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Faecal immunochemical testing (FIT): sources of result variation based on three years of routine testing of symptomatic patients in English primary care

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ABSTRACT

Introduction: We aimed to determine the analytical capabilities of a commonly used faecal immunochemical test (FIT) to detect faecal haemoglobin (Hb) in symptomatic people attending primary care in the context of the English NICE DG30 guidance.
Materials and Methods: Data obtained from independent verification studies and clinical testing of the HM-JACKarc FIT method in routine primary care practice were analysed to derive performance characteristics.
Results: Detection capabilities for the FIT method were 0.5 μg/g (limit of blank), 1.3 μg/g (limit of detection) and 3.0 μg/g (limit of quantitation). Of 33 non-homogenized specimens, 31 (93.9%) analysed in triplicate were consistently categorized relative to 10 μg/g, compared to all 33 (100%) homogenized specimens. Imprecision was higher (median 27.8%, (range 20.5% to 48.6%)) in non-homogenized specimens than in homogenized specimens (10.2%, (7.0 to 13.5%)). Considerable variation was observed in sequential clinical specimens from individual patients but no positive or negative trend in specimen degradation was observed over time (p = 0.26).
Discussion: The FIT immunoassay evaluated is capable of detecting faecal Hb at concentrations well below the DG30 threshold of 10 μg/g and is suitable for application in this context. The greatest practical challenge to FIT performance is reproducible sampling, the pre-analytical step associated with most variability. Further research should focus on reducing sampling variability, particularly as post-COVID-19 guidance recommends greater FIT utilization.

Introduction

Colorectal cancer is globally the third most commonly occurring malignancy [1]. Early disease is surgically treatable with good long-term outcomes [2]. Most developed countries, including the UK, operate colorectal screening programmes using tests for the presence of haemoglobin. Screen detected cancers benefit from early diagnosis and treatment, and are associated with improved survival [2]. The faecal immunochemical test (FIT) has largely replaced traditional guaiac-based faecal occult blood tests for many reasons including increased analytical specificity [3].

To complement the NHS England Bowel Cancer Screening Programme (UKBCSP) [4], and the 2015 National Institute for Health and Care Excellence (NICE) NG12 Suspected Cancer guidelines [5], the 2017 DG30 NICE guidance [6] recommended the use of FIT for faecal haemoglobin (Hb) detection in patients presenting to primary care with low-risk abdominal symptoms suggestive of gastrointestinal cancer. The adoption of FIT in primary care was initially slow, with notable variation in uptake and implementation across England [7]. During the COVID-19 pandemic, FIT has been rapidly implemented across the

NHS to triage the highest risk symptomatic patients to a reduced colonoscopy resource [8,9].

The Oxford University Hospitals NHS Foundation Trust (OUH) adopted FIT in 2016 in response to NG12 [5]. This coincided with a desire from the clinical laboratory to move away from the poorly performing guaiac-based method [10]. Following a study by this group comparing the accuracy of the guaiac and FIT methods in symptomatic primary care patients, FIT was commissioned by Oxfordshire Clinical Commissioning Group (OCCG) as a direct access test for General Practitioners [11]. Evaluation of the clinical performance of FIT between 2016 and 2019 in nearly 10,000 patients demonstrated the sensitivity for colorectal cancer to be 90.5%, specificity 91.3% with an associated negative predictive value of 99.9% [12].

Despite increasing uptake of FIT, there remains a clear need to understand FIT method characteristics [13]. FIT testing, whether undertaken for screening asymptomatic or testing symptomatic populations, is dependent on the performance of the laboratory procedures used including both analytical and pre-analytical factors such as sampling [14]. Whilst there has been significant work undertaken in the context of

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screening [15] there is less information available in the symptomatic population. This is an important omission as the characteristics of the screening population, and so the associated specimens, differ from symptomatic patients. For example, the age-range of the symptomatic population is broader and specimen characteristics, including faecal consistency, will differ in patients with changes in bowel habit. This may affect sampling, and sampling integrity.

In this study, we present our observations of FIT method performance since introducing it into routine clinical practice for a symptomatic primary care population. We include estimates of analytical performance, observations on longer term method precision, sampling reproducibility in homogenized compared to non-homogenized material, and consistency in sequential specimens from the same patients.

Material and methods

This study presents data from a single laboratory, within the Oxford University Hospitals NHS Foundation Trust (OUH) undertaking centralized analysis of FIT requests, mainly from primary care, for the 680,000 population of Oxfordshire. The assessment of FIT method performance was registered as a service evaluation on the OUH Datix register (CSS-BIO-3 4730).

