Choice of faecal immunochemical test matters: Comparison of OC-Sensor and HM-JACKarc, in the assessment of patients at high risk of colorectal cancer.

Short title: Comparison of 2 FIT systems in a clinical pathway

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Conflicts of interest: none

Word count: 3253

Tables: 4

Figures: 1

References 31

Supplementary files 1 (5 figures, 3 tables)

Keywords: FIT; faecal immunochemical test; symptomatic; colorectal cancer; bowel cancer; faecal haemoglobin; risk stratification; diagnostic accuracy

List of abbreviations:

CV: coefficient of variation

Hb: haemoglobin
f-Hb: faecal haemoglobin concentration
FIT: faecal immunochemical test
SD: standard deviation
HM-J: HM-JACKarc
OC-S: OC-Sensor
LoD: Limit of Detection
LoQ: Limit of Quantification

Acknowledgements:

We thank the FIT system manufacturers and suppliers (OC-Sensor: Eiken Chemical Co., Ltd, Tokyo, Japan and Mast Diagnostics Division, Bootle, UK; and HM-JACKarc: Hitachi Chemical Diagnostics Systems Co., Ltd,, Tokyo, Japan and Alpha Laboratories Ltd, Eastleigh, UK) for supplying some of the consumables and the analysers used in this study.

Abstract

Objectives:

Currently NICE recommends the use of faecal immunochemical test (FIT) at faecal haemoglobin concentrations (f-Hb) of 10 µg Hb/g faeces to stratify for colorectal cancer (CRC) risk in symptomatic populations. This f-Hb cut-off is advised across all analysers, despite the fact that a direct comparison of analyser performance, in a clinical setting, has not been performed.

Methods:

Two specimen collection devices (OC-Sensor, OC-S; HM-JACKarc, HM-J) were sent to 914 consecutive individuals referred for follow up due to their increased risk of CRC. Agreement of f-Hb around cut-offs of 4, 10 and 150 µg Hb/g faeces and CRC detection rates were assessed. Two OC-S devices were sent to a further 114 individuals, for within test comparisons.

Results:

732 (80.1%) individuals correctly completed and returned two different FIT devices, with 38 (5.2%) CRCs detected. Median f-Hb for individuals diagnosed with and without CRC were 258.5 and 1.8 µg Hb/g faeces for OC-S and 318.1 and 1.0 µg Hb/g faeces for HM-J respectively.

Correlation of f-Hb results between OC-S/HM-J over the full range was rho=0.74, $p<0.001$. Using a f-Hb of 4 µg Hb/g faeces for both tests found an agreement of 88.1%, at 10 µg Hb/g faeces 91.7% and at 150 µg Hb/g faeces 96.3%.

114 individuals completed and returned two OC-S devices; correlation across the full range was rho=0.98, $p<0.001$.

Conclusion:

We found large variations in f-Hb when different FIT devices were used, but a smaller variation when the same FIT device was used. Our data suggest that analyser-specific f-Hb cut-offs are applied with regard to clinical decision making, especially at lower f-Hb.

[Words 262/250, NB additional words needed in order to address reviewers comments]

Introduction

Developing and refining the performance of diagnostics tests is crucial in improving both the efficiency of clinical care pathways and the patient experience.

The use of faecal immunochemical testing (FIT) for detecting occult blood in faeces is currently recommended for both symptomatic testing and asymptomatic colorectal cancer (CRC) screening, as its superior sensitivity and specificity compared to previous methodologies of detecting occult blood is increasingly evidenced[1–5].

There are a number of manufacturers who produce FIT assay kits to measure faecal haemoglobin concentration (f-Hb), and these all have unique patented systems. Differences in their ability to produce the same result, even when calibrators and controls are employed, are not surprising as there is no primary reference material for FIT and a lack of standardization[6]. Each system has its own collection device, with a different sample picker, a different stabilisation buffer within the sampling device, and different analytical methods.

Only a few studies have been carried out that directly compare analyser performance in healthcare settings. These have been performed within population screening programmes in Europe, and the importance of comparing quantitative FIT tests before selecting one for population screening has been highlighted[7]. These studies, that compared results from HM-J vs OC-S in Umbria[5,8] and OC-S vs FOBgold in the Netherlands[9], showed clear differences in cancer detection rates.

However, there have been no such studies performed in a symptomatic primary or secondary care setting. It is surprising therefore that a single cut-off of 10 µg Hb/g faeces, across all analyser manufacturers was suggested by NICE in the UK, for referral for CRC from UK primary care[10].

Previous publications from our group identified the benefits of using FIT to risk stratify patients at high-risk of CRC within the rapid access CRC 2-week-wait (2WW) pathway[4,11]. The 2WW care pathways are used in England to facilitate rapid

assessment of patients, referred from primary to secondary care, who are deemed at high-risk of a cancer diagnosis. In CRC the 2WW pathway is designed to facilitate expedited access to diagnostic services (typically colonoscopy) and treatment for patients with CRC symptoms. Given the capacity issues faced by colonoscopy services and the potential unnecessary requirement for invasive investigation for many patients the use of FIT to risk stratify is highly beneficial. However, pathways of this type are being recommended and implemented across the country with little understanding of the optimal cut-offs for referral or the potential differences in referral patterns the use of different assays may create. Recently this has been expedited into practice with the recommendation to use FIT for prioritisation during the Covid-19 pandemic[12,13].

