TITLE : **''Evaluation of the BÜHLMANN fCAL Turbo Faecal Calprotectin Assay on the Abbott Architect C8000. Comparison to the Phadia EliA and the IBDoc® home test kit.''**

MSc Research Project Scientific Paper - Biomedical Science

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Evaluation of the BÜHLMANN f CAL Turbo Faecal Calprotectin Assay on the Abbott Architect C8000. Comparison to the Phadia ELiA and the IBDoc® home test kit.

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Short Running Title : f CAL Turbo on Abbott Architect C8000.

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STATEMENT OF CONTRIBUTION :

In the studies comprising this paper, I undertook all the work on the BÜHLMANN f CAL Turbo assay. This included the extraction of the Faecal Samples, preparation of the f CAL Turbo reagents on the Abbott Architect and performing the test. I also extracted all the IBDoc samples. I undertook all statistical analyses reported in this project.

"I Mary O'Connell, hereby confirm that I am the major Author of this paper and worked described therein and that this work has been conducted using and following humane and ethical procedures, in accordance with the Research Ethics and Governance Policies and Procedures and other Research Policies and Procedures at The Mercy University Hospital, Cork, Republic of Ireland, including the code of practice for Professional Integrity in the Conduct of Research. I also confirm that this work is not plagiarised, does not infringe copyright, and provides a full disclosure of relevant interests."

ABSTRACT

Background: Faecal Calprotectin is a valuable marker for Inflammatory Bowel Disease and has resulted in significant cost savings. ELISA is the standard assay. There are a few considerations with this method including that it is labour intensive, time consuming and requires specific laboratory equipment. A major advantage of the BÜHLMANN f CAL turbo assay is that it can be adapted to any of the current open clinical chemistry analysers. This will permit more clinical laboratories to perform testing on site and reduce the turn-around time of the test.

Method: f CAL turbo assay was evaluated on the Abbott Architect C8000 analyser, with comparison to the Phadia 250 ELiA . A total of 60 faecal samples were measured for comparison. The f CAL turbo was also compared to the IBDoc® home test.

Results: Precision of the assay achieved coefficient of variation 0.74% to 3.3%. Linearity is 16µg/g to 1922µg/g (highest calibrator) with an option of an automatic 1:10 dilution for values greater than this. Correlation coefficient of 0.930 in comparison to the Phadia 250, with a statistical significance (p=0), with the f CAL turbo reading higher. A review of participant medical charts confirmed the accuracy of the f CAL turbo. A p=0.226 for f CAL turbo versus IBDoc® home test, and p=0.003 for IBDoc® versus the Phadia. Calibration is stable for 60 days.

Conclusion: f CAL turbo is very adaptable to the Abbott Architect C8000 analyser. The CALEX ® cap extraction devices are easy to use and practical. Inter-assay variability demonstrated, and recommends users to participate in the UK NEQAS Scheme . No interference to any of the other chemistries.

KEYWORDS: BÜHLMANN f CAL turbo, Faecal Calprotectin, IBD.

ABBREVIATIONS

CLiA: Chemiluminescent immuno-assay

CRP: C Reactive Protein

CV: Coefficient of Variation

ECCO : European Crohn's and Colitis Organisation.

EliA : Enzyme linked immunoassay

ELISA : Enzyme Linked Immuno-Sorbent Assay.

EQA : External Quality Assessment

ESR: Erythrocyte Sedimentation Rate.

FC: Faecal Calprotectin

GIT: Gastrointestinal tract

IBD: Inflammatory Bowel Disease

IBS: Irritable Bowel Syndrome

NICE: National Institute for Health and Care Excellence.

PETIA : Particle Enhanced Turbidimetric Immunoassay.

RCF : Relative Centrifugal Force

UKNEQAS : United Kingdom National External Quality Assessment Scheme

INTRODUCTION

Faecal Calprotectin (FC) is well established as a biomarker for Inflammatory Bowel Disease (Manz *et al.,* 2012; Du *et al.,* 2016). Inflammatory Bowel Disease(IBD) is the general term given to a group of conditions that involves inflammation of the gastrointestinal tract (GIT) with Crohn's Disease and Ulcerative Colitis being the two most common (Waugh *et al*., 2013). IBD is more common in white people than in African/Asian origin with the prevalence of Ulcerative colitis 0.1-0.2 % of the population and 0.05-0.1% for Crohn's disease (NICE guidelines 2013). Ulcerative colitis is characterised by inflammation of the colon, whereas Crohn's can affect any part of the GIT. Both diseases are relapsing and remitting inflammatory processes which could result in surgery and an increased risk of colorectal cancers.

Calprotectin is a calcium binding heterodimer that is abundant in the cytoplasm of neutrophils. Calprotectin is a protein that is released by neutrophils due to infiltration of the intestinal mucosa during inflammation and thus is released into the intestinal lumen (Bressler *et al*., 2015). Faecal measurements are a much more specific biomarker for IBD than other biomarkers such as plasma CRP and ESR and is stable and not degraded by bacteria in the faeces (Chang *et al*., 2014). Calprotectin release is proportionate to the degree of inflammation (Chang *et al*., 2014; Dhaliwal *et al*., 2015).

FC testing has been well documented for differentiating between inflammatory and non-inflammatory bowel disease (ECCO guidelines 2016; NICE guidelines 2013). This is an important factor to the medical providers as they strive to provide appropriate treatment plans for their clients. Irritable Bowel Syndrome (IBS) is a bowel disorder characterised by abdominal pain, bowel disturbances and bloating but no inflammation and therefore do not require medical treatment. Direct endoscopic examination of the mucosal in the bowel is the gold standard for diagnosing IBD (Chang *et al.,* 2014; Bressler *et al.,* 2015). However, colonoscopies are very invasive, expensive and time consuming. In most cases a negative FC (<50µg/g) rules out IBD (Waugh *et al.,* 2013). A positive FC test indicates IBD and patients are referred for Colonoscopies. ECCO (European Crohn's and Colitis Organisation) report that low FC levels have a 99% NPV for IBD (Gomollón *et al.,* 2017). This saves the IBS patient from having unnecessary colonoscopies and therefore has resulted in significant cost savings by the healthcare providers (Mindemark & Larsson, 2012). FC levels, also correlate with disease activity and is a surrogate marker of mucosal healing in response to treatment (Louis, 2014). Therefore the validity of measuring FC levels has two functions : (1) Distinguish IBD from IBS and ; (2) Monitoring of IBD patients.

