbo assay on the Vitros 5600 automated chemistry analyzer in a pediatric population. We find that the fCal Turbo assay exhibits acceptable intra-assay (0.3–3.2%) and inter-assay (5.2–8.9%) precision and is linear across clinically relevant concentrations of fecal calprotectin. In method comparison studies with the Buhlmann fCal ELISA, the fCal Turbo assay performed well, with excellent correlation between methods and very minor positive proportional bias relative to results from ELISA.

Fecal calprotectin has been reported to be a remarkably stable analyte, with stability up to one week at room temperature or two and

Table 1
Within-run and between-day precision studies.

	Within-run	Between-day
mean	%CV	%CV
57.8	3.2%	6.0%
88.8	1.4%	8.9%
175.9	1.6%	5.2%
670.4	0.3%	6.9%
1539.5	0.6%	8.4%
6446.2	0.6%	7.9%

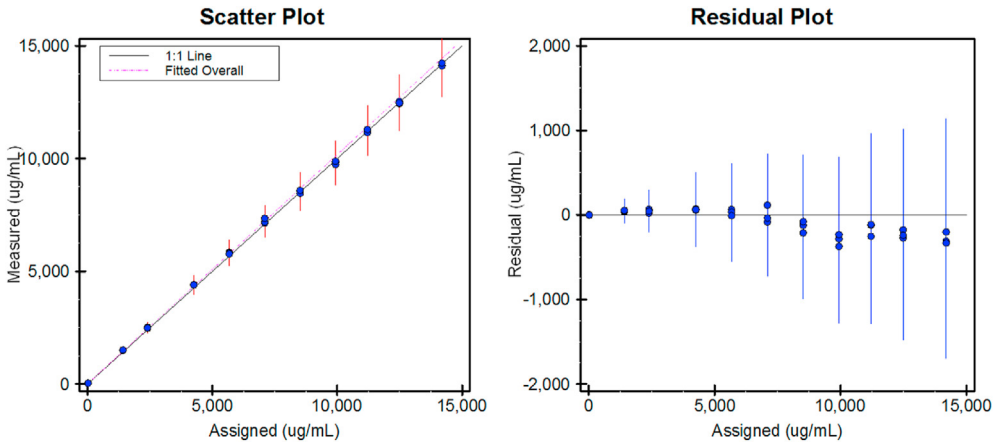

Fig. 1. Scatter and residual plots for linearity studies of the fCal Turbo by Vitros 5600. Samples used for these studies were provided by the manufacturer with assigned values.

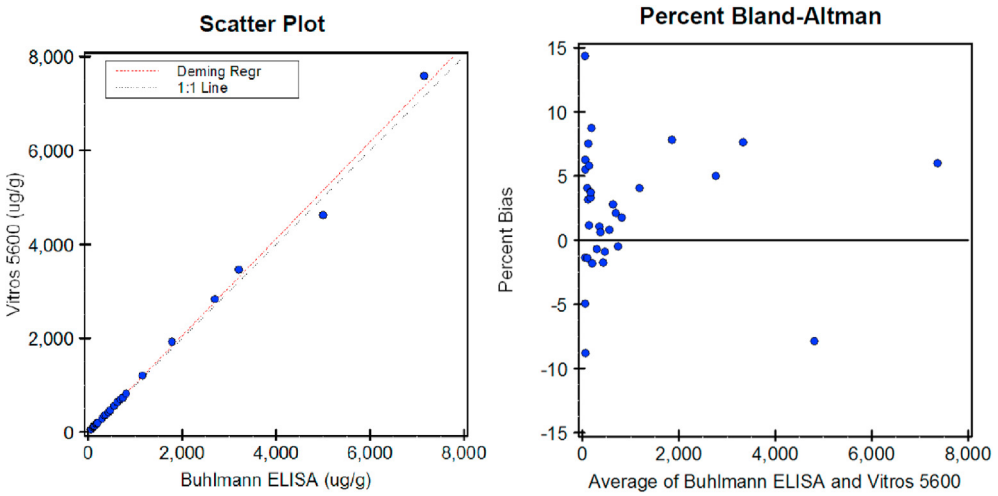

Fig. 2. Deming regression and percent Bland-Altman plots for method comparison of the fCal Turbo by Vitros 5600 and fCal ELISA assays.