Utilising the "Getting FIT" study[4] we aimed to determine a) the diagnostic yield for CRC of pre-specified cut-offs for two commonly used FIT assays; and b) the inter-assay f-Hb variability.

Material and Methods

Participants

During the twelve month period from September 2016, the use of FIT within the Nottingham University Hospitals Trust 2WW pathway for symptomatic CRC was piloted[14] and the pathway described in detail previously[4].

In brief, the first 1000 patients referred through the CRC 2WW pathway were eligible for the study and identified to the Eastern Hub of the NHS-England's Bowel Cancer Screening Programme (BSCP). In addition to standard clinical care patients were sent two FIT sampling devices through the postal service, these were either from two different manufacturers or two from the same manufacturer (OC-S only). Sampling kits included two FIT packs (with instructions for use) and information about the purpose of the study. Completed FIT kits were returned by pre-paid post.

FIT assays

Each test kit posted to participants included two manufacturer prepared collection devices containing the specified quantity of Hb-stabilising buffer solution. The instructions asked participants for each collection device to remove the lid which contained an integrated collection probe and to scrape the probe across the same collected bowel motion. They were then asked to check that all the grooves (OC-S) and or dimples (HM-J) on the collection probe were filled before returning it to the collection device. This was repeated for the second kit on the same bowel movement. There were ~900 OC-S/HM-J and 100 OC-S/OC-S kits available.

Both FIT assays were analysed in a UKAS ISO:15189 accredited medical laboratory, (No.8361) based within the laboratory of the Eastern Hub Bowel Cancer Screening Programme, Nottingham University Hospitals NHS Trust, Nottingham, UK.

The assays used were:

- OC-Sensor (OC-S) test kit was analysed using the standard sampling system (third generation buffer) and the OC-Sensor Diana analyser (Eiken Chemical, Japan) supplied by Mast Diagnostics, UK.
- HM-JACKarc (HM-J) test kit was analysed using the standard sampling system and the HM-JACKarc analyser (Hitachi Chemical Diagnostic Systems Co., Ltd, Tokyo, Japan) and supplied by Alpha Labs, UK.

Faecal haemoglobin concentrations (f-Hb) were determined according to analysis on the FIT systems and reported as µg Hb/g faeces. All returned samples were logged prospectively at the receiving laboratory and analysed once for f-Hb according to manufacturer's protocols, alongside f-Hb controls.

The analysers were calibrated once a month, and 2 levels of controls were validated at the beginning and end of each run. Returned samples were stored in a refrigerator at 4°C upon arrival until analysis. All samples were analysed within 1 week of receipt.

If f-Hb were above the upper measurement limit of the assay (200 and 400 µg Hb/g faeces for OC-S and HM-J respectively) they were diluted in respective calibration diluent (1 in 15 and 1 in 250 for OC-S or 1 in 10 and 1 in 100 for HM-J) to obtain a quantitative result.

The Limit of Detection (LoD) and Limit of Quantification (LoQ) for each assay is reported as: OC-S (Diana) analyser, LoD 2.0 µg Hb/g faeces and LoQ 2.4 µg Hb/g faeces[15] and HM-J analyser, LoD 1.3µg Hb/g faeces and LoQ 7.0 µg Hb/g faeces[16]. Our laboratory LoD analysis was calculated at analyser installation, as 2.0 µg Hb/g faeces and 1.9 µg Hb/g faeces for OC-S and HM-J respectively. LoQ was not assessed for this study.

Covariates

Age and sex of patients was collated from the test referral request information.

Outcome

Patients were investigated as usual through the 2WW pathway. CRC was determined from medical record review from histology following colonoscopy, and additional investigations (e.g. radiology) as determined appropriate by the clinical team.

Analysis

Analysis was undertaken on all patients returning two analysable test kits and who had completed clinical investigation. 95% CI were calculated using the Clopper and Pearson method. Tests of significance were considered significant if a $p<0.05$. All statistics were performed using R (version 4.0.2).

For this study both OC-S and HM-J lower assigned cut-off was taken as 4 µg Hb/g faeces (corresponding to lower clinically prescribed cut-offs as described previously[4,11] For comparison purposes however any measurable f-Hb was recorded. Values over 20,000 µg Hb/g faeces were censored at this upper limit. Median values of f-Hb were compared by age and gender using Wilcoxon signed ranked test (skewed data).

Univariate analysis of the inter-assay agreement was undertaken using Pearson's correlation and multivariable analyses using linear regression adjusted for age and sex. Agreement was assessed both overall and around predefined cut-offs of interest where either measure fell in the specified range (excluding values >5x range upper limit): 4 µg Hb/g faeces (range 0-10), 10 µg Hb/g faeces (range 4-20), 100 µg Hb/g faeces (range 20-200).

The positive predictive values (PPV) and negative predictive values (NPV) for the diagnosis of CRC were also reported for the predefined cut-offs of 4 µg Hb/g faeces, 10 µg Hb/g faeces and 150 µg Hb/g faeces. Diagnostic accuracy statistics were calculated using the ROCR package. The inter-assay agreement for the same cut-offs was assessed using Kappa coefficients.

To support any variability in results found between the 2-assays being related to the assay and not the faecal sample a sensitivity analysis was undertaken amongst patients receiving two OC-S devices only.

