Review Article

Interpretation of faecal haemoglobin concentration data in colorectal cancer screening and in assessment of symptomatic patients

Callum G. Fraser

Centre for Research into Cancer Prevention and Screening, University of Dundee, Ninewells Hospital and Medical School, Dundee, Scotland, UK
Correspondence to: Professor Callum G. Fraser. Centre for Research into Cancer Prevention and Screening, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland, UK. Email: callum.fraser@nhs.net.

Abstract: Faecal immunochemical tests for haemoglobin (FIT) are used in asymptomatic colorectal cancer screening and in assessment of patients presenting in primary care with lower gastrointestinal symptoms. Many qualitative and quantitative FIT are available. Qualitative FIT give dichotomous results and the most important characteristic for interpretation of test results is the C_{50}, often described as the cut-off. Qualitative FIT have different C_{50} and thus give different clinical outcomes in screening. These are often stated to be simple, but both their performance and interpretation of results are fraught with difficulties. Quantitative FIT have many advantages. In screening, the faecal haemoglobin concentration (f-Hb) used as cut-off can be decided for each endeavour. As the f-Hb cut-off is increased, positivity rate, neoplasia detection rate and sensitivity decrease, while positive predictive value, specificity and interval cancer proportion increase. Different FIT give different outcomes and comparisons are best done using a consistent positivity rate. Many factors affect f-Hb and, although controversial, it has been suggested that sex and age stratified f-Hb cut-offs would have advantages. In consequence, current attention is becoming more focused on risk-scoring approaches incorporating f-Hb and on application of precision medicine strategies. FIT must have a low f-Hb in assessment of the symptomatic. The detection capability of FIT requires harmonisation with regard to terminology, determination and application in both academic research and routine clinical practice. The limit of quantitation is the lowest f-Hb that can be determined when some predefined analytical performance specifications (APS) for the characteristics of bias and imprecision (or total error) are satisfied. Proposals for interim APS are documented. The reporting of numerical data on f-Hb requires further consideration and creation and promulgation of consensus guidelines by international professional bodies.

Keywords: Colorectal cancer (CRC); colorectal disease; faecal haemoglobin; faecal immunochemical test; faecal occult blood test; screening

Received: 15 November 2017; Accepted: 30 November 2017; Published: 18 December 2017.
doi: 10.21037/jlpm.2017.11.01
View this article at: http://dx.doi.org/10.21037/jlpm.2017.11.01

Introduction: faecal immunochemical tests for haemoglobin (FIT)

FIT are now widely used in both opportunistic and programmatic asymptomatic population colorectal cancer (CRC) screening efforts as the best available non-invasive approach (1). In addition, FIT are now becoming applied for assessment of patients presenting in primary care with lower gastrointestinal symptoms (2). FIT are obtainable in two constructs (3), qualitative, which are generally based on immunochromatographic test strips or cassettes, and quantitative, which are usually based on automated immunoturbidimetry, although a variety of other techniques, including enzyme-linked immunosorbent assays (ELISA), are also available (4).

Both qualitative and quantitative FIT usually have easy

to use, hygienic faecal specimen collection devices (5) in which a probe (sometimes termed a stick) attached to the cap of the device is used to collect faeces into dimples or grooves at the end of the probe by either multiple insertions into a single faeces or by scraping across the surface of the faecal sample. Then, the probe is reinserted into the device (sometimes termed a bottle or a tube), which contains a volume of buffer: these buffers are constituted with the aim of conferring stability on any haemoglobin (Hb) present in the faeces. Most devices have collars which remove excess sampled faeces from the probe before its insertion into the buffer

Qualitative FIT provide dichotomous results, i.e., positive/negative or present/absent. In contrast, quantitative FIT provide estimates of the faecal haemoglobin concentration (f-Hb) in the sample (6). Until relatively recently, all data concerning f-Hb were documented using units of ng Hb/mL buffer and, unfortunately, some still adhere to this obsolete approach. Since the different FIT specimen collection devices gather different amounts of faeces into different volumes of buffer, results expressed in these units are not transferable between different FIT. Now, as a result of efforts by the Expert Working Group (EWG) on FIT for Screening, CRC Screening Committee, World Endoscopy Organization, significant global harmonisation has occurred with the wide adoption of µg Hb/g faeces units to report f-Hb (7) although, since the specimen collection devices actually collect volumes of faeces rather than masses, units of µg Hb/mL faeces are more correct, at least theoretically (8). Units of µg Hb/g faeces will be used throughout this review.

