

Experience of adopting Faecal Immunochemical Testing to meet the NICE 2015 colorectal cancer referral criteria in Oxfordshire, UK.

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Abstract

Objective: To compare the diagnostic performance of guaiac faecal occult blood (gFOB) testing with faecal immunochemical test (FIT) in a low-risk symptomatic primary care population, to provide objective data on which to base local referral guidelines.

Design: Stool samples from routine primary care practice sent for faecal occult blood testing were analysed by a standard gFOB method and the HM-JACKarc FIT between January and March 2016. Symptoms described on the test request were recorded. Patients were followed up over 21 months for evidence of serious gastrointestinal pathology including colorectal adenocarcinoma.

Results: In 238 patients the sensitivity and specificity for colorectal adenocarcinoma detection using gFOB were 85.7% and 65.8% respectively compared to 85.7% and 89.2% for FIT. The Positive Predictive Value (PPV) for gFOB was 7.1% and Negative Predictive Value (NPV) 99.3%. Comparatively, the PPV for FIT was 19.4% and NPV 99.5%. The improved performance of FIT over gFOB was due to a lower false positive rate (10.8 vs 34.2, $p = <0.01$) with no increase in the false negatives rate. For any significant colorectal disease, the PPV for FIT increased to 35.5% with a reduction in NPV to 95.7%.

Conclusion: In this low risk symptomatic patient group the proportion of samples considered positive by FIT was considerably lower than gFOB with the same rate of colorectal adenocarcinoma detection. One in three of those with positive FIT had a significant colorectal disease. This supports NICE recommendation that FIT can be reliably used as a triage test in primary care without overburdening endoscopy resources.

What is already known on this subject?

Faecal Occult Blood testing (FOB) is recommended by NICE as a triage test before further colorectal cancer investigation in patients with low-risk abdominal symptoms presenting to primary care. No study has assessed the comparative diagnostic accuracy of guaiac FOB (gFOB) and Faecal Immunochemical Testing (FIT) in this setting.

What are the new findings?

In a consecutive sample of FOBs submitted by General Practitioners to a large UK laboratory following local implementation of the NICE FOBT criteria, gFOB and FIT had equivalent sensitivities and high negative predictive values for colorectal adenocarcinoma. However, the performance of FIT was far superior due to a significantly reduced false positive rate, resulting in higher positive predictive values and specificity.

How might it impact on clinical practice in the foreseeable future?

Our results show that FIT can be reliably used as a triage test without overburdening endoscopy resources, supporting widespread implementation of the NICE recommendations for its use in low-risk patients in primary care

Introduction

The 2015 National Institute of Health and Care Excellence (NICE) recommended that UK General Practitioners (GPs) use faecal occult blood (FOB) testing to investigate low risk symptomatic patients in primary care with positive results triggering an urgent referral for investigation of colorectal cancer (1). At the time of the NICE guidance many UK laboratories had ceased offering FOB to primary care services: only 54% of GPs had direct access to any FOB test (2). Test demand had steadily reduced prior to this point reflecting changes in practice that arose following the availability of the National Bowel Cancer Screening Programme, concerns over the diagnostic accuracy, and well documented analytical performance issues with the standard FOB method, which detects haemoglobin through its ability to oxidise the dye guaiac (gFOB) (3).

The 2015 NICE NG12 guidance recommended GPs used FOB to decide whether the following patients should be referred for urgent investigation for colorectal cancer: aged ≥ 50 years with abdominal pain or weight loss, < 60 years with a change in bowel habit or iron deficiency anaemia, or aged ≥ 60 years with any type of anaemia. The guidance generated significant debate (4, 5), which included concerns around FOB delaying cancer diagnosis due to false negatives and its potential to increase demand on already stretched endoscopy service due to false positives, a common problem associated with the gFOB method (3). Although the 2015 guidance recommended FOB, the method of FOB detection had not been defined. This was subsequently clarified in July 2017 NICE DG30 and the more specific immunoassay based method for human haemoglobin, the Faecal Immunochemical Test (FIT), is now recommended over gFOB “to guide referral for suspected colorectal cancer in people without rectal bleeding who have unexplained symptoms but do not meet the criteria for a suspected cancer pathway” (6).