The first research studies measuring FC began 20 years ago. Roseth *et al. (*1997), compared FC levels to histological findings in Ulcerative Colitis patients, and Tibble *et al.* (2000), measured FC levels in Crohn's disease patients and explored the potential to use it as a screening test to distinguish between Crohn's disease and IBS. In adults there is a prevalence of IBS in 10-20% of the general population. (NICE guidelines 2013). ELISA is the standard method to measure FC levels (Bressler *et al.* 2015). ELISA's are performed on micro-titre plates with the optical densities read manually on a plate reader. This method is intensive and time consuming and also require specific laboratory equipment.

BÜHLMANN Laboratories in Switzerland, have recently produced an assay which they have hypothesised can be adapted to any open clinical chemistry analyser. The fCAL turbo is a new Particle Enhanced Turbidimetric Immuno-assay (PETIA), which allows random access sample handling and a result within 20 minutes (Schallberger *et al*., 2016). This assay has the huge advantage that it can be adapted to any of the current open clinical chemistry analysers. This will permit more clinical Laboratories to perform FC testing on site and reduce the turn-around time of the test. BÜHLMANN Laboratories also developed their own extraction device, the CALEX® Cap to speed up and standardise the extraction step (Schallberger *et al.*, 2016). Initially extracting Calprotectin from the faeces sample was manual and involved weighing out a specific amount of stool in grams. Manual weighing of the sample is the reference extraction method (Louis 2014). Commercial extraction devices were then developed to aid this step and most assay providers have their own extraction device.

The aim of this study is

1. To evaluate the f CAL turbo assay on the Abbott Architect C8000 analyser with comparison to the Thermo-Scientific Phadia 250 ELiA . The Phadia 250 is an automated quantitative Fluorenzyme-Immunoassay.(Phadia 250 ELiA kit insert 2015)
2. To compare the IBDoc® home self-test kit to the f CAL turbo and the Phadia 250 EliA. IBDoc® is a Calprotectin home self-test for that was launched in 2015 by BÜHLMANN Laboratories and became the first CE marked Calprotectin Home Test (BÜHLMANN Laboratories , 2015). IBDoc® is a simple to use technology which combines the CALEX® extraction device, a calprotectin test strip and the IBDoc® smart phone app to read the test result by state of the art image processing. The core of this test is the IBDoc® web portal for managing patient data. The result is sent back digitally to the patient as well as to the Clinician. It is designed with very high security standards for patient confidentially. The CalApp® can be downloaded to the majority of smartphones and is available in many languages (Alpha Laboratories, 2015). An independent study of IBDoc has never been performed.

MATERIALS AND METHODS

Samples

All participant samples were selected randomly. Faecal samples collected into Universal containers that were routinely sent to the Laboratory from the IBD Clinic of the hospital for external FC testing were used in this study.
The IBDoc participants sent their samples directly to the Laboratory from home with informed consent. All were current IBD patients.
This study was approved by the local Clinical Research Ethics Committee, University College Cork, which is recognised under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children

Extraction Device

All samples were extracted using the BÜHLMANN Calex® Cap Extraction device ((BÜHLMANN Laboratories, 2017). The CALEX® Cap is a single tube filled with 5mls of extraction buffer. The 6 grooves of the sampling pin were completely filled by rotating it in the faecal sample. The sampling pin was reintroduced into the tube with excess faeces being removed via the funnel. The tube was homogenized using a vortex and allowed to stand for ten minutes, ensuring all the sample was homogenised from the grooves. Final dilution of samples was 1:500**.**
The tubes were centrifuged at 4300 RCF for ten minutes with the sampling pin side of the tube inverted. The extracts were analysed directly or stored at -20°C in the tubes prior to analysis. The original faecal sample with the exception of the IBDoc samples, were then sent at 4°C to the referral Laboratory to be extracted and measured for Calprotectin on the Phadia 250 ELiA. The IBDoc faecal samples were also extracted using the Thermo- Scientific ELiA stool extraction kit (Thermo Scientific, 2012). These extracts were stored in fresh tubes at -20°C, and sent in a batch to the referral Laboratory at-20°C to be analysed directly on the Phadia 250 ELiA.

Reagents

BÜHLMANN f CAL® Turbo PETIA kit (BÜHLMANN Laboratories, 2015).
R1 – Reaction Buffer MOPS Buffered Saline. 1 vial - 35mls.(Ref code B-KCAL-R1)
R2 – Immunoparticles which are polystyrene beads coated with Avian antibodies against human calprotectin. 1 vial – 7mls. (Ref B-KCAL-R2)

BÜHLMANN f CAL® Turbo Calibrator kit. (Ref B-KCAL-CASET) 1-6 calibrators x 1ml.

BÜHLMANN f CAL® Turbo Control Kit. (Ref B-KCAL-CONSET) Low and high control 3 x 2 1ml vials

The reagents were supplied by Brennan & Company which are the distributors for BÜHLMANN Laboratories in the Republic of Ireland. (www.brennanco.ie)

Instrumentation

Abbott Architect C8000 Analyser.

Parameters

Assay Type	Photometric
Reaction mode	End up
Sample Volume	11.2 ul
R1 Volume	150 ul
R2 Volume	30 ul
Primary Wavelength	548 nm
Blank read time	19-19
Main read time	24-25
Sample Blank type	Self

Statistical analysis.

All statistical analysis was performed using the statistical package IBM SPSS version 23. The paired samples t-Test was used for method comparison to both the Phadia 250 and the IBDoc. Differences were considered statistically significant at $p < 0.05$. Correlation coefficients were assessed by Pearson's correlation. Scatter plots with linear regression analysis were also used to demonstrate method comparison.

RESULTS

Precision of the f CAL Turbo Assay

Precision was performed on the low and high controls both within run (10 times) and between run over 10 days. Table 1 and Table 2 details the means, standard deviations, CV's and Uncertainty of measurement. CV's ranged from 0.74% to 3.3%

Precision of the extraction step.

A participant sample was extracted ten times within run and ten times between run. (Some of the between run extracts were stored frozen at -20°C prior to analysis). Table 3 details the means, standard deviations and CV's. A CV of 7.5% and 9.4% was achieved.

Limit of Quantitation (LoQ)

The LoQ observed was 16µg/g . This was calculated by diluting the lowest calibrator (48µg/g) with the zero calibrator. It was determined where the total error was <20 %. The total error was 3.1 % at 16µg/g.