This work fell under the remit of service improvement, and evaluation and therefore did not require ethical approval from the local NHS Research Ethics Committee. All individuals were not required to complete the test and informed that the results would not be used in their care pathway.

Results

Patient characteristics

Two FIT kits were sent to a 1030 individuals (914, OCS/HM-J, 116 OC-S/OC-S) investigated within a two week wait setting as described previously[4]. An overall return rate for at least 1 device was 82.6%. 735 (80.4%) individuals correctly completed and returned two different FIT devices of which 732 had full clinical outcomes available and formed the main analysis cohort. In addition 114 (98.3%) who were sent two OC-S devices correctly completed and returned both devices formed the sensitivity cohort; clinical outcomes were not assessed in this subset.

In the analysis cohort three results were >upper limit of measurement and were censored at 20,000 µg Hb/g faeces. The median age of participants was 71.1 years (interquartile range (IQR) 62.5-78.7years) and 43.9% (321) were male.

Median f-Hb levels were all below the LoQ at and 2.0 (0-16.9) and 1.2 (IQR 0.3-9.6) for OC-S and HM-J respectively ($p<0.001$). Overall males had higher levels than females for both assays and older patients had higher levels. In general OC-S produced higher values than HM-J ($p<0.001$) (table 1).

Colorectal cancer detection

During the study period 38/732 (5.2%) colorectal cancers were diagnosed. Median f-Hb levels for individuals diagnosed with CRC were 258.5 µg Hb/g faeces and 318.1 µg Hb/g faeces for OC-S and HM-J respectively ($p=0.695$) and for those without CRC they were 1.8 µg Hb/g faeces and 1.0 µg Hb/g faeces for OC-S and HM-J respectively ($p<0.001$) (table 2).

The area under the receiver operating curves were 0.91 (95%CI 0.87-0.94) for OC-S and 0.90 (95%CI 0.84-0.95) for HM-J. The optimal f-Hb cut-offs for the diagnosis of CRC were 18.2 µg Hb/g faeces (sensitivity=0.87, 95%CI 0.72-0.96, specificity=0.79, 95%CI 0.76-0.82) and 22.6 µg Hb/g faeces (sensitivity=0.82, 95%CI 0.66-0.92,

specificity=0.81, 95%CI 0.78-0.84) for OC-S and HM-J respectively (supplementary figures 1 and 2).

Using the pre-specified cut-offs, both assays performed similarly for the detection of CRC. Using a f-Hb of 4 µg Hb/g faeces for positivity OC-S would have identified 37 (97.5%) and HM-J 35 (92.1%) cancers. Using only a FIT cut off of 10 µg Hb/g faeces for positivity OC-S would have identified 34 (89.5%) and HM-J 32 (84.2%) cancers. Using only a FIT cut off of 150 µg Hb/g faeces for positivity OC-S would have identified 24 (63.2%) and HM-J 22 (57.9%) cancers. Full measures of diagnostic accuracy for CRC are in table 3 and supplementary tables 1 and 2.

Inter-assay concordance (OC-S vs HM-J)

539 participants had at least one measure in range around 4 µg Hb/g faeces , 156 participants around 10 µg Hb/g faeces and 134 participants around 100 µg Hb/g faeces.

Correlation between the OC-S/HM-J f-Hb over the full range was rho=0.74 (95%CI 0.70-0.77), p<0.001. However this fell when analysis was restricted to measurements around 4 µg Hb/g faeces, 10 µg Hb/g faeces and 100 µg Hb/g faeces with rho =0.47, 0.26 and 0.28 respectively. When assessing by the presence of CRC the correlation in those without CRC was 0.48 and with CRC was 0.94. There were 77 (10.5%) individuals with measurement differences of >50 µg Hb/g faeces and 55 (5.7%) individuals with measurement differences of >100 µg Hb/g faeces (supplementary figures 3 and 4).

Using a cut-off of 4 µg Hb/g faeces for both tests found an agreement of 88.1% and a Cohen's Kappa of 0.74. Using a cut-off of 10 µg Hb/g faeces for both tests found an agreement of 91.7% and a Cohen's Kappa of 0.79. Using a cut-off of 150µg µg Hb/g faeces for both tests found an agreement of 96.3% and a Cohen's Kappa of 0.76.

The results of linear regression analyses are shown in table 4 and figure 1.

Sensitivity analysis

114 additional patients returned 2x OC-S collection devices. Over the full range of results correlation between the 2 measures was high (rho=0.99, p<0.001). Using cut-offs of 4 µg Hb/g faeces, 10 µg Hb/g faeces and 150 µg Hb/g faeces for both tests found an agreement of 90.4% (Cohen's Kappa =0.80), 96.5% (Cohen's Kappa =0.91) and 100% (Cohen's kappa =1.00) respectively. There were 9 (7.9%) individuals with measurement differences of >50 µg Hb/g faeces and 5 (4.4%) individuals with measurement differences of >100 µg Hb/g faeces (supplementary figure 5).

Discussion

This study is the first 'real world' UK symptomatic bowel cancer pathway study comparing different FITs, where participants were asked to sample their own bowel motion. When assessed over the full measurement scale there was adequate agreement between the two analysers, however this fell when examined around key cut-points.