Increasing evidence demonstrates improved performance of FIT compared to gFOB in colorectal cancer screening programmes (7-9) but there is limited evidence to demonstrate the benefits of FIT in symptomatic primary care patients. NICE expressed concerns about the applicability of all ten of the studies used to underpin this updated recommendation: none reported data on people with low risk symptoms of colorectal cancer (6) and only one study was conducted in primary care (10). A threshold of 10 $\mu\text{g/g}$ faeces was recommended to trigger referral but the literature reported a range of thresholds. A recent report combining three studies of FIT diagnostic accuracy supported its use in primary care but included patient populations referred for colonoscopy to assess referral guidance and not patients with low risk symptoms tested in the primary care setting in line with the NICE guidance (11).

In early 2016, when considering how to react to the initial NICE guidance, and to inform the redesign of the diagnostic pathways for colorectal cancer investigation in Oxfordshire, we analysed samples referred for FOB testing by both a conventional gFOB and by one of the new automated FIT methods and compared results obtained with subsequent diagnostic outcomes. We present data from this assessment together with the impact of changing FOB technique on demand test positive rates and outcomes.

Methods

The Oxford University Hospitals NHS Foundation Trust (OUHT) Clinical Biochemistry laboratory serves all primary care clinicians in the county of Oxfordshire with a population of approximately 660,000. The department is one of the largest in the United Kingdom, performing over 8 million tests a year, and has an 'M'-based laboratory information management system. All FOB testing is undertaken in a single laboratory at the John Radcliffe Hospital.

The patients included in the evaluation were consecutive samples sent to the laboratory from primary care in the period January to March 2016 for investigation of FOB. Leading up to this period, the change in NICE guidance and the indications for FOB testing were communicated to GPs in Oxfordshire by email and newsletter from the Oxfordshire Clinical Commissioning Group. To accurately reflect the clinical requesting patterns and standard laboratory practice no samples were excluded. The assessment was registered as a service evaluation on our hospitals Datix register (CSS-BIO-3 4730).

Samples were collected into standard stool pots by patients in primary care and referred in for a standard FOB testing using a conventional in house method, the gFOB, utilising hydrogen peroxide and guaiac solution (12). Results were reported as either positive or negative dependent on blue/green colour development detected visually with the method quality monitored through participation in an external quality assessment programme. Only the gFOB result was reported to clinicians, consistent with historical practice and this was used to guide subsequent patient management.

For the evaluation period samples were additionally analysed for FIT using the HM-JACKarc analyser (Kyowa Medex Co., Ltd., Tokyo, Japan) a method that has been independently evaluated with respect to analytical performance (13) and is now recommended in the context of use for samples from primary care (6). The method had a calibration range of 7 to 450 $\mu\text{g Hb/g faeces}$ and reproducibility across this range was determined to be between 2.2% and 7.3% when expressed as a percentage coefficient of variation. Sample preparation prior to analysis on the FIT instrument utilized the Extel Hemo-Auto MC device, a process which introduced additional analytical variation. Assessment of total variability, including the sampling stage, was determined to be 14.9% at 16 $\mu\text{g/g}$ and 30.7% at 2 $\mu\text{g/g}$. External quality assurance samples were analysed to confirm test performance. The selection of the Hb concentration considered positive was made before the NICE recommendation to use 10 $\mu\text{g/g}$ and was based on the methods lowest calibrant value of 7 $\mu\text{g/g}$.

In selecting the approach to faecal sample handling we balanced the requirements to minimize sampling errors which may give rise to false positives if undertaken by patients with stability concerns (14) and we have highlighted this issue and balancing these risks in our contribution to the NICE FIT adoption resource (6). Where more than one sample result was available for any individual patient, any positive result within those samples tested was considered a positive outcome on the basis that a single positive would trigger referral. Where multiple samples on a single patient were collected these were on sequential days which precluded assessment of changes in FOB test results with disease progression.

To confirm the presence or absence of disease, OUHT clinical and diagnostic databases were searched for evidence of pathology for between 21 and 23 months following the FOB testing for all patients. Histology, endoscopy and CT colonoscopy reports were retrieved by searching by both hospital and NHS number. Patients were classified individually then by discussion between members of the research team (BS, BN, TJ, JE) as having adenocarcinoma; having significant colorectal disease (adenocarcinoma, high risk adenoma polyps larger than 10mm and inflammatory bowel disease and one patient with neuroendocrine tumour),

upper GI pathology; no significant pathology identified on endoscopy or CT colonoscopy; or no further follow up investigation for 21 to 23 months. Patients who had no further investigation were categorised as negative for serious pathology as any serious pathology would be expected to have presented to secondary care within this time period.