Linearity

This was performed by running a series of 6 samples of known concentration (low to high) randomly in duplicate and plotting against the target values. See Table 4 for results and % recovery, and Figure1 for the corresponding scatter plot with a linear fit. A coefficient of determination of 1.00 with a slope of 0.99 was achieved. The results demonstrate the assay is linear from LoQ to the highest calibrator. (16µg/g to 1922µg/g)

Calibration curve stability

The calibration curve was stable for 60 days as recommended by the manufacturers. Two reagent kits (different lot numbers) were used in this study. Each kit was in use over 60 days and both controls always read within 1-2 SD of the target. The performance of the low and high control are summarised as

	Target	Running Mean	SD	%CV
Low Control :	76.6µg/g	77.7µg/g	3.1	4.0
High Control:	250.5µg/g	247.8µg/g	3.2	1.3

Method Comparison to Phadia 250

A total of 60 sample in the range of "<15 to >3000 µg/g" were measured on both the f CAL turbo assay on the Abbott Architect C8000 and on the Phadia 250 ELiA. The results are tabulated in Table 5 with the clinical interpretation. The results span the entire measuring range. The Phadia 250 has a measuring range of 15 to >3000µg/g. The f CAL turbo has a measuring range of 16 to 1922µg/g with the option of an automatic 1:10 of all values >1922 and therefore can measure up to 19,220µg/g. Both assays quote the same reference range of

Faecal Calprotectin < 50µg/g : Normal

50-200µg/g : Mild organic disease. Suggest repeat in 4-6 weeks

>200 µg/g : Indicative of active organic disease

(BÜhlmann Laboratories ,2015) (Phadia 250 ELiA, 2015). See Figure 2 for scatterplot of the results of the Phadia 250 versus Architect C8000 f CAL turbo with linear regression. A coefficient of determination of 0.864 was achieved with a slope of 1.15 and an intercept of 192.0.
See Figure 3 for paired samples t -Test of the Phadia 250 vs Architect f CAL Turbo. A Pearson correlation coefficient of 0.930 was achieved with a difference of 245 between the two means. A statistical significance demonstrated between the two methods as p=0.
See Table 6 for analysis of the discrepant results. Patient charts were reviewed for colonoscopy reports if performed and clinical presentation to determine which of the two assays produced the most accurate results as colonoscopy is the gold standard test. Sample number 35 was also included here due to the huge difference in positivity of the result.

Method Comparison to IBDoc and Phadia.

15 specimens were received from IBDoc participants for this part of the study. Only 13 results were available on the Phadia 250. Table 7 details the results comparison of the three methods. Figure 6 demonstrates that the f CAL turbo correlated better than the Phadia to the IBDoc, with a Pearson correlation coefficient of 0.904 for f CAL turbo versus IBDoc, and 0.806 for Phadia versus IBDoc. No statistical significance for f CAL Turbo versus the IBDoc as p=0.226. Whereas a p= 0.03 for the Phadia versus IBDoc demonstrates a statistical significance between these two methods.
See Figure 4 for scatterplot of f CAL Turbo versus IBDoc with linear regression. A coefficient of determination of 0.817 was achieved with a slope of 1.47. Figure 5 shows the scatterplot of the Phadia 250 versus IBDoc. A coefficient of determination of 0.650 was achieved with a slope of 0.32 which also demonstrates poorer correlation. .

Stability of Faecal Calprotectin.

Stability of faecal samples stored at 4°C is 3 to 4 days. A participant sample was extracted 3 times after being stored for 5 days at 4°C. 70% recovery was achieved. (A mean of 476µg/g with a target of 683µg/g) Another participant sample was extracted after 4 days stored at 4°C and a recovery of 91% was achieved (1370 µg/g for 1502µg/g). The extracts are stable stored at 4°C for 5 days (117% recovery with a mean of 798ug/g for a target of 683µg/g). Extracts are stable for a minimum of 6 weeks stored at -20°C. 5 extracts of the same sample were frozen at -20°C for 6 weeks and the % recovery was 103%. (A mean of 702µg/g with a target of 683µg/g). In conclusion it is recommended to extract the faecal samples as soon as possible. All faecal samples should be stored at 4°C, with extracts stable for 5 days at 4°C, and up to 6 weeks stored at-20°C.

UK NEQAS Report for Faecal Markers of Inflammation

Table 8 details the UK NEQAS report for all participants for August 2017. The mean of all methods is given as well as the mean for each method. This table demonstrates the inter –assay variability between all of the different methods. Note the large value for SD and %CV. There are only 7 participants using the BÜHLMANN f CAL turbo method and this study correlated very well with these users. The f CAL turbo means for samples 159A and 159B are higher than the total mean and also are higher than the Phadia 250 ELiA.(referred to as Thermo ELiA in the table).

DISCUSSION

Faecal Calprotectin is a valuable marker in the diagnosis and monitoring of IBD including a huge cost saving benefit. The aim of this study was two- fold. Firstly, and most importantly, to evaluate the novel f CAL turbo assay on the Abbott Architect C8000 with comparison to the Phadia 250 ELiA and secondly to compare to the IBDoc home test. Traditionally FC assays were ELISA, which were time consuming and favoured a batch system and therefore resulted in an increased turn-around time. Automated immuno-assays then became available such as the Phadia 250 which is a Fluoroenzyme-Immunoassay. Newer FC assays include the Diasorin® Liaison® calprotectin assay which is also an automated Chemiluminescent immuno-assay (Delefortrie *et al.,* 2016*).* However even though these automated assays may have a quicker turn- around time, they are still stand- alone specific analysers. This is the huge advantage of the f CAL turbo assay as BÜHLMANN claim that it can be adapted to any open clinical chemistry analyser. This gives access to more Clinical Laboratories to perform FC testing on site. On site testing hugely enhances and improves the service to the patient. The advantages include early detection of flare ups, earlier clinical decisions and intervention, optimises clinical outcomes and increases patient satisfaction.