Both tests appear fit for purpose in terms of their efficacy for detecting occult blood, and ease of use as the majority of people returned both devices, used correctly. It is clear however that employing the same cut-off levels for the two tests investigated here will lead to different referral practices with the use of OC-S leading to referral of higher numbers of patients. However, consequently OC-S detected more cancers than HM-J for the same cut offs. At the NICE advised cut-off of 10 µg Hb/g faeces for CRC referral[10], the sensitivity of OC-S vs HM-J was 89.0% vs 84.0% respectively.

This study has a notable strength of being undertaken in a routine clinical pathway and compares two of the most commonly used FIT assays which have not be directly compared in such a setting before. The advantage of using real participants in a 2WW cancer diagnosis pathway avoids the selection bias with formal research studies. Limitations include overall sample size, a relatively small number of CRC diagnoses and lack of detail on whether patients sampled the same bowel motion at the same time with different kits.

Results were reported guided by the FITTER guidelines for reporting f-Hb levels[17–19] and STARD guidelines (supplementary table 3), quality control materials were utilised, and quality management procedures were in line with UKAS 15189 standards. Manufacturer ranges, alongside manufacturers analyser set up details, were accepted as accurate for this paper. The lower LOD of both assays is 2 µg Hb/g faeces, as a result it could be argued that all measures below LOD be given the same value e.g. 0.0. However, we chose to use the actual values as this reflects all the currently available information. Any results reported as <2 µg Hb/g faeces must be interpreted in this

context and not considered accurate, i.e considered only as <2 µg Hb/g faeces in clinical practice.

Linear regression analyses demonstrated that whilst over the full range of measurements of f-Hb were similar between the 2 assays, when more focussed assessment around cut-offs of interest was undertaken they were not comparable (table 4) and became more disparate as values increased (figure 1). These results suggest that the relationship between OC-S and HM-J f-Hb are not directly linear, preventing the determination of an accurate conversion factor. To determine the true relationship more studies are needed using high value FIT tests as we had a paucity of these.

It is known that occult blood is not evenly distributed in faeces[20]. It is unclear how much of the variation in f-Hb from a single bowel motion between analysers seen here is the result of faecal sampling variation or analytic bias. Previous studies have attempted to mitigate this issue by using i) artificial systems with homogenised faecal samples, either with previously frozen faecal samples[21] or using small sample numbers[22], or ii) using 'artificial biological samples' (where Hb was added to Hb-free specimens)[8]. However this does not reflect what occurs in a 'real world' setting when an individual is exhibiting symptoms that could be associated with bowel cancer. Our sensitivity analysis using two OC-S tests on a single sample had a much closer agreement of f-Hb measurements than with OC-S vs HM-J suggesting that differences are not simply due to sampling variation within the bowel motion. Wide variations did however still occur, in both settings, highlighting the importance of repeat testing if concerns still exist.

Now, more than ever, detailed understanding of how to operationalise FIT testing in the diagnosis of symptomatic CRC is needed. During the Covid-19 pandemic endoscopy capacity for colonoscopy was reduced by 90% in the UK[23], with evidence suggesting similar findings globally[24–26]. With a significant backlog of patients waiting for assessment there have been numerous calls for the incorporation of FIT testing into clinical practice to expedite those at greatest risk[12,13,27,28] – but it needs to be taken into account that risk will vary depending on the FIT device used.

International efforts are being made to standardise assays so that results obtained of different analysers can be directly compared. Our study supports this and the work of others[21] in confirming that there is heterogeneity between different FIT analysers. Currently no single assay in the UK is recommended with choice locally determined; based on a wide number of factors including but not limited to negotiated prices, cut-off evidence and local access. Our results mean that it is important to establish local limits, and analyser performance when introducing new assays into routine practice[18,29]. We clearly demonstrate that when results are compared around potential cut-off values (4 µg Hb/g faeces and 10 µg Hb/g faeces) results from different tests are not directly comparable and that is not wholly attributable to sampling variation. It is therefore essential, to determine these criteria in conjunction with local hospital referral capacity, with cut-offs being regularly reviewed and refined as new information emerges. Consequently laboratories cannot switch between FIT tests without discussions with clinical users. Care should also be taken when comparing different publications for these reasons described. Ultimately either bespoke cut-offs for each platform or adjustment factors should be used to align the analysers. Standardisation of FIT is needed[7,30] to allow accurate use of the device and to support decision makers in understanding the true clinical impacts of utilising FIT. This is one of the aims of the Working Group on FIT of the Scientific Division of the International Federation of Clinical Chemistry and Laboratory Medicine[31].

Acknowledgements

Nottingham Colorectal Service: Acheson Austin, Sarah Thomson, Bev Harwood, Sarah Blower, Tara Dorn, Helen Andrews, Julian Williams, John Scholefield, John Abercrombie, Charles Maxwell-Armstrong, Austin Acheson, Katie Walter, Bala Bharathan, Khalid Mohiuddin, Kathryn Thomas, Alastair Simpson, Arifa Siddika, Christopher Thompson, Barrie Keeler, Ebrahim Dalwai, Rachael Briggs, Rachel Spencer & Christopher Thomas.

Nottinghamshire Bowel Cancer Screening Hub staff.

Thanks to the Department of Gastroenterology Nottingham University Hospitals; Nottingham City Hospital Endoscopy Service and Department of Radiology, Nottingham University Hospitals.