a half months at −20 °C [3]. This property enables significant flexibility in storage and processing of specimens for fecal calprotectin testing. In our studies, frozen specimens were used for method comparison studies to enable batching of specimens over longer periods of time. In our daily workflow, specimens are refrigerated prior to testing.

We verified a reference range of 33.1–60 μg/g fecal calprotectin in our pediatric population. This differs slightly from the widely-cited cutoff of 50 μg/g fecal calprotectin, however, fecal calprotectin concentrations are reported to be higher in children under 5 years of age [11]. Our reference range verification included subjects as young as age 2, which may influence our higher cutoff. Partitioning of reference ranges based on age may be a useful avenue for further study, to allow better accuracy in classification of young patients.

One European group has previously published their validation of the fCal Turbo assay under similar conditions [12]. Our results align closely with these previously published data. Noebauer et al. reported LOQ of 21 μg/g and linearity between 20 and 2100 μg/g, with intra-assay imprecision of 2.15–6.63% and inter-assay imprecision of 0.01–6.05%. They also performed method comparison to fCal ELISA, and reported similarly excellent correlation between methods (R = 0.99, proportional bias of 7.2%).

The fCal Turbo assay appears to perform similarly on the Vitros 5600 relative to other reports of this assay on other automated chemistry analyzers. On the Roche cobas c501 (Basel, Switzerland), three reports have described performance of the fCal Turbo assay, indicating intra-assay imprecision of 1.1–2.9% [13] and 1.4–5.6% [7], inter-assay imprecision of 0.9–2.8% [13] or <4.5% [14], and limit of quantitation of 20 μg/g with linearity up to 1955 μg/g [14]. In contrast to the Vitros 5600, however, the cobas c501 has been reported to exhibit significant bias against the fCal ELISA (proportional bias of −22%) [13]. Relative to the fCal ELISA, the precision of the fCal Turbo assay is also favorable, with intra-assay CV of the ELISA reported at 8.1–3.8%, and inter-assay CV of 9.1–12.4%. Linearity of the two assays are similar (1800 μg/g for ELISA vs. 2200 μg/g for Turbo) [3].

There is little information on interference with the fCal Turbo assay. We identified significant positive interference from protein (above 66 mg/dL), conjugated bilirubin (above 50 μg/dL), and from triglyceride-rich lipid (above 340 μg/dL) when spiked into fecal

extract. In practical terms, we determined that any visually detectable degree of turbidity produced positive interference. This perhaps can be expected, as the fCal Turbo assay operates on a turbidimetric detection scheme. Nilsen et al. performed interference studies on the BS-380 analyzer (Mindray, Shenzhen, China) with a variety of drugs and other compounds and did not observe significant interference with any tested substance [14]. We did not evaluate hemolysis as a source of interference, as the manufacturer's sample integrity criteria indicate that bloody or mucoid stool should be rejected. Future studies may evaluate to what degree these contaminants affect measurements with the fCal Turbo assay.

While the fCal Turbo is a rapid and automated assay, some workflow considerations remain. Our technical staff were much less familiar with evaluation of stool specimens for sample integrity than urine or plasma/serum, and we needed to ensure adequate equipment was available for manual specimen processing steps. In particular, vigorous and extended vortexing of specimens was required for complete specimen extraction from the Calex cap, and we found that use of a tube holder on our vortex unit facilitated this process. However, even with these processing steps, the time to result is still far less than by ELISA. In our laboratory, the process from receipt to result can be accomplished in approximately 30 min.