Diagnostic accuracy of gFOB and FIT was summarised using sensitivity, specificity, negative and positive predictive values and exact 95% confidence intervals. Total test demand and numbers of positive results for FOB testing were assessed through reported results extracted from the LIMS results reporting system. Assessment of the change of positive rates was done using an interrupted time series analysis. We used a Poisson regression, corrected for overdispersion to model rate of positive tests in both the FOB era (1st Jan 2016 to 31st December 2016 and the FIT era (1st Feb 2017 to 31st Oct 2017). The model was specified so that we could test a step change in the number of positives as well as a longer-term change in trend. A sensitivity analysis was preformed to remove patients with rectal bleeding as these patients have a potentially higher risk of colorectal adenocarcinoma and are excluded from the NICE guideline criteria.

Results

FOB testing by both FIT and gFOB was undertaken on 332 samples from 238 patients, median age 58 years, range 19 to 93 years, of whom 103 (43%) were male and 135 (57%) were female.

Clinical details.

The majority of FOB requests had one or more clinical details (table 1) confirming lower abdominal symptoms (1,2): change in bowel habit (n=59), anaemia (62), abdominal pain/discomfort (45), blood in stools (23), rectal bleeding (9), weight loss (4) symptoms. It was not possible to categorise the remaining requests (n=46) due to absent or uninterpretable clinical information. Significant colorectal disease was detected in 20 patients, seven of which had adenocarcinoma (full details: Appendix 1). Subsequent investigations including gastroscopy only (n=17), colonoscopy only (30), gastroscopy and colonoscopy (13), and CT colonography (32).

gFOB/FIT concordance.

Figure 1 & Appendix 2 present results by diagnostic group by FIT concentration and by FOB positivity. The analytical agreement between gFOB and FIT was 77%, 202 tests being positive and 36 negative by both methods. In 65 samples the gFOB was positive and the FIT negative compared to 36 samples that were gFOB negative and FIT positive. The overall positive rate in this direct comparison was 30.4% (n=101) for gFOB compared to 14.2% for FIT (n=47). 74 patients had more than one sample collected (from 2 up to 5 samples). For FOB there was complete concordance in all samples in 73% of patients compared to 85% for FIT.

Diagnostic accuracy.

Table 2 presents the diagnostic accuracy of FIT and gFOB for adenocarcinoma and significant colorectal disease. Figure 2 presents receiver operator characteristics of the relationship between FIT threshold and the impact on diagnostic accuracy for both adenocarcinoma and significant colorectal disease.

At the haemoglobin concentration of 7 µg/g used for defining a FIT positive result a sensitivity and specificity for detection of colorectal adenocarcinoma was 85.7% and 89.2% compared to 85.7% and 65.8% for gFOB. The Positive Predictive Value (PPV) for FIT was 19.4%, and Negative Predictive Value (NPV) 99.5%. Comparatively PPV for gFOB was 7.1% and NPV 99.3%. None of the patients with upper GI pathology (including one cancer) had a FIT above our threshold of 7 µg/g.

Changing the threshold for FIT to 10µg/g had little effect on the diagnostic characteristics of FIT: sensitivity 85.7%; specificity 90.5%; PPV 21.4% and NPV of 99.5%. Increasing the threshold further to a commonly used screening threshold (50µg/g) increased specificity and PPV at the expense of sensitivity but with minimal impact on the NPV.

The diagnostic performance to detect any significant colorectal disease for gFOB were 65.0% sensitivity and 67.0% specificity compared to 55.0% and 90.8% for FIT. The PPV for gFOB was 15.3% and NPV 95.4% compared to a PPV of 35.5% and NPV of 95.7% for FIT. The AUC for FIT reduced from 89.5 (76.0-100.0) for colorectal adenocarcinoma to 79.8 (68.2-91.4) for any significant colorectal disease.

A sensitivity analysis removing the nine patients that had been referred with rectal bleeding resulted in no change in the sensitivity or specificity of gFOB or FIT for colorectal adenocarcinoma and a marginal decrease in the sensitivity and specificity of gFOB and FIT for the detection of significant colorectal disease (Appendix 3).