Funding

DJH is funded by a National Institute for Health Research post-doctoral fellowship.

JRM is funded by a Medical Research Council Clinician Scientist Fellowship [grant number MR/P008348/1].

OC Sensor FIT kits were produced by EIKEN Chemical Co Ltd, and provided by Mast (Mast Group Ltd . Mast House, Derby Road, Bootle, England, L20 1EA)) for the study

HM-JACK FIT kits were produced by Hitachi Chemical Diagnostic Systems Co., Ltd (Tokyo, Japan), and provided by Alpha Laboratories (40 Parham Dr, Eastleigh SO50 4NU) for the study.

Author contributions

CC, AB, DH, RF were involved in the conceptual design of the study. CC, AJ, BA, HD, RL, SO and JM were involved in design and collection of data for evaluation. CC and JM produced the first draft and all authors made critical contributions and approved the final version.

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Tables and figures

Table 1 Faecal haemoglobin concentrations by age and sex for those with full clinical follow up

	n	OC-S µg Hb/g faeces	HM-J µg Hb/g faeces	p
All	732	2.0 (0-16.9)	1.2 (0.3-9.6)	<0.001
Sex				
Male	321	2.4 (0-23.8)	1.7 (0.4-15.7)	0.231
Female	411	1.8 (0-13.3)	0.9 (0.2-6.2)	<0.001
Age group				
18-59years	144	0.4 (0-4.3)	0.6 (0.2-3.5)	0.411
60-79years	432	2.2 (0-15.0)	1.0 (0.2-9.2)	<0.001
≥80years	153	4.1 (1.0-36.6)	2.6 (0.7-38.5)	0.589

Values are median (IQR)

Table 2 Faecal haemoglobin concentrations in patients with and without colorectal cancer by age and sex

	n	OC-S µg Hb/g faeces	HM-J µg Hb/g faeces	p
No colorectal cancer				
All	694	1.8 (0-11.6)	1.0 (0.2-6.7)	<0.001
Male	295	2.0 (0-11.8)	1.2 (0.3-9.4)	0.043
Female	399	1.6 (0-11.2)	0.9 (0.2-4.6)	<0.001
18-59years	142	0.4 (0-4.1)	0.6 (0.2-3.2)	0.364
60-79years	412	1.9 (0-12.1)	0.9 (0.2-7.4)	<0.001
≥80years	140	3.0 (0.6-26.5)	2.1 (0.5-16.8)	0.210
Colorectal cancer				
All	38	258.5 (43.3-1434.1)	318.1 (29.9-1352.2)	0.695
Male	26	258.5 (43.3-991.8)	336.5 (48.1-1352.2)	0.247
Female	12	290.1 (52.0-2608.5)	184.5 (28.3-864.8)	0.012
18-59years	2	39.8 (23.8-55.8)	55.0 (31.0-78.9)	1.000
60-79years	20	284.0 (23.9-1278.3)	163.9 (20.3-1289.2)	0.095
≥80years	16	317.9 (57.9-249.6)	541.8 (159.0-1658.5)	0.433

Values are median (IQR)

Table 3 Sensitivity, specificity, positive and negative predictive values (95%CI) of FIT tests for colorectal cancer at predefined cut-offs

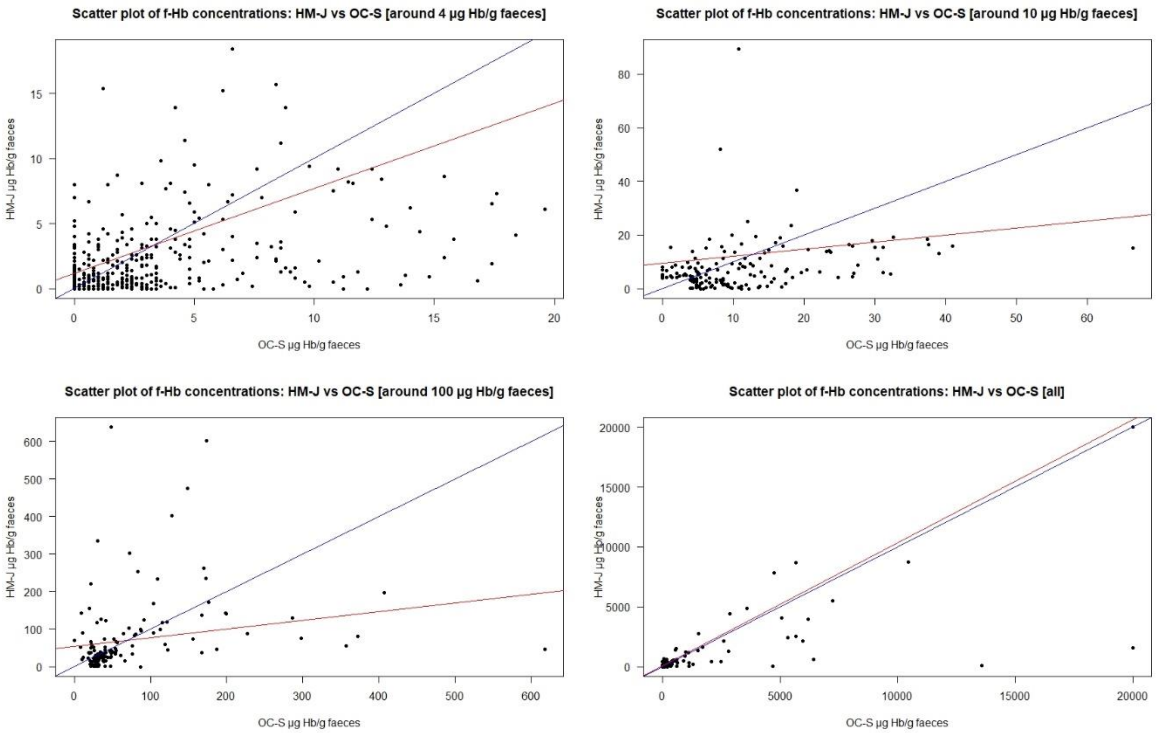
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Cut-off: 4 µg Hb/g faeces				
OC-S	0.97 (0.86-1.00)	0.64 (0.60-0.68)	0.13 (0.09-0.17)	1.00 (0.99-1.00)
HM-J	0.92 (0.79-0.98)	0.70 (0.66-0.73)	0.14 (0.10-0.19)	0.99 (0.98-1.00)
Cut-off: 10 µg Hb/g faeces				
OC-S	0.89 (0.75-0.97)	0.74 (0.70-0.77)	0.16 (0.11-0.21)	0.99 (0.98-1.00)
HM-J	0.84 (0.69-0.94)	0.78 (0.75-0.81)	0.18 (0.12-0.24)	0.99 (0.98-1.00)
Cut-off: 150 µg Hb/g faeces				
OC-S	0.63 (0.46-0.78)	0.94 (0.92-0.96)	0.37 (0.25-0.50)	0.98 (0.97-0.99)
HM-J	0.58 (0.41-0.74)	0.95 (0.93-0.96)	0.37 (0.25-0.50)	0.98 (0.96-0.99)