This study has demonstrated that the f CAL turbo assay is adaptable to the Abbott Architect C8000 clinical chemistry analyser. The assay did compare well to the Phadia 250 with a correlation coefficient of 0.930. The f CAL turbo results are higher than the Phadia 250. This correlates with many other studies that demonstrate the Inter-Assay variability with FC. Labaere *et al.* (2014), compared six different FC assays. Three were rapid Immuno-Chromatography assays, two ELISA's and the final was the Phadia 250 ELiA. The correlation coefficients ranged from 0.65 to 0.93 and there was up to 5-fold quantitative differences between the assays. However most of the assays had similar sensitivities and specificities and therefore comparable clinical performance. Amcoff *et al.(*2017*),* compared the BÜHLMANN ELISA, the Immunodiagnostik ELISA and the Phadia, Again BÜHLMANN read up to 7 times higher than the Immunodiagnostik, with the Phadia reading in

between the two. Vendel *et al. (*2015), reported a correlation coefficient of 0.86 for the BÜHLMANN ELISA when compared to the Calpro ELISA. This present study demonstrates the same with the paired samples t- Test demonstrating a statistical significance between the two assays. Looking at the results in Table 5, there are twelve matching positives, twenty matching negatives, six matching borderlines and twenty two discrepant results. To determine which of these two assays is more accurate, the patient charts of the discrepant results were reviewed for clinical presentation and colonoscopy reports if available as colonoscopy is the gold standard in diagnosing IBD (See Table 6). The most significant discrepancies were the five that were reported positive on the f CAL turbo and negative on the Phadia 250. Sample number 1 which had the biggest quantitative difference, had a positive colonoscopy report which correlated with the f CAL turbo result. Sample 31 did not have a colonoscopy but clinically presented as being active for the disease and therefore correlated with f CAL turbo. Sample 52 also clinically correlated with the f CAL turbo result while samples 30 and 53 had no colonoscopy report and therefore not conclusive, however the quantitative difference was not so significant. Sample number 35 was positive by both methods but the f CAL turbo result was up to 6 times higher and again this patient also clinically correlated with the f CAL turbo result as the patient had active advanced IBD. There were eleven positive on the f CAL turbo and borderline on the Phadia 250. These were: sample 46 which reported a positive colonoscopy that correlated with f CAL turbo, : sample number 27, 36, 50 and 55 also clinically correlated with this FC method, : sample number 21 had a colonoscopy that reported query small bowel disease and therefore clinically correlated with f CAL turbo and : samples 23, 33, 40, 43 and 48 were inconclusive, however overall (with the exception of sample 33) the quantitative difference was not too significant. Sample number 4, which was borderline on the f CAL turbo and negative on the Phadia 250, had a colonoscopy that correlated with the f CAL turbo as the participant had mild active inflammation.

To confirm the validity of this study all of the 60 results compared on both methods were reviewed by the IBD medical team of the hospital. They concluded that the f CAL turbo correlated very well with the participants clinically presentation and stage of IBD. They confirmed that for diagnosis the over-all clinical presentation along with the FC result is considered before referral for colonoscopy. They confirmed the validity of the negatives and the positives. They were not too concerned by the borderline results as those results always warrant a repeat test in 4-6 weeks to monitor the progress. They were very happy with the study findings and very supportive in using f CAL turbo in the future on-site and will adapt to the higher readings of the f CAL turbo assay.

The big variable between all of the FC methods is the extraction step and is a contributing factor to the inter-assay variability. This study has also demonstrated this with CV's of 7.5 % and 9.4% for precision of the extraction step. Most assay providers supply their own commercial extraction devices. The CALEX® cap extraction device used in this study is easy to use and practical as the same tube can be used directly on the analyser. It also has a septum to remove excess faeces and

therefore reduces error. This study also confirmed the stability of the extracts stored at 2-8°C for 5 days and for up to 6 weeks stored at -20°C. For storage of the extracts at -20°C, this study recommends removal of the supernatant extract to fresh tubes. Previous studies report that FC is stable in the faeces sample for up to 6 days when stored at 2-8°C (Van Rheenen *et al*,. 2010; Merna, 2013). However this study found the stability to be up to 3-4 days with an exponential decrease in recovery with the days stored. More work will have to be performed to validate this finding as this study did not have enough reagents to carry this out..

The IBDoc part of this study had 15 participants. It was hoped to recruit more, but probably due to the nature of the specimens, participants declined. This study reports better correlation between the f CAL turbo and the IBDoc® with no statistical significance between the two methods. Again there was a statistical significance between the f CAL turbo and the Phadia 250 , with the f CAL turbo reading higher. Some of the IBD patients at this hospital were set up to use IBDoc® due to the long turn-around times from the referral laboratory. IBDoc® is only suitable to patients that are confident and comfortable to use it and the smart phone app and therefore limited to a small percentage of the patient population. Again the IBD medical team were very happy with this finding which gives more confidence in the IBDoc® test.

This study did not observe any problems with running faecal samples on the routine clinical chemistry analyser (Abbott Architect C8000). The samples are diluted 1:500 on extraction and when centrifuged, the supernatants are clear with no debris. A quality control was run for all the routine chemistries after running the FC samples and no errors were observed.

In conclusion this study has demonstrated the f CAL turbo assay is very adaptable and suited for rapid analysis on the Abbott Architect C8000 Clinical Chemistry Analyser. A result is generated within 12 minutes post sampling. It also demonstrated the inter –assay variability for FC and recommends, users to participate in the UKNEQAS EQA scheme, which allows participants to compare their assay performance to all other users of the same assay. See Table 8 for the results for all participants for the distribution for August 2017 (UK NEQAS,2017) .UKNEQAS in 2012, introduced a Faecal Marker of Inflammation EQA programme to monitor FC assays (Whitehead *et al*,. 2015*).* This was the first step towards standardising FC assays. Patients for serial measurements of FC should be performed by the same assay and site. This study also demonstrated good correlation with the IBDoc® home test. This laboratory is going to introduce this f CAL turbo assay for on- site testing of FC which will have huge benefits to the patient and the medical IBD team as well as a cost saving benefit.

APPROVAL

This study was fully approved by The Clinical Research Ethics Committee of the Cork Teaching Hospitals ,University College Cork, Republic of Ireland, which is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products.

DECLARATION OF INTEREST

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Table 1. Precision of the low control, within and between run

	Within Run µg/g Reagent lot 2005	Between -Run µg/g (over 3 months)	Reagent Lot
	77.7	77.8	2005
	83.3	78.7	2005
	81.3	82.2	2005
	81.3	79.7	2005
	79.7	77.8	2005
	81.3	79.2	2005
	74.1	77.3	2005
	81.8	75.1	3606
	81.8	77.7	3606
	79.2	73.5	3606
Mean	**80.1**	**77.9**	
SD	**2.65**	**2.4**	
%CV	**3.3**	**3.1**	

Uncertainty of Measurement : 3.6µg/g

Table 2. Precision of the high control, within and between run.