FIT analysis was undertaken using the HM-JACKarc analyser (Hitachi Chemical Diagnostics Systems Co., Ltd, Tokyo, Japan and distributed in the UK by Alpha Labs Ltd, Eastleigh, Hants) which has an analytical range of 7 to 400 µg Hb/g faeces (manufacturers data).

All specimens were requested by NHS primary care clinicians and collected into standard faecal pots by patients, a sampling approach taken due to concerns about sampling capability when undertaken by (often elderly) symptomatic patients [11]. Clinician advice from OUH and OCCG included guidance on delivering the sample to the laboratory on the same day as collection as Hb can degrade over time [16]. Despite issuing guidance recommending that only a single specimen was required for FIT, many primary care clinicians continued to request more than one. This may have been due to clinicians being unaware of revised guidance as the standard practice for the guaiac method was to collect serial samples, the additional reassurance multiple specimens might provide, or because there remained doubt about test credibility [17,18].

On arrival in the laboratory the faecal specimens were sampled by laboratory staff using a sampling device, Extel Hemo-Auto MC (Alpha Labs Ltd, Eastleigh, Hants) designed for application with the HM-JACKarc. The whole sampling procedure was undertaken in a fume cupboard by staff with standard laboratory protective equipment and involved a 30 second vortex mix which for most specimens was adequate for release of the faecal pellet collected into the dimples/grooves of the specimen collection device. All specimens were visually inspected to ensure complete suspension of the specimen and if it was noted any residual specimen adherence mixing continued until all material was removed.

The FIT method limit of blank (LOB), limit of detection (LOD) and limit of quantitation (LOQ) were estimated using standard approaches [13,19].

The LOB was calculated as the mean FIT result of the sample blank run as part of the method internal quality control (IQC) over 100 consecutive batches over 11 months and using three different reagent batches of specimen collection devices with no sample collected into the buffer.

The LOD was calculated using the LOB + 1.645 (SD) of samples with 'very low concentrations', considered to be those giving FIT results above the manufacturers quoted LOB of 0.6 µg/g. We used the average SD of 47 specimens analysed in duplicate between 0.5 µg/g and 1.0 µg/g.

LOQ was estimated from an imprecision profile constructed from faecal samples analysed in duplicate across a wide FIT concentration range of naturally Hb positive material and was taken as the concentration at which the percent coefficient of variation (CV) was 10%. A 10% CV was selected as this has been recommended as an appropriate imprecision threshold for FIT method LOQ evaluation [13]. The imprecision profile was constructed using data from 132 paired, within-batch replicates, analysed in seven different batches over a one-week period.

The on-instrument intermediate imprecision (*not* including sampling into the collection device) was estimated from two IQC materials provided by the manufacturer over several months to include the effects of different analysts, reagent and calibration material lot numbers. As the manufacturer's two IQC materials both had concentrations above 10 µg/g (the threshold used to define a positive result in the NICE DG30 guidelines) we also explored precision characteristics near to the LOD. This was prepared using a 1 in 40 dilution of the manufacturer's high IQC in sample device buffer.

It is unclear whether Hb is evenly distributed in faeces therefore we investigated the Hb concentration before and after sample homogenization. A total of 33 randomly selected faecal specimens arriving in standard faecal containers were sampled in triplicate, homogenized and then resampled a further three times. This resulted in six estimates for each faecal sample (three non-homogenized replicates and three homogenized replicates). Imprecision of sampling was calculated as the CV, % for each set of three replicates. Individual faecal specimen replicates were sampled by the same member of staff but, overall, three different members of laboratory staff were involved in this part of the study. Homogenization involved using a disposable wooden applicator stick (VWR International Ltd, Lutterworth, UK) to mix all faecal material within the stool container to consistency.

To verify the stability of Hb in the buffered collection device, a selection of 13 specimens were analysed at three time points (at time zero, at 6 days and at 21 days) after storage at 4°C. Samples were selected to represent concentrations just below and above the 10 µg/g decision threshold.

Sequential samples delivered to the lab on the same day allowed assessment of delayed analysis on Hb concentration. These sequential specimens represented maximum within patient error as Hb concentrations reflected total variability (between day biological variation, analyte instability, sampling error and immunoassay method imprecision).

Differences between the imprecision of sampling between non-homogenized and homogenized sampling were assessed using a Wilcoxon rank test with continuity correction, the null hypothesis being that the distribution of x – y (CV, % in homogenized group – CV, % in non-homogenized group) is symmetric about 0.