Table 4 Linear regression analysis of the relationship between OC-S and HM-J faecal haemoglobin concentrations.

	n	Beta coeff (HM-J)	SE	R^2	p
All					
Unadjusted	732	1.026	0.035	0.546	<0.001
Adjusted*	732	1.029	0.035	0.546	<0.001
0-10 µg Hb/g faeces					
Unadjusted	539	0.653	0.053	0.216	<0.001
Adjusted*	539	0.651	0.054	0.224	<0.001
4-20 µg Hb/g faeces					
Unadjusted	156	0.263	0.080	0.059	0.001
Adjusted*	156	0.258	0.081	0.057	0.002
20-200 µg Hb/g faeces					
Unadjusted	134	0.229	0.070	0.069	0.001
Adjusted*	134	0.235	0.072	0.057	0.001

*Adjusted for age and sex

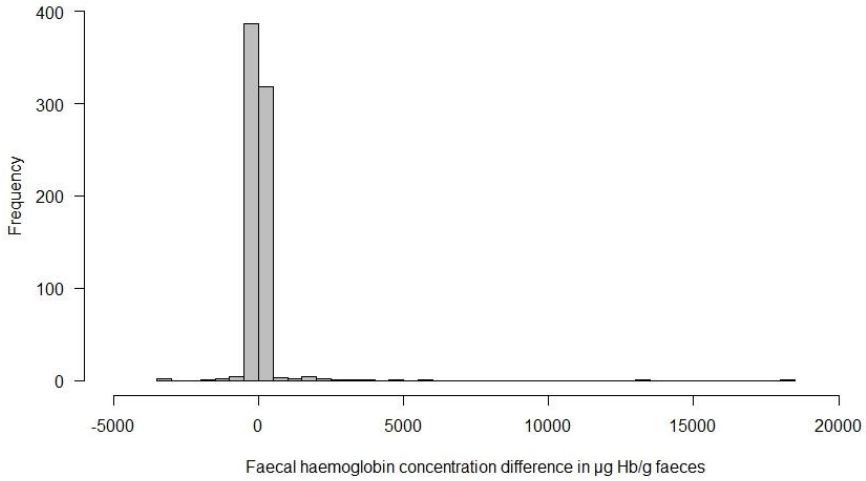
Figure 1. Scatterplots of the relationship between OC-S and HM-J faecal haemoglobin concentrations.



Blue line indicates line of equality
Red line indicates line of best fit from linear regression (unadjusted)

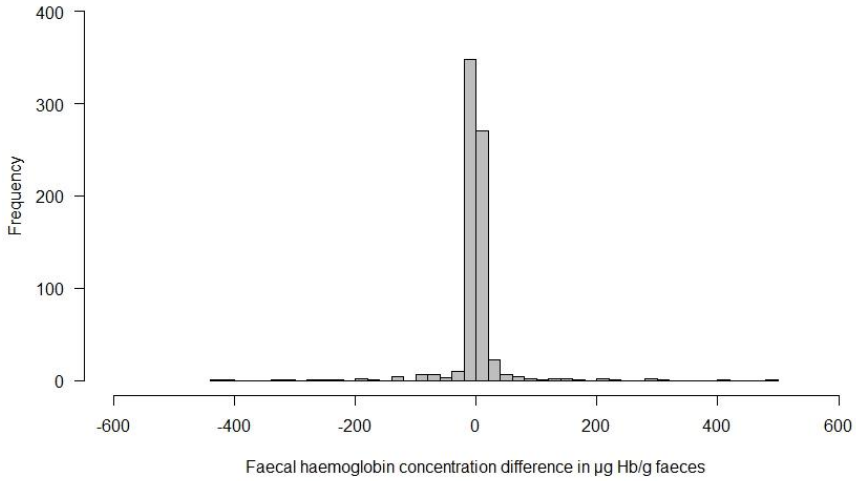
Supplementary material

Supplementary figure 1. Differences in faecal haemoglobin concentration OC-S vs HM-J (all participants)