5. Conclusions

The newly FDA-cleared fCal Turbo assay, with the Calex cap for sample extraction, represented a much more rapid and facile workflow for our laboratory than referring samples for analysis by ELISA. We determined that the fCal Turbo assay on the Vitros 5600 analyzer offered excellent performance relative to ELISA, was reproducible, and was not excessively prone to analytical interference. We anticipate that it will be a useful tool for identification of IBD in our patient population.

CRediT author contribution statement

Emily Garnett: Data curation, Writing - original draft, put all data together and Garnett and Devaraj-wrote manuscript. The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors. **Jayson Pagaduan:** Data curation, and. **Deepthi Rajapakshe:** Methodology, Validation, Method validation. **Estella Tam:** Methodology, Validation, and. **Richard Kellermeyer:** Conceptualization, and. **Sridevi Devaraj:** Conceptualization, Writing - original draft, conceptualized study.

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References

[1] T. Sairenji, K.L. Collins, D.V. Evans, An update on inflammatory bowel disease, Prim Care 44 (4) (Dec 2017) 673–692.
[2] S. Nasiri, M.E. Kuenzig, E.I. Benchimol, Long-term outcomes of pediatric inflammatory bowel disease, Semin. Pediatr. Surg. 26 (6) (Dec 2017) 398–404.
[3] S. Kittanakom, M.S. Shajib, K. Garvie, et al., Comparison of fecal calprotectin methods for predicting relapse of pediatric inflammatory bowel disease, Chin. J. Gastroenterol. Hepatol. 2017 (2017) 1450970.
[4] H. Manceau, V. Chicha-Cattoir, H. Puy, K. Peoc'h, Fecal calprotectin in inflammatory bowel diseases: update and perspectives, Clin. Chem. Lab. Med. 55 (4) (Mar 1 2017) 474–483.
[5] K. Queliza, M.K. Wang, R. Kellermayer, Compliance with fecal calprotectin testing in pediatric patients with inflammatory bowel disease, *J Pediatr Gastroenterol Nutr.* Jun 66 (6) (2018) 932–933.
[6] N. Waugh, E. Cummins, P. Royle, et al., Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation, *Health Technol Assess.* Nov 17 (55) (2013) 1–211, xv-xix.
[7] M. Oyaert, A. Boel, J. Jacobs, et al., Analytical performance and diagnostic accuracy of six different faecal calprotectin assays in inflammatory bowel disease, Clin. Chem. Lab. Med. 55 (10) (2017) 1564.
[8] Buhlmann fCal ELISA Calprotectin Instructions for Use, Buhlmann Laboratories AG, 2018.
[9] Buhlmann fCAL Turbo Calprotectin Turbidimetric Assay Reagent Kit, Buhlmann Laboratories AG, 2019.
[10] E. Klingberg, H. Carlsten, E. Hilme, M. Hedberg, H. Forsblad-d'Elia, Calprotectin in ankylosing spondylitis–frequently elevated in feces, but normal in serum, Scand. J. Gastroenterol. 47 (4) (Apr 2012) 435–444.
[11] S.J. Jeong, The role of fecal calprotectin in pediatric disease, Korean J Pediatr 62 (8) (2019) 287–291.
[12] B. Noebauer, L. Ramic, A. Konstantin, C. Zachbauer, E. Einwallner, Analytical evaluation of a fully automated immunoassay for faecal calprotectin in a paediatric setting, Biochem. Med. 27 (3) (Oct 15 2017), 030710.
[13] M.M.W. De Sloovere, D. De Smet, F.J. Baert, J. Debrabandere, H.J.M. Vanpoucke, Analytical and diagnostic performance of two automated fecal calprotectin immunoassays for detection of inflammatory bowel disease, Clin. Chem. Lab. Med. 55 (9) (Aug 28 2017) 1435–1446.
[14] T. Nilsen, K. Sunde, L.O. Hansson, A.M. Havelka, A. Larsson, A novel turbidimetric immunoassay for fecal calprotectin optimized for routine chemistry analyzers, J. Clin. Lab. Anal. 31 (4) (Jul 2017).