Changes in workload

Workload increased following the 2015 NICE guidance and the rate of positive results fell both in a slope and a step wise manner when the FIT replaced gFOB (figure 3). Poisson regression modelling allowing for overdispersion indicated a significant step change in the number of positive tests after the introduction of FIT (rate ratio (RR) = 0.53, $p=0.004$) and a borderline significant change in the long-term trend (RR = 0.95, $p=0.04992$). Furthermore, the false positive rate of gFOB (34.2) was three times that of FIT (10.8, $p<0.01$) with no increase in the false negative rate.

Discussion

The NICE guidance DG30 (6) highlighted the need for further research into the use of FIT including auditing the use of the FIT in primary care. It has also been noted that the current evidence base for use of diagnostic tests in primary care for diagnosis of colorectal cancer is lacking (15). Our early experience with adopting FIT shows equivalent detection rates and high NPV for colorectal adenocarcinoma for gFOB and FIT in a population of low risk symptomatic primary care patients. However, gFOB had many more false positive results: one in five patients with a positive FIT had cancer detected compared to one in fourteen positive gFOBs, and one in three patients with positive FIT had serious colorectal disease detected compared to one in six positive gFOBs.

At the end of our initial evaluation we selected a cut off for 7 µg/g as this related to the lowest calibrant value for the FIT method used. Subsequently NICE has recommended a value of 10 µg/g (6). Our analysis suggests the difference in threshold had little impact on overall test performance, but this should be explored in the future as larger data sets become available as more laboratories start using this methodology.

The patient cohort included several patients with an upper GI pathology including one patient with cancer and none of these patients had FIT above our cut off. This is consistent with a recent analysis from large Dutch FIT cohort that supports not performing upper GI investigations in patients with a positive FIT test (16) as blood from an upper GI lesion does not remain intact through the GI tract and loses FIT assay immunoreactivity.

One patient in our group had a neuroendocrine tumour (NET) and this did not produce a positive FIT result. There is limited literature on tests of occult blood in the context of NET but a recent report has suggested that their detection is possible through organised colorectal cancer screening (17) however we have limited evidence to confirm the characteristics of bleeding in these tumours..

Limitations

There are several potential limitations with the data presented. Firstly, we have assumed that the samples referred from primary care accurately reflect the low risk symptomatic population. The clinical details that GPs entered onto the FOB request suggest this is the case for most patients tested. As single symptoms, only rectal bleeding and anaemia would qualify for urgent investigation for colorectal cancer, but it can be assumed that GPs assessed the cases tested with FOB to be lower risk or they did not qualify for fast-track referral due to age. It is also likely that this is the population that the NICE guidance is aimed at as it accurately reflects the FOB requests received from routine primary care practice.

Secondly, the majority of patients did not have a gold standard investigation to rule-in or rule-out serious colorectal disease as this was not a controlled study, but a retrospective assessment of diagnostic performance using routinely collected clinical data. However, all patients have been followed up for evidence of subsequent pathology in hospital clinical, laboratory, radiology, endoscopy and pathology databases for between 21 and 23 months after initial testing - a period that would reasonably allow significant pathology to be identified. The clearly defined geographical catchment area and the single central laboratory used in this study minimises the likelihood that serious disease was diagnosed during the study period and not captured.

Finally, there was a delay between faecal specimen collection and sampling into the devices provided by the supplier to preserve haemoglobin immunoreactivity. One report using exogenous haemoglobin added to faecal material showed that significant sample degradation can occur on storage (18), however, endogenous haemoglobin is more heterogeneous in this respect and most samples retain measurable quantities (14), above the 10 µg/g threshold, even 7 days after collection compared to using the suppliers sampling device. It has also been noted (14) that when patients use the sampling device it can lead to false positive results, presumably due to poor sampling technique. This is consistent with our observation that even when undertaken by experienced laboratory staff, use of the sampling device is the major factor contributing to pre-analytical variability. We feel our current approach of advising laboratory delivery on the day of specimen collection mitigates degradation risk and should avoid the potential of false positives associated with patient collection. The current preliminary evaluation aimed to take a pragmatic approach in this respect but further work on sampling technique and identification of the optimum procedure using the collection device should be undertaken in future larger studies.