For patients with multiple specimens and discordant results (at least one result greater than, and at least one result less than 10 ug/g), we examined whether there was an association between the likelihood of a positive result and delay in processing the specimen in the laboratory of 48 or 72 hours. In this group, we also examined whether there was an association between a positive result and the sequence number of the specimen. Both were assessed using the Chi-squared test.

Results

The distribution of blank results was non-gaussian and was therefore calculated with two non-parametric approaches. The 95th centile the LOB was 0.5 µg/g (95% CI, 0.4–0.6) and through log transformation of the blank data (zero replaced by 0.1) which also derived a LOB of 0.5 µg/g. The LOD was determined as 1.3 µg/g and LOQ was 3 µg/g.

Method reproducibility for the low IQC material (Table 1) across four different lot numbers with mean concentrations between 24 and 27 µg/g was between 6.4% to 8.8 CV,%. Comparatively, the high IQC, at concentrations between 90 and 100 µg/g ranged from 4.4% to 6.8%. Imprecision characteristics of a highly diluted IQC material, targeted to give a concentration near the LOD were mean 1.5 µg/g, SD 0.5 µg/g, CV, % 33.9%.

Of 33 non-homogenized samples, 31 (93.9%) were consistent with respect to their categorization above or below the 10 µg/g threshold (Figure 1).

The mean result was below 10 µg/g (negative) in 27 (82%), and six (18%) had mean results above (positive). Four of the six positive results had all three replicates above the threshold and imprecision estimates were calculated: median CV, % of 27.8%, range 20.5% to 48.6%. Two further samples had discrepant result relative to the 10 µg/g NICE threshold. The first had replicates of <1.1, <1.1, 34.5 µg/g (two below the LOD and one above the LOQ). Clinical details of this patient were rectal bleeding and endoscopy showed an 8 mm polyp in the sigmoid. Histology revealed the polyp to be non-dysplastic and hyper-plastic. This patient was discharged. The second of these discrepant results had replicates <1.1, <1.1, 14.7 µg/g (two below the LOD and one positive relative to the NICE threshold of 10 µg/g). This patient was being followed up by an established vague symptoms pathway [20] and whilst nothing abnormal was detected after 9 months of follow-up, at the time of patient assessment they were noted to be taking non-steroidal anti-inflammatory drugs.

In the homogenized specimen group, all 33 (100%) specimens were consistently above or below the 10 µg/g threshold (Figure 1). Of the 33 groups, 28 had a mean result below 10 µg/g (negative) and 5 had mean results above (positive). Categorization as positive or negative was consistent in all replicates relative to a threshold of 10 µg/g. Four of the five positive specimens in the homogenized set had imprecision estimates calculated: median CV, % of 10.2%, range 7.0 to 13.5%. This was lower than, but not statistically significant from (p = 0.10), the imprecision observed in the non-homogenized samples. The fifth positive result had values above the limit of linearity which precluded the calculation of imprecision.

Thirteen specimens selected as their initial result were above the LOQ, (5 below the 10 µg/g threshold and 8 above) were repeat tested. Mean concentration, day 1 was 15.1 µg/g (range 3.1 to 30.9); storage for 6 days showed no significant change (p = 0.24), mean 17.1 µg/g (2.3 to 41.6) and neither did storage for 21 days (p = 0.79), mean 14.6 (range 2.3 to 32.3). All 13 specimens had consistent categorization (positive/negative) when repeat tested at 6 days however at 21 days one specimen would have been re-categorized as negative as it had fallen from 25 to 6 µg/g.

More than one sample was sent within a 3-month period by 527 patients: 302 returned two specimens; 222 returned three; two returned four; and one

Table 1. Imprecision estimates over a 12 month period.