	Within Run µg/g Reagent lot 2005	Between -Run µg/g (over 3 months)	Reagent Lot
	251	247	2005
	248	241	2005
	249	254	2005
	251	245	2005
	246	246	2005
	249	248	2005
	251	252	3606
	249	245	3606
	246	246	3606
	249	250	3606
Mean	**249**	**247**	
SD	**1.85**	**3.78**	
%CV	**0.74**	**1.53**	

Uncertainty of Measurement : 4.2µg/g

Table 3. Precision of Extracted Faecal Calprotectin

	Within Run µg/g Reagent lot 2005	Between Run µg/g (over 6 weeks)	Reagent lot number
	741	751	2005
	702	844	2005
	637	767	2005
	730	858	2005
	641	798	2005
	637	681	3606
	639	688	3606
	754	792	3606
	627	682	3606
	722	667	3606
Mean	**683**	**751**	
SD	**51.17**	**70.6**	
% CV	**7.5**	**9.4**	

Uncertainty of Measurement : 87 µg/g

Table 4. Linearity Check

Sample	f CAL turbo Result µg/g	Target µg/g	Recovery (%)
5	961	961	100.0
1	<15	0	-------
3	194	192	101.0
2	50	48	104.2
4	480	480	100.0
6	1915	1922	99.6
5	960	961	99.9
4	481	480	100.2
2	51	48	106.2
6	1909	1922	99.3
3	194	192	101.0
1	<15	0	-------

Figure 1. Scatter plot of Linearity check with linear fit.

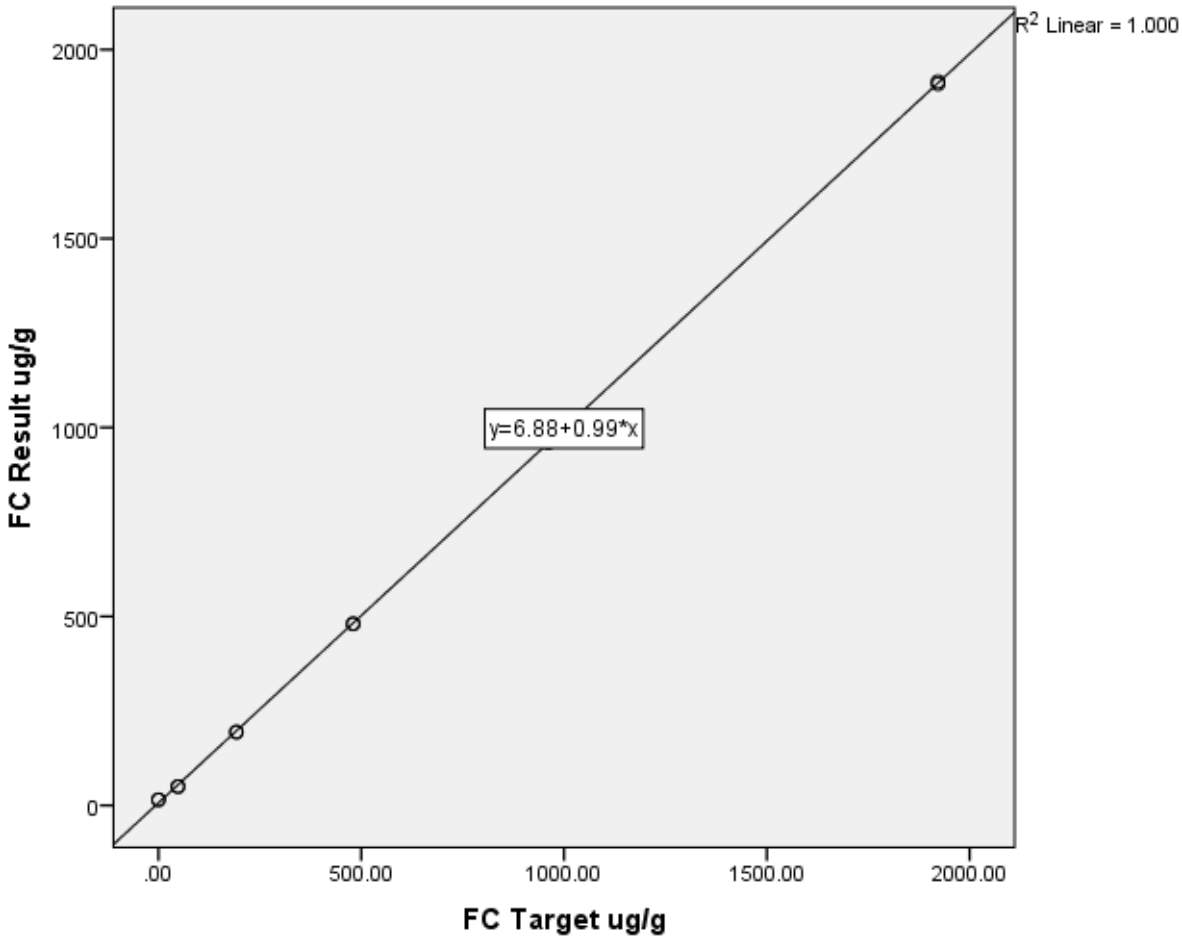

Table 5 Faecal Calprotectin results f CAL Turbo versus Phadia 250

Sample Number	f CAL Turbo µg/g	Phadia 250 µg/g	Interpretation
1	1820	29	Positive-Negative
2	<20	15	Negative
3	122	48	Borderline-Negative
4	188	24	Borderline-Negative
5	<20	<15	Negative
6	41	15	Negative
7	167	35	Borderline-Negative
8	41	45	Negative
9	1703	606	Positive
10	89	70	Borderline
11	3563	>3000	Positive
12	722	332	Positive
13	35	<15	Negative
14	1502	1795	Positive
15	4255	>3000	Positive
16	<20	<15	Negative
17	26	<15	Negative
18	54	35	Borderline-Negative
19	26	20	Negative
20	14183	>3000	Positive
21	238	72	Positive-Borderline
22	282	244	Positive
23	319	145	Positive-Borderline
24	3728	2860	Positive
25	157	54	Borderline
26	20	15	Negative
27	571	120	Positive-Borderline
28	3695	>3000	Positive
29	93	93	Borderline
30	371	44	Positive-Negative
31	519	47	Positive-Negative
32	28	16	Negative
33	543	112	Positive-Borderline
34	168	50	Borderline
35	1879	302	Positive
36	749	51	Positive-Borderline
37	37	17	Negative
38	78	<15	Borderline -Negative
39	<20	<15	Negative
40	362	110	Positive-Borderline
41	34	<15	Negative
42	52	159	Borderline
43	385	143	Positive-Borderline
44	<20	62	Negative-Borderline
45	21	<15	Negative
46	893	59	Positive-Borderline
47	40	<15	Negative
48	388	98	Positive-Borderline
49	<20	<15	Negative

50	554	113	Positive-Borderline
51	19	<15	Negative
52	345	37	Positive-Negative
53	288	43	Positive-Negative
54	166	72	Borderline
55	365	56	Positive-Borderline
56	19	15	Negative
57	510	509	Positive
58	<16	<15	Negative
59	<16	<15	Negative
60	618	341	Positive

The reference range for both Faecal Calprotectin assays is

<50 µg/g : Negative.