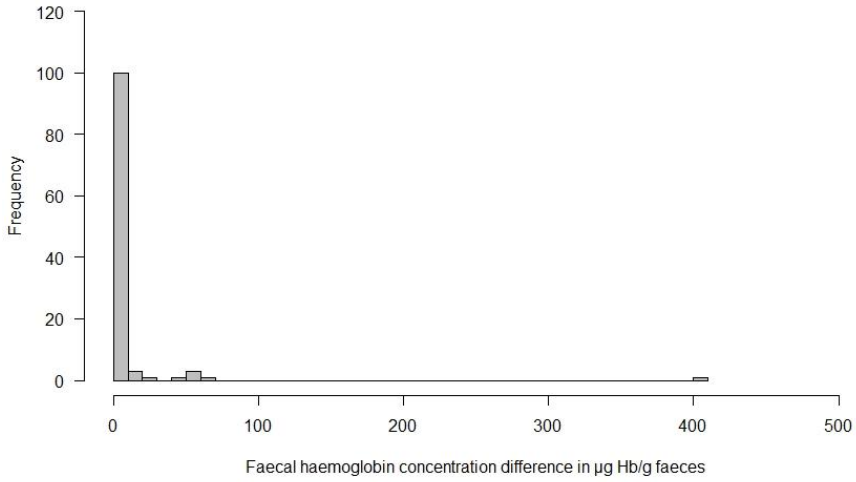
Positive values indicate OC-S>HM-J
N=732

Supplementary figure 2. Differences in faecal haemoglobin concentration OC-S vs HM-J (difference <500 µg Hb/g faeces)



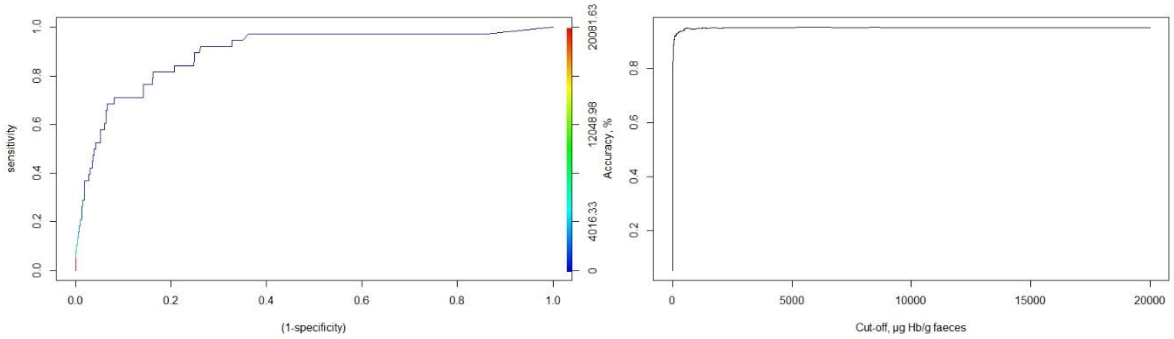
Positive values indicate OC-S>HM-J
N=705

Supplementary figure 3. Differences in faecal haemoglobin concentration OC-S vs OC-S (difference <500 µg Hb/g faeces)

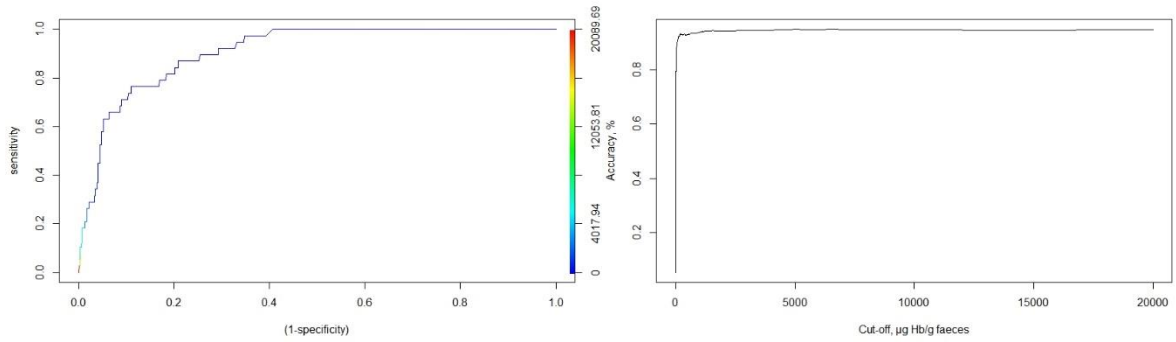


N=110

Supplementary figure 4. Receiver operator and diagnostic accuracy curves for OC-S faecal haemoglobin concentrations in the diagnosis of colorectal cancer



Supplementary figure 5. Receiver operator and diagnostic accuracy curves for HM-J faecal haemoglobin concentrations in the diagnosis of colorectal cancer



Supplementary table 1. Comparable HM-J f-Hb cut-off when compared to OC-S for the predetermined cut-offs, whilst maintaining **sensitivity**.