QC Lot number	Period (n)	Low QC			High QC		
		Mean (µg/g)	SD (µg/g)	CV, %	Mean (µg/g)	SD (µg/g)	CV, %
168AHG	7 weeks (57)	24.7	1.6	6.4	90.0	4.0	4.4
169AHJ	36 weeks (259)	27.2	2.4	8.8	91.4	4.3	4.7
170AIA	5 weeks (32)	25.9	2.0	7.7	97.7	6.7	6.8
171AIE	13 weeks (117)	26.2	2.0	7.6	97.9	6.7	6.8
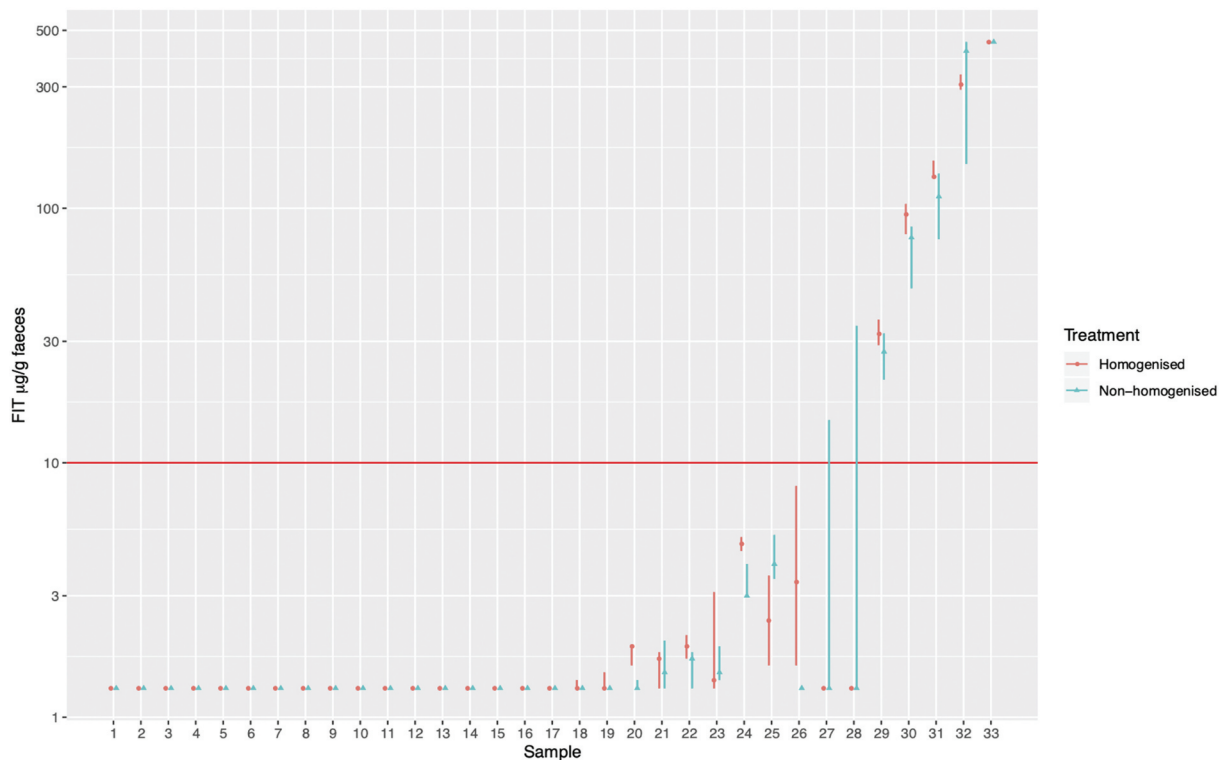
Figure 1. Specimen preparation effects. 33 specimens analysed in triplicate prior to and after homogenization. Results show the observed concentration range (lowest to highest) with each specimen presented sequentially with homogenized (circles) and non-homogenized (triangles) results adjacent.

returned five. Of the 225 patients with three or more specimens: 197 (87.6%) had concordant results for all specimens (188 negative, 9 positive). 28 (12.4%) had discordant results, that is, at least one value more, and at least one value less, than 10 μg/g. There was no obvious trend on visual review of plots of individual patient's FIT values over time and no association between the likelihood of a positive result based on a delay of more than 2 days in the specimen reaching the laboratory ($p = 0.84$, Chi-squared test). There was no association between the likelihood of a positive result and the number in the sequence of a particular patient's specimens ($p = 0.21$, Chi-squared test). Figure 2 shows the serial FIT concentrations in the 28 patients whose serial results were inconsistently categorized relative to the 10 μg/g threshold.

Discussion

In this study the LOB (0.5 μg/g), LOD (1.3 μg/g) and LOQ (3.0 μg/g) for the HM-JACKarc FIT method were lower than the current NICE threshold of 10 μg/g and we conclude this method is suitable for application in this context. The detection capabilities are also marginally better than the manufacturers claims of LOB 0.6 μg/g, LOD 2.0 μg/g, LOQ 7.0 μg/g. This may have future relevance as a recent large study examining the use of FIT in the two-week wait cancer pathway proposed using the manufacturer reported LOD of the HM-JARKarc at 2.0 μg/g as the cut off for referral for to secondary care. [21]

Method imprecision was consistently <9% and although the concentration of Hb present in the two IQC materials (25 μg/g and 95 μg/g) are satisfactory for method control at positive concentrations this approach does not provide adequate confidence <10 μg/g, where most clinical results are observed. We suggest an additional IQC material between 5 and 10 μg/g is introduced.