Comparison with existing literature

Several studies have reported the diagnostic accuracy of FIT in symptomatic primary care patients. Three studies have been reported by the same group from Dundee, Scotland, who combined these studies data resulting in a sensitivity for colorectal cancer at 93.3% and a NPV of 99.7% (10). A significant difference in our population was that it had not already been referred to secondary care for investigation and all patients were tested using a single FIT technique. As we have observed similar specificity, 90.5%, and NPV, 99.5%, it would endorse the use of FIT in this context, although gFOB performed similarly. Where FIT outperforms gFOB in our study is in sensitivity and PPV which appears to be due to a reduced number of false positive results. For the detection of any significant colorectal disease we observed very similar NPV at 95.7% compared to the Dundee group with an overall NPV of 96.0%. The sensitivity in our study was lower at 55.0% compared to 63.2%, possibly because the patients included in our study of routine practice may be a lower risk symptomatic group.

In a Dutch cross-sectional diagnostic accuracy study (19), 810 patients referred for colonoscopy for suspicion of significant colorectal disease were investigated with point-of-care FIT using a haemoglobin threshold of >6µg/g. The sensitivity for significant colorectal disease (colorectal cancer, inflammatory bowel disease, diverticulitis, or advanced adenoma > 1 cm) was 67%; specificity 84%; PPV 47%; and NPV 92%. In a further prospective English study of 430 non-consecutive patients (20) referred for urgent lower gastrointestinal investigation, the sensitivity for colorectal cancer was 84% and specificity 93%, similar to our data at 85.7% and 89.2%. Our data would suggest gFOB to be inferior in most measures of diagnostic performance to FIT. The difference in performance of FIT found in our study is most likely to be due to our study including a lower risk spectrum of unselected symptomatic primary care patients - the population that NICE intended GPs to use FOB testing.

Implications

Adoption of the NICE guidance NG12 (1) for colorectal with respect to use of FOB in the patient pathway for colorectal cancer has been slow, probably due to the uncertainty of test methodology. The July 2017 (6) guidance supporting the use of FIT methodologies to detect blood clarified this uncertainty but there is limited data to show what impact the switch will have in a routine clinical setting.

It is possible that the initial reaction to NG12 (4, 5) may have influenced clinician behaviour. However, following the guidance we have seen a rise in laboratory demand for FOB testing and the switch to FIT, with its lower rate of positive result, may attenuate the impact this workload growth may have on referral numbers for endoscopy. Within our service the current laboratory cost of the FIT method is around £11 per sample, which is more expensive than gFOB (costing in the region of £7). This is low compared to the alternative options including colonoscopy, which costs £458 at 2017 NHS national tariffs, and enables fast tracking of those at greater risk of colorectal cancer. At a stable rate of 300 FOB samples per month, and assuming all positive FOB results progress to colonoscopy, compared to gFOB a FIT strategy would save approximately £13,380 per month (£160,560 per year). A formal economic evaluation is underway at our institution.

Historically, more than one sample was recommended for FOB testing (3) to overcome false positives associated with ingestion of a range of peroxidase containing dietary material. For FIT this consideration is not relevant since it is a more specific analytical test based on immunoassay for human haemoglobin. Our observations that when more than one sample is taken the degree of concordance between samples is higher (73% for FOB and 85% for FIT) suggests greater test consistency, but clearly there is still some variability in the presence of blood in stools and the optimum number of samples for colorectal cancer detection also needs further consideration.

Areas of uncertainty where future research is warranted include: whether the utility of FIT can be improved in combination with symptoms associated with colorectal cancer (21); how FIT can be used to influence the progression on to imaging techniques (22); and how GP initiated FIT testing may complement bowel cancer screening programmes as there is evidence that longer intervals between symptomatic presentation to primary care testing can lead to more advanced stage cancer (23).

Conclusion

To date, published studies have focused on those patients already referred for follow up of lower gastrointestinal symptoms. Our data, albeit a small comparative study, provides confidence that the diagnostic accuracy of FIT in routine primary care practice translates successfully in the context of the lower risk population considered in NG12. We conclude that FIT offers a significant and safe methodological transition with improved diagnostic ability to reduce pressure on urgent referral pathways by identifying patients who do not require further investigation for colorectal adenocarcinoma, thereby controlling colonoscopy demand and reducing costs.

Contributorship Statement

BDN, TJ, DG, JEE and BS conceived the study. TJ, BS, MP and SJ collected the data. BDN, JO, BS and TJ analysed the data. TJ and BDN prepared the first draft of the manuscript. All authors provided critical revisions to the manuscript.