	Cut-off	Sensitivity	Specificity
OC-S	4	0.97	0.64
HM-J	1	**0.97**	0.64
OC-S	10	0.89	0.74
HM-J	6	**0.89**	0.26
OC-S	150	0.63	0.94
HM-J	123.4	**0.63**	0.63

Supplementary table 2. Comparable OC-S f-Hb cut-off when compared to HM-J for the predetermined cut-offs whilst maintaining **sensitivity**.

	Cut-off	Sensitivity	Specificity
HM-J	4	0.92	0.70
OC-S	7	**0.92**	0.31
HM-J	10	0.84	0.78
OC-S	18	**0.84**	0.21
HM-J	150	0.58	0.95
OC-S	208	**0.58**	0.05

Supplementary table 3. STARD checklist (based on FITTER checklist recommendations by the Colorectal Cancer Screening Committee, World Endoscopy Organization)

https://www.worldendo.org/wp-content/uploads/2016/08/weo_expert_working_group_fit_discussion_doc_no5_pu.pdf

Specimen collection and handling		
Name of specimen collection device and supplier	OC-Sensor (Mast Diagnostics, UK)	HM-JACKarc (Alpha Labs, UK)
Description of specimen collection	Plastic probe with grooves, inserted into collection tube with twist and push lid.	Plastic probe with 2 small dimples, inserted into collection tube with screw-on lid.
Description of specimens used if an in vivo study	Single faecal sample	Single faecal sample
Details of faecal collection method	Instructions asked participants for each device to remove the lid which contained an integrated collection probe and to scrape the probe across the collected bowel motion (2 test kits per bowel motion) and replace in the device. Pictoral and written instructions were included.	
Who collected the specimens from the samples	Patient	Patient
Number of faecal specimens used in the study	1,030	914
Mean mass of faeces	~10 mg	~2mg

collected		
Volume of buffer into which specimen is taken by probe	2.0 mL	2.0 mL
Time and storage conditions of faecal specimen from "passing" to sampling	Participants were advised to date the sample and post envelope without delay after collection. Once received into the laboratory, if not tested immediately the samples were refrigerated and brought to room temperature before analysis.	
Time and storage of collection devices from specimen collection to analysis	Completed test kits were returned using the Royal Mail postal system, and stored at 4°C upon arrival until analysis. All samples were analysed within 1 week of receipt and within 14 days of sample collection.	
Analysis		
Name of analyser, model, supplier (address), number of systems if more than one used.	1 OC-Sensor Diana analyser, manufactured by Eiken Chemical (Japan) and supplied by Mast Diagnostics (UK)	1 HM-JACKarc analyser, manufactured by Hitachi Chemical Diagnostics Systems Co. Ltd (Japan) and supplied by Alpha Labs (UK)
Number of times each sample was analysed	Once	Once
Analytical working ranges and whether samples outside this range were diluted (factor) and re-assayed	Up to 200 µg Hb/g faeces Samples were diluted 1 in 15 and 1 in 250.	Up to 400 µg Hb/g faeces Samples were diluted 1 in 10 and 1 in 100.

Source of calibrators and details of calibration process including frequency	Calibrators supplied by Mast Diagnostics, UK Single level of calibrant auto-diluted to seven levels Calibrated once per month according to manufacturer's specifications	Calibrators supplied by Alpha Labs, UK 2 levels of calibrant requiring reconstitution Calibrated once per month according to manufacturer's specifications
Analytical imprecision.	Analytical imprecision was taken as according to manufacturer's specifications and at analyser set up by the manufacturer. An additional 25 faecal samples, in sampling devices, were used to confirm in house LoD and analytical imprecision.	
Quality management		
Source, or description of IQC materials, rules for acceptance and rejection of analytical runs.	IQC material supplied by Mast Diagnostics, UK 2 levels of QC (liquid material) 1-2s rule used for acceptance or rejection of analytical runs	IQC material supplied by Alpha Labs, UK 2 levels of QC (reconstitution required) 1-2s rule used for acceptance or rejection of analytical runs
Participation in external quality assessment schemes, frequency, performance attained	UK NEQAS for Faecal Haemoglobin, PO Box 3909, Birmingham B15 2UE, UK; Monthly distribution Acceptable performance but results influenced by pre-analytical variables	
Accreditation held by the	The laboratory is accredited by the UK Accreditation Service (ISO 15189), Ref 8361. At the time of the study	

analytical facility (address)	neither of the analysers had been accredited. The OC-Sensor Diana was subsequently added to the accreditation schedule.	
Number, training and expertise of persons performing the analyses and recording the results	The processes were overseen, and results reported by 2 HCPC registered BMS staff. The kits were sent out and the analysers were run by 3 additional trainee BMS staff.	
Result handling		
Mode of collection of data	Single readings manually recorded	
Units used	ng/mL converted manually to µg/g, conversion factor 0.20 used	ng/mL converted manually to µg/g, conversion factor 1.0 used
Cut-off concentration used	Locally defined cut-offs of 4 µg Hb/g faeces, 10 µg Hb/g faeces and 150 µg Hb/g faeces	
Were the analysts blinded to the results of the reference investigation and other clinical information?	Yes	