Imprecision rises when faecal sampling is taken into consideration. Imprecision in homogenized specimens (median 10.2%, upper estimate 13.5%) was lower than non-homogenized specimens (median 27.8%, 48.6%). Importantly two samples in the non-homogenized group were inconsistently classified relative to the 10 μg/g threshold. This would suggest that Hb is not uniformly distributed in the faecal specimen, a finding consistent with other faecal constituents [22].

The manufacturer recommends stabilization of Hb within collection devices used by the patient using non-homogenized material, a process that in a small number of cases may cause false-negative or false-positive results. However, the possibility that home sampling may miss pockets of faecal Hb must be balanced against the risk that Hb degrades over time if faecal samples are not stabilized in buffer [16]. The stability of Hb in the sampling device buffer appeared reasonable for most specimens up to 21 days although one specimen showed notable deterioration from above to below the 10 μg/g threshold. A weekly FIT batch analysis
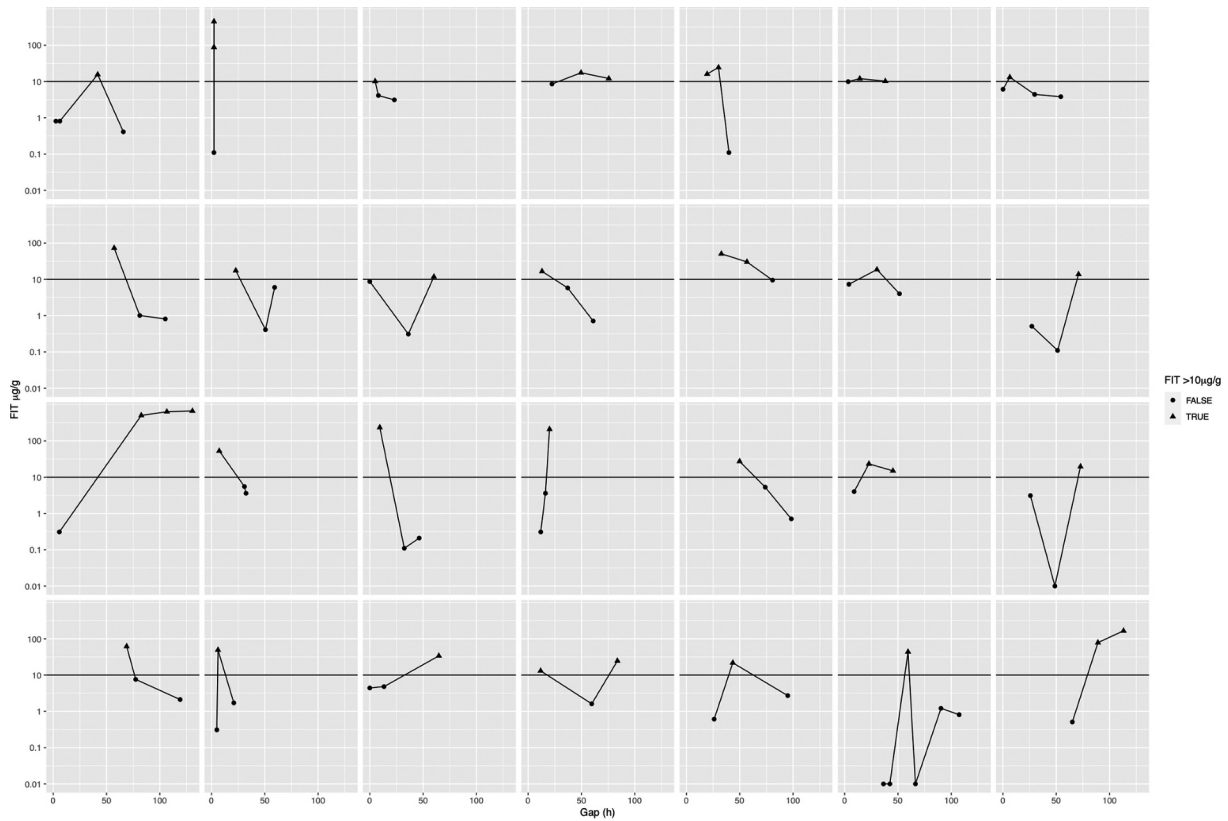
Figure 2. Variability of sequential results the 28 patients whose specimens had discordant categorization relative to a 10 μg/g threshold. Each panel represents a single patient, time (hours) vs concentration (μg/g) profile showing the inconsistent patterns observed: round points represent those results categorized as negative and triangle points positive. The horizontal line on each panel is the 10 μg/g threshold defining a positive or negative result.