50 -200 µg/g : Grey area and warrants monitoring and therefore is suggested to repeat in 3-4 weeks.

>200 µg/g : Positive for active inflammation (disease).

In summary, Twelve: Positive, Twenty: Negative, Six: Borderline and Twenty Two : Discrepancies. The patient charts of the discrepant results to be reviewed to determine which method is the most accurate. Over all the f CAL turbo results are higher.

Figure 2. Scatterplot of Phadia 250 EliA vs f CAL Turbo with linear regression

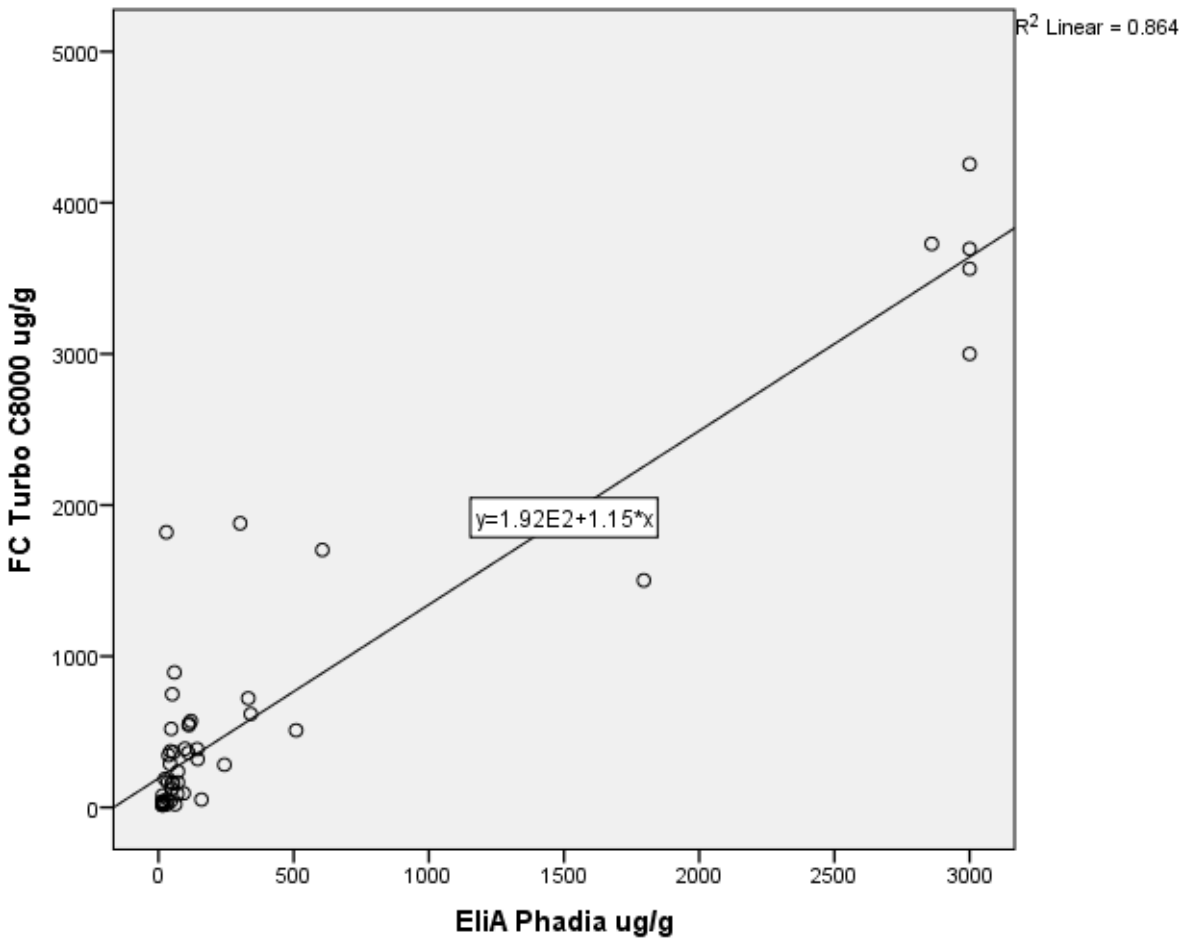

Figure 3. Paired Samples t –Test of Phadia 250 vs Architect C8000 f CAL Turbo

(A) Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Elia ug/g	355.63	60	833.700	107.630
	FC Turbo C8000 ug/g	600.67	60	1031.404	133.154

(B) Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Elia ug/g & FC Turbo C8000 ug/g	60	.930	.000

(C) Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Elia ug/g – FC Turbo C8000 ug/g	-245.033	400.312	51.680	-348.445	-141.622	-4.741	59	.000

Figure 3. Paired samples t –Test of Phadia 250 vs Architect C8000 f CAL Turbo

(A) The paired samples statistics demonstrates the over-all mean of each method with the SD and the standard error of the mean. The f CAL Turbo values are higher than the Phadia.

(B) The paired samples correlation shows the bivariate Pearson correlation coefficient of 0.930.

(C) The paired samples t -Test shows the average difference between the two means is 245 and the difference between the standard deviations is 400. The p value of 0.0 which is <0.05 demonstrates that there is a statistical significance between the two methods and therefore reject the Null Hypothesis.

Table 6. Colonoscopy report and or Clinical Presentation of Discrepant results.