Interpretation of results generated with qualitative FIT

Qualitative FIT provide a dichotomous result, usually termed positive or negative in the asymptomatic population screening setting. Such FIT are used to identify, usually in an age range selected population, those who are most likely to have colorectal neoplasia and would benefit from colonoscopy. In this setting, FIT are used as a rule-in test. A positive result means that an increased risk of CRC is present in that participant and further investigation is warranted. A negative result means the participant should be re-invited after the screening interval, currently one year in some countries and two years in others. In contrast, in assessment of the symptomatic, FIT are used to assist, usually along with the results of other investigations, in deciding in patients of any age presenting in primary care with lower abdominal disease warning signs who would be unlikely to benefit from referral to secondary care for colonoscopy. This is FIT applied as a rule-out test. A negative result means there is considerable reassurance that significant colorectal disease (SCD) [CRC + higher-risk adenoma (HRA) + inflammatory bowel disease (IBD)] is not present. A positive result means that the patient may warrant further investigation. But the germane question is—what do positive and negative test results mean?

Sensitivity, detection limit, threshold and other similar terms are often used for the f-Hb at which 50% of qualitative test results are positive and 50% are negative, the f-Hb which effectively leads to further investigation or not. The terminology and the methodology to derive this performance characteristic should always be that of the Clinical Laboratory Standards Institute (CLSI) EP12-A2: Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition (9). The recommended nomenclature for this f-Hb "cut-off" is the C_{50}. It would be of real advantage if all manufacturers applied the methods detailed in this guideline to generate C_{50}, along with confidence intervals, and that they and their suppliers, and then all users, used this terminology and only this terminology.

There are many tests available for the detection of small amounts of blood in faeces: the FDA CLIA test categorization database at July 2017 includes 134 test systems for occult blood in faeces with 129 non-automated methods (10). However large-scale comparative studies of different FIT to detect CRC and advanced adenoma in a screening setting, overall and by stage, are sparse (11), although a number of comparisons of qualitative FIT have been performed. The published comparisons do show that interpretation of the results obtained with qualitative FIT is fraught with difficulties. For example, pairs of overall sensitivity and specificity of six qualitative FIT ranged from 66% and 96% to 92% and 62%, respectively (11). Moreover, it has been stated that, although about two-thirds of the commonly used FIT in the United States performed acceptably on samples spiked with human Hb, some had low sensitivity and specificity and probably should not be used for population-based or other screening (12,13). Interestingly, in an evaluation of the performance of two qualitative FIT at five different f-Hb cut-offs for positivity (14), it was demonstrated that these

yielded similar sensitivities at comparable specificity and, with appropriate adjustment of f-Hb cut-offs, qualitative FIT might be an economic strategy and a comparable alternative to quantitative FIT. One potential consequence is that manufacturers could provide qualitative FIT with performance characteristics as delineated by screening programme organisers. Finally, although manufacturers may document the C_{50} of their qualitative FIT correctly, this can vary from lot to lot of ostensibly identical product giving different screening outcomes over time (15). There are few studies on the use of qualitative FIT in assessment of patients with symptoms (16,17): these have been described in detail previously (18). This might be a fruitful topic for further research.

Irrespective of their clinical application, interpretation of the results of qualitative FIT is often said to be easy and the analysis simple to perform. However, there are many real difficulties in practice, as recently documented (18). The faecal specimen collection is not straightforward since Hb in faeces is unstable and thus, immediate sampling of passed faeces into the FIT specimen collection device is required. Later analysis of a sample collected in the traditional faecal pots widely used in laboratory medicine is unacceptable, since false negative results will occur (19). In addition, the colour development of the lines on the cassettes or strips of qualitative immunochromatographic FIT is very dynamic so that early reading will lead to false negative results and late reading to false positive results: in consequence, accurate timing is required. In addition, the coloured lines are sometimes not easy to interpret, especially when very weak positive test lines are present, unless the analyses are done following adequate training and in adequate light, preferably by those with good visual acuity.

f-Hb cut-offs in CRC screening

Using quantitative FIT, the f-Hb cut-off to be applied in asymptomatic population-based CRC screening can be decided by programme organisers based, for example, on deliberation of the colonoscopy capacity available. The f-Hb cut-off currently used in CRC screening varies considerably among countries, ranging from 10 µg Hb/g faeces, through the most widely used cut-off f-Hb of 20 µg Hb/g faeces (1) to 40 µg Hb/g faeces in New Zealand (20), 47 µg Hb/g faeces in The Netherlands (21) to 80 µg Hb/g faeces in a large pilot evaluation in Scotland (22). Moreover, even in one country, different f-Hb cut-offs may be applied in different regions, such as in Canada (23).