would minimize the potential of this degradation to misclassify results, however, daily analysis would provide more appropriate turnaround times for 2-week wait testing.

Our serial sample data provide insight into sample variability and stability when a standard collection pot without buffer is used. We found no association between the likelihood of a positive or negative result based on a delay >2 days in the specimen reaching the laboratory in serial samples from individual patients. Time intervals between specimens were variable and the magnitude of degradation in individual specimens remains unknown. However, it is possible that the variability between days (a component of biological variability) and from sampling (a component of analytical variability) may be larger than the changes in concentration associated with degradation. If degradation were the dominant variable the results of the time series analysis would be significant.

In a previous diagnostic accuracy study of one vs two specimens, it was concluded that two offered no advantage over one [23] despite sequential results in some individuals being 400 μg/g and then <10 μg/g. Another study of two specimen strategies for colorectal cancer in a symptomatic population noted 39.2% non-concordance between the first and the maximum FIT result [24]. More recently FIT testing in

a symptomatic population in a 2 week wait pathway found 12.5% of colorectal cancers were missed using a single, patient collected, specimen [25]. The method utilized the OC-Sensor™ with a reported detection limit of 4 μg/g and also reported the majority of FIT results from patients with colorectal cancer had very high values (>150 μg/g). It is not certain what the relative contribution of biological variation, inconsistent bleeding or sampling imprecision have on FIT results but these and other studies [7] show whatever strategy is used a small number of false negatives occur.

Collection of samples from patients with a change in bowel habit presents specific additional challenges since faecal water content can vary between 60 and 82% [26]. This dilutional effect will impact on quantitative measures and the lack of form may affect entrapment of the specimen within the manufacturer's collection device. Furthermore, concern about faecal-oral or aerosol transmission of COVID-19 [27] during sample manipulation and the need for a period of very high throughput need to be balanced against analytic accuracy.

In the last year, both the NICE FIT steering group and our own centre have reported diagnostic accuracy estimates in large symptomatic populations. The NICE FIT group reported an area under the receiver operating

characteristic curve (AUC) of 0.93 (95% CI 0.92 to 0.95) in a multicentre setting utilizing specimens taken into collection devices by the patient in 9822 individuals [21]. Comparatively at our single centre in 9896 patients, where sampling was undertaken by laboratory staff, the AUC was 0.94 (0.91 to 0.97) [12]. This would suggest that whilst sampling strategies are important the impact on outcome of each approach are similar.

In summary, we have found that a commonly used FIT method shows good consistency of categorizing Hb results against the DG30 threshold of 10 μg/g, and therefore appears suitable for this clinical application. However, there appear numerous sources of variability that require further investigation and optimization and the International Federation of Clinical Chemistry have set up a working group to address these issues [28]. Further studies should investigate the relative contributions of biological variation, sampling technique, Hb stability and method performance in relation to false-negative results. It would be particularly valuable to accurately assess the mass of faeces collected into collection devices when sampling is undertaken by patients, rather than laboratory staff, as this is the setting in which most specimens will be collected. Sources of variation become even more important as FIT is now recommended to reprioritise patients with lower GI cancer symptoms whose tests have been delayed by the COVID-19 pandemic.

This work represents an advance in biomedical science as it provides independent FIT method verification data in a symptomatic primary care setting and identifies sources of variation of importance to colorectal cancer detection.

Summary table

What is known about this subject:
- Faecal immunochemical testing (FIT) is increasingly used to detect haemoglobin (Hb) at low concentrations in specimens from symptomatic primary care patients.
- Several methods are available for FIT but there are limited extended method evaluations in this context against the NICE recommended threshold of 10 μg/g.

What this paper adds:
- The FIT method assessed demonstrated good analytical performance characteristics against the requirements of NICE DG30.
- Sampling technique is subject to high imprecision and appears to be higher in non-homogenised faecal specimens
- Biological variability also appears to contribute to result outcome

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Disclosure statement

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