Sample Number	F CAL Turbo ug/g	Phadia ug/g	Colonoscopy report/Clinical Presentation
1	1820	29	Colonoscopy –Active surface Inflammation of Terminal Ileum(Ulcerative Colitis)
3	122	48	Ulcerative Colitis. On Humira. No Colonoscopy
4	188	24	Colonoscopy- Mild active inflammation of Large Bowel
7	167	35	Crohn's Disease- Ileocolitis. No Colonoscopy
18	54	35	For Colonoscopy-? Eosinophilis Enteritis
21	238	72	Colonoscopy – No evidence of Colitis. Severe acute Gastroenteritis & Abdominal cramps. ? Small Bowel disease. MRI Enterography to follow.
23	319	145	Ulcerative Colitis patient. On Infliximab. No Colonoscopy
27	571	120	IBD patient. No colonoscopy. IBDOC 520ug/g same date.
30	371	44	Crohn's Disease. Feels well. No Colonoscopy
31	519	47	Crohn's Disease. Possibly Active, Low Humira levels. No Colonoscopy
33	543	112	Ulcerative Colitis. No Colonoscopy. Feels well at present
35	1879	302	Active advanced IBD. Failed drugs, non –compliant patient
36	749	51	For Colonoscopy. Inflammatory mass in sigmoid colon, combination of IBD and Diverticulosis. Very symptomatic.
38	78	<15	Probably not GI. ? Fatty liver disease. For liver biopsy.
40	362	110	Crohn's Disease, Feels well. No colonoscopy.
43	385	143	Could not locate the chart
44	<20	62	Crohn's Disease, Possible flare up. No colonoscopy.
46	893	59	Colonoscopy-Mild to Moderate active Ileal inflammation
48	388	98	Crohn's Disease, No significant pain. No Colonoscopy,
50	554	113	Severe Crohn's Disease affecting Colon + Perianal area. Resistant to standard Medical Therapy.
52	345	37	Diverticulosis + Hyperplastic Poly. Left sided Abdominal pain. Diarrhoea 5-6 times per day. No extra-articular features of IBD. ? Superimposed IBS.
53	288	43	Ulcerative Colitis. On 6-Mercaptopurine + Infliximab Infusions. No Colonoscopy
55	365	56	Crohn's disease of terminal Ileum diagnosed in 2011. Some central abdominal pain and occasional diarrhoea with bright red blood. No current colonoscopy.

Table 6 summaries the clinical presentation and colonoscopy report if performed on the discrepant results. Most of these participants are known IBD patients. Colonoscopy is only performed on new patients and if the clinician decides the patient warrants further investigation looking at the over- all clinical presentation. Five of the samples were positive on the f CAL Turbo and negative on the Phadia. Five were borderline on the f CAL turbo and negative on the Phadia. One negative on f CAL turbo and borderline on the Phadia. Eleven were positive on the f CAL Turbo and borderline on the Phadia.

Table 7. FC Method comparison of Architect f CAL Turbo versus IBDoc versus Phadia

Sample Number	f CAL Turbo ug/g	IBDoc ug/g	Phadia ug/
1	618	545	341
2	<20	<30	<15
3	1342	947	245
4	1592	728	337
5	166	413	72
6	<20	<30	<15
7	436	570	133
8	337	160	116
9	28	<30	No result
10	119	117	15
11	<16	<30	15
12	571	520	120
13	429	380	42
14	<16	<30	15
15	<16	<30	No result

Out of the 3 methods, the f CAL Turbo results all read higher (except for sample no 5) and the Phadia results all read lower. Only 13 results available on the Phadia 250

Figure 4. Scatter plot of Architect C8000 f CAL Turbo versus IBDoc with linear regression