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Competing Interests

There are no competing interests to declare.

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Figure legends

Figure 1. FIT and gFOB results of patients by outcome category

Figure 2: Receiver operating characteristic (ROC) curves of FIT for the detection of adenocarcinoma (solid line) and significant colorectal disease (dashed line). Black dots represent a single estimated true positive/false positive rate of gFOB for adenocarcinoma and significant colorectal disease. Cross-hatches correspond to the true positive /false positive rate estimates for thresholds of (7,10,20 and 50 µg/g). AUC = area under the curve.

Figure 3: Changes in workload and positivity rates before and after the transition to FIT testing. The total number of tests (top panel) and the number of tests that were positive (bottom panel) for the period in which gFOB was used and the period in which FIT replaced gFOB. Lines in bottom panel are fitted trend lines estimated by Poisson regression. Shaded area represents the month in which the change from gFOB to FIT occurred.

Appendix 1. FIT results by FOB result and outcome category

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Table 1: Clinical details of patients tested.

*38 patients had >1 clinical symptom noted

Clinical Details		Number of male:female patients	Median Age (Range)	gFOB positive	FIT positive
Symptoms/Indications*					
Rectal bleeding		8:1	44 (33 to 78)	3	1
Change in bowel habit		25:34	58 (19 to 88)	21	2
Weight loss		2:2	46.5 (38 to 83)	0	0
Anaemia		22:40	64 (38 to 93)	22	11
Abdominal pain/discomfort		15:30	56 (25 to 80)	6	2
Blood in stools		14:9	56 (24 to 91)	11	6
Family history		5:4	54 (35 to 76)	1	1
Absent or unclear symptoms recorded		19:27	60 (30 to 90)	23	9
Diagnosis					
Significant colorectal disease	Adenocarcinoma	2:5	80 (53 to 86)	6	6
	Other significant colorectal disease (Polyp >10mm or inflammatory disease)	8:5	56 (26 to 86)	7	5
Non-significant colorectal disease (Polyp <10mm/Diverticulitis)		14:11	63 (41 to 91)	12	72
Upper GI	Upper GI pathology	3:7	59 (46 to 85)	5	0
	Upper GI malignancy	1:0	61	1	0
No significant pathology		3:17	58 (30 to 86)	14	1
No further investigation		72:90	57 (19 to 93)	34	17
Total		103:135	58 (19 to 93)	85	31

		Sensitivity	Specificity	PPV	NPV
Adenocarcinoma	FOB	85.7 (42.1 to 99.6)	65.8 (59.3 to 71.9)	7.1 (2.6 to 14.7)	99.3 (96.4 to 100.0)
	FIT at 7µg/g	85.7 (42.1 to 99.6)	89.2 (84.4 to 92.9)	19.4 (7.5 to 37.5)	99.5 (97.3 to 100.0)
	FIT at 10µg/g	85.7 (42.1 to 99.6)	90.5 (85.9 to 93.9)	21.4 (8.3 to 41.0)	99.5 (97.4 to 100.0)
	FIT at 20µg/g	71.4 (29.0 to 96.3)	92.6 (88.5 to 95.7)	22.7 (7.8 to 45.4)	99.1 (96.7 to 99.9)
	FIT at 50µg/g	57.1 (18.4 to 90.1)	95.7 (92.2 to 97.9)	28.6 (8.4 to 58.1)	98.7 (96.1 to 99.7)
Significant colorectal disease	FOB	65.0 (40.8 to 84.6)	67.0 (60.3 to 73.2)	15.3 (8.4 to 24.7)	95.4 (90.8 to 98.1)
	FIT at 7µg/g	55.0 (31.5 to 76.9)	90.8 (86.2 to 94.3)	35.5 (19.2 to 54.6)	95.7 (91.9 to 98.0)
	FIT at 10 µg/g	50.0 (27.2 to 72.8)	91.7 (87.3 to 95.0)	35.7 (18.6 to 55.9)	95.2 (91.4 to 97.7)
	FIT at 20µg/g	45.0 (23.1 to 68.5)	94.0 (90.0 to 96.8)	40.9 (20.7 to 63.6)	94.9 (91.1 to 97.4)
	FIT at 50µg/g	40.0 (19.1 to 63.9)	97.2 (94.1 to 99.0)	57.1 (28.9 to 82.3)	94.6 (90.8 to 97.2)

Table 2. Comparison of diagnostic accuracy of gFOB and FIT at different concentration thresholds for detection of adenocarcinoma and significant colorectal disease (adenocarcinoma; high risk adenoma; inflammatory bowel disease)

Appendix 2 – Detailed breakdown of adenocarcinoma and serious colorectal disease cases.