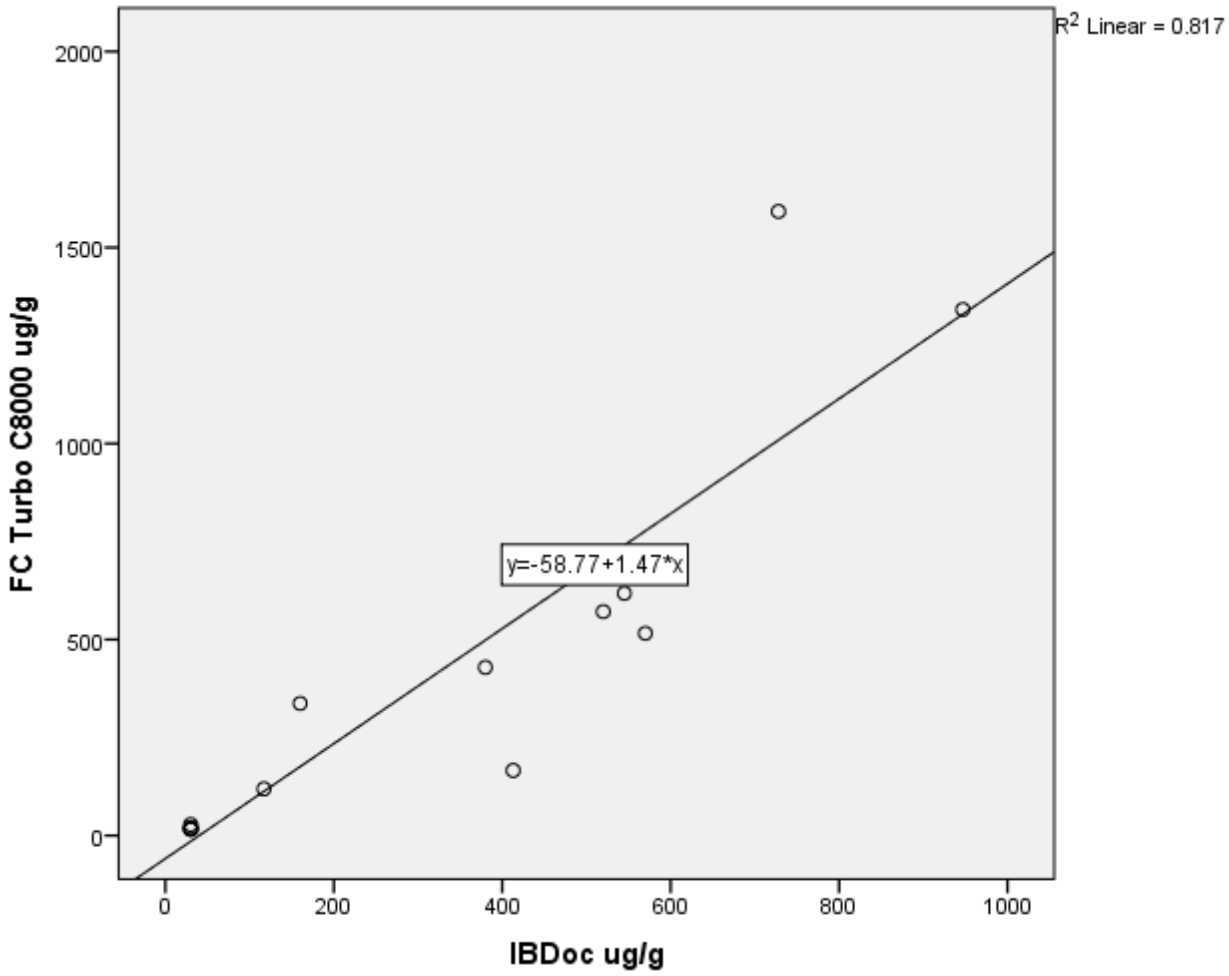

Figure 5. Scatter plot of FC Phadia 250 versus IBDoc with linear regression

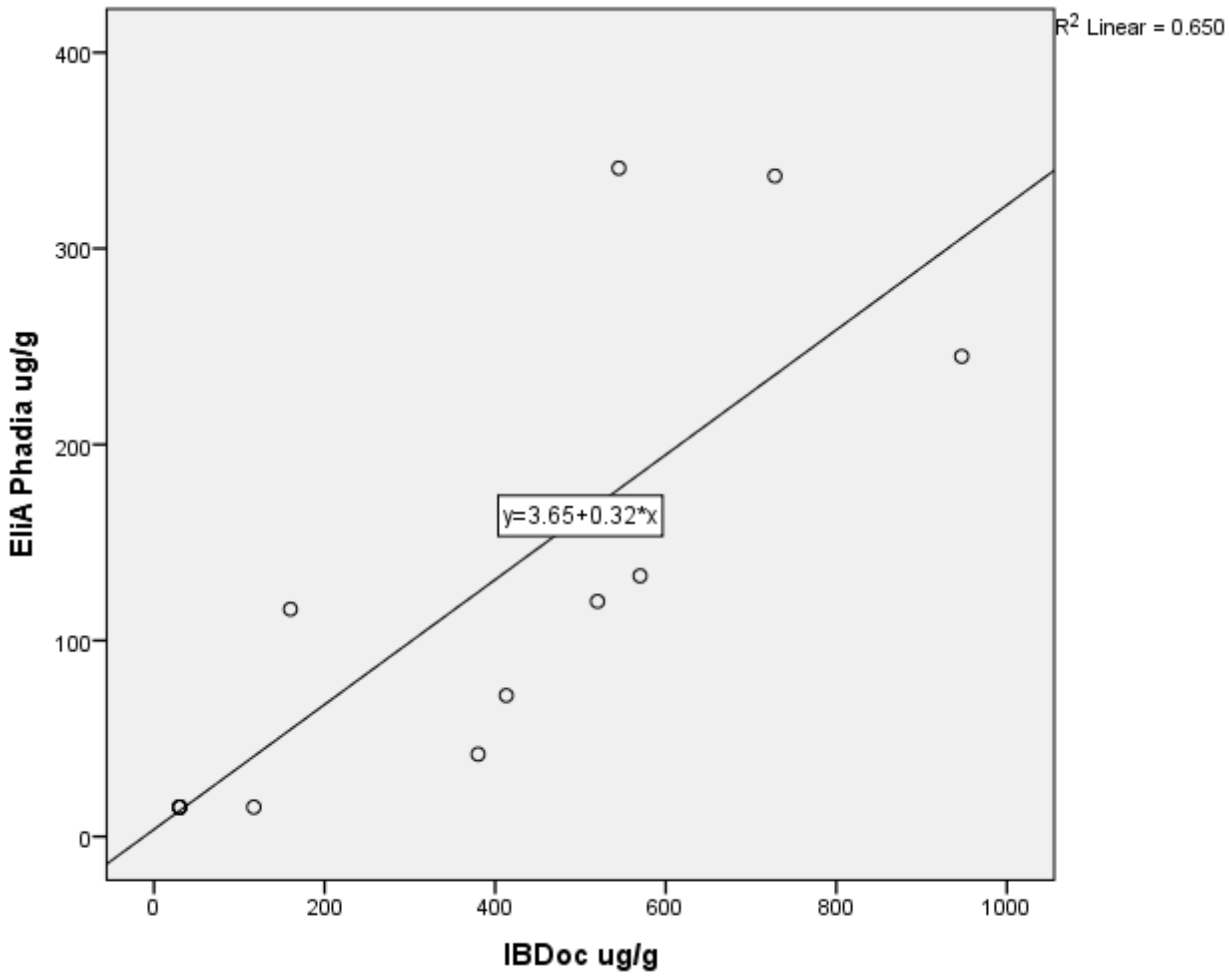

Figure 6. FC Paired samples t -Test f CAL Turbo vs IBDoc vs Phadia EliA.

(A) Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	FC Turbo C8000 ug/g	387.07	15	493.097	127.317
	IBDoc ug/g	304.00	15	303.849	78.453
Pair 2	EliA Phadia ug/g	113.92	13	120.683	33.471
	IBDoc ug/g	346.15	13	305.407	84.705

(B)Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	FC Turbo C8000 ug/g & IBDoc ug/g	15	.904	.000
Pair 2	EliA Phadia ug/g & IBDoc ug/g	13	.806	.001

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	FC Turbo C8000 ug/g - IBDoc ug/g	83.067	254.306	65.662	-57.763	223.897	1.265	14	.226
Pair 2	EliA Phadia ug/g - IBDoc ug/g	-232.231	220.025	61.024	-365.190	-99.271	-3.806	12	.003

Figure 6. FC Paired samples T test f CAL Turbo vs IBDoc vs Phadia EliA.

(A) The paired samples statistics demonstrates the over-all mean of each method with the SD and the standard error of the mean. The f CAL Turbo results are higher and the Phadia are the lowest.
(B) The paired samples correlation shows the bivariate Pearson correlation coefficient of 0.904 for f CAL turbo vs IBDoc® and 0.806 for Phadia vs IBDoc®.
(C) The paired samples t- Test shows the average difference between the two means is 83 for FC Turbo vs IBDoc® and 232 for the Phadia vs IBDoc®. The p =0.226 for f CAL turbo vs IBDoc® demonstrates no statistical significance between the two methods and therefore accept the Null Hypothesis. A p= 0.003 for the Phadia 250 vs IBDoc® demonstrates a statistical significance between the two methods and therefore reject the Null Hypothesis.

Table 8. UK NEQAS Report for Faecal Calprotectin August 2017 (www.birminghamquality.org.uk)

	n	159 A. Mean FC µg/g	n	159 B Mean FC µg/g	n	159 C Mean FC µg/g
All Methods	59	1248	84	88.5	15	8.0
All ELISA	49	1172	69	78.0	14	8.4
Accusay	4	581	5	43.2	2	5.9
Buhlmann	4	1442	22	138	0	NA
Immunodiagnostik	4	870	6	120	1	2.0
Thermo ELiA Calpro 2	17	1715	17	45.8	4	6.3
Thermo ELiA	11	1028	6	22.8	2	44.5
Buhlmann f CAL Turbo	5	1610	7	165	0	NA
This study	1	1828	1	167	1	<16
SD		576		62.0		5.9
% CV		46.1		70.1		73.7

n = number of participants