Age	Gender	Max FIT (µg/g)	Symptoms	Conclusion
83	Female	450	None reported	Adenocarcinoma
55	Female	450	Iron deficiency anaemia	Lower GI: Inflammatory disease
53	Female	450	Anaemia	Adenocarcinoma
52	Male	389.5	Rectal bleeding	Lower GI: Large polyp (>10 mm)
56	Male	213.9	Diarrhoea with blood in stool	Lower GI: Inflammatory disease
85	Male	210.1	Iron deficiency anaemia	Adenocarcinoma
46	Male	119.1	Melena	Lower GI: Inflammatory disease
80	Female	63.9	Change in bowel habit	Adenocarcinoma
83	Female	38	Anaemia	Adenocarcinoma
55	Female	14.8	Change of bowel habit	Adenocarcinoma
51	Female	8.7	Iron deficiency anaemia	Lower GI: Large polyp (>10 mm)
60	Male	6.2	Anaemia	Lower GI: Inflammatory disease
50	Female	1.9	Abdominal pain	Lower GI: Large polyp (>10 mm)
80	Male	1.7	Anaemia	Lower GI: Inflammatory disease
74	Female	1.3	New onset constipation	Lower GI: Large polyp (>10 mm)
86	Male	1.2	Change in bowel habit	Lower GI: Inflammatory disease
80	Male	1	Change in bowel habit	Adenocarcinoma
77	Male	1	Loose stools	Neural endocrine tumour
45	Female	0.5	Loose stools and abdominal pains	Lower GI: Large polyp (>10 mm)
26	Male	0.5	Loose stools	Lower GI: Large polyp (>10 mm)

Appendix 3. Comparison of diagnostic accuracy of gFOB and FIT at different concentration thresholds for detection of adenocarcinoma and significant colorectal disease (adenocarcinoma; high risk adenoma; inflammatory bowel disease) excluding nine patients with rectal bleeding.

		Sensitivity	Specificity	PPV	NPV
Adenocarcinoma	FOB	85.7 (42.1 to 99.6)	65.8 (59.1 to 72.0)	7.3 (2.7 to 15.2)	99.3 (96.3 to 100.0)
	FIT at 7µg/g	85.7 (42.1 to 99.6)	89.2 (84.3 to 92.9)	20.0 (7.7 to 38.6)	99.5 (97.2 to 100.0)
	FIT at 10µg/g	85.7 (42.1 to 99.6)	90.5 (85.9 to 94.0)	22.2 (8.6 to 42.3)	99.5 (97.3 to 100.0)
	FIT at 20µg/g	71.4 (29.0 to 96.3)	92.8 (88.6 to 95.8)	23.8 (8.2 to 47.2)	99.0 (96.6 to 99.9)
	FIT at 50µg/g	57.1 (18.4 to 90.1)	95.9 (92.4 to 98.1)	30.8 (9.1 to 61.4)	98.6 (96.0 to 99.7)
Significant colorectal disease	FOB	63.2 (38.4 to 83.7)	66.7 (59.9 to 73.0)	14.6 (7.8 to 24.2)	95.2 (90.4 to 98.1)
	FIT at 7µg/g	52.6 (28.9 to 75.6)	90.5 (85.7 to 94.1)	33.3 (17.3 to 52.8)	95.5 (91.6 to 97.9)
	FIT at 10 µg/g	47.4 (24.4 to 71.1)	91.4 (86.8 to 94.8)	33.3 (16.5 to 54.0)	95.0 (91.1 to 97.6)
	FIT at 20µg/g	42.1 (20.3 to 66.5)	93.8 (89.6 to 96.7)	38.1 (18.1 to 61.6)	94.7 (90.7 to 97.3)
	FIT at 50µg/g	36.8 (16.3 to 61.6)	97.1 (93.9 to 98.9)	53.8 (25.1 to 80.8)	94.4 (90.5 to 97.1)