When selecting or monitoring a quantitative FIT in CRC screening, programme organisers and others may wish overall information on the key performance indicators, including uptake (which might depend on the type of specimen collection device used), positivity rate, CRC detection rate and stage detected, adenoma detection rate, positive predictive value for CRC and HRA, and sensitivity and specificity if possible. Most screening programmes use one only f-Hb cut-off to decide which of the participants warrant further investigation, usually colonoscopy. It has been shown that f-Hb is related to the severity of colorectal disease (24). In addition, it has been documented (25) that median f-Hb is higher in those with CRC than those with no or non-neoplastic pathology and those with low-risk adenoma (LRA), and polyp CRC cancers have lower f-Hb than more advanced stage cancers. Higher f-Hb is also found in those with HRA than with LRA, large (>10 mm) compared with small adenoma, and also adenoma displaying high-grade dysplasia compared with low-grade dysplasia. Thus, it is hardly surprising that changing the f-Hb leads to changes in many of the key performance indicators in CRC screening (26-28). As the f-Hb cut-off is increased, the positivity rate decreases, as do CRC and adenoma detection rates and sensitivity, while positive predictive value and specificity increase. Further, as the f-Hb cut-of is increased, the interval cancer proportion, that is the number of CRC found in participants who had a negative screening test result but had a diagnosis before the next screening episode was scheduled, rises (29).

Since it is well known that more men than women get CRC and older than younger people also suffer from CRC, there has been significant work on investigating consequences of sex, in particular, on the key performance indicators. A recent review concluded that the influence of sex on the comparative performance of tests for detecting advanced colorectal neoplasia (AN: CRC + HRA) has not been investigated with sufficient power in any of the studies conducted to date (30). However, as recently comprehensively documented by Arana-Arri *et al.* (31), a considerable body of work exists showing the differences between the sexes. For example, in the Basque Colorectal Cancer Screening Programme, results were obtained on 17,387 positive participants: men had a positivity rate of 8.3% and women 4.8%. The detection rate for AN was 44.0‰ for men and 15.9‰ for women. The number of colonoscopies required decreased in both sexes and all age

groups as the f-Hb cut-off increased. In an earlier study, van Turenhout *et al.* (32) concluded that FIT have a higher sensitivity and lower specificity for CRC in men compared to women and that different f-Hb cut-offs should be used in screening programmes. However, others disagree (33).

In large part, the differences between the sexes and age groups can be explained by the fact that f-Hb is higher in men than in women and increases with age (34,35). Moreover, the differences vary from country to country (36). In addition, f-Hb is dependent on deprivation, or socioeconomic status, higher f-Hb being found in the more deprived (35,37). Further, the distribution of f-Hb depends on the screening round, higher overall f-Hb being found in the initial prevalence screening round as compared to subsequent incidence screening rounds (38). In consequence, the following strategy has been proposed to better use the numerical f-Hb generated in screening programmes (39):

- Examine the f-Hb distributions in pilot participants, or very early in the programme, by age and sex;
- Determine positivity at different f-Hb cut-off(s) by age and sex;
- Assess the characteristics of the invited population in determining the f-Hb cut-off(s) to be used to obtain the positivity required;
- Change the f-Hb cut-off(s) where necessary, using the f-Hb distributions to set these objectively;
- Use examination of the f-Hb to investigate problems;
- Perform this assessment regularly as the programme evolves.

Since f-Hb is dependent of a number of factors, some have advocated that the use of numerical f-Hb could be much improved by incorporation of the f-Hb of an individual into some type of "risk score": this has been addressed in a recent editorial in this journal (40). A summary follows. Chen *et al.* showed that f-Hb at first screening predicts subsequent risk of incident colorectal neoplasia (41) and that f-Hb is an independent predictor of the risk of colorectal neoplasia (42). A recent study showed that f-Hb is related to detection of AN in the next screening round (43).

It has been suggested that a personalised approach to screening could enable those at greatest risk to be referred for colonoscopy and a recent opinion piece detailed some ideas and documented all the work supporting this potentially useful risk-scoring approach (44). In addition, a systematic review of risk prediction models that could be used for personalisation in CRC screening has been recently published: few to date actually incorporate f-Hb (45). These concepts have been superbly elaborated and much supported in a recent study of Grobbee *et al.* (46) discussed in detail in the recent editorial (40): risk assessment based on f-Hb is here to stay and further developments are awaited with much interest!

The outcomes of screening using quantitative FIT depend on the f-Hb cut-off(s) applied, whether single, multiple or in risk-scoring algorithms. Manufacturers of quantitative FIT provide detailed instructions for use. In these, data are given on the range in which numerical estimates of f-Hb can be reported. This is often termed the analytical working range: this somewhat unclear concept will be expanded upon in a later section of this review. The working range has been defined by one authoritative body as: the interval over which the method provides results with an acceptable uncertainty (47). It is important to note that, as superbly shown in a recent work on comparison of nine quantitative FIT (4), manufactures of quantitative FIT have very different analytical ranges, irrespective of how these have been determined and documented, and advocate very different f-Hb cut-offs for screening as shown in *Table 1*. The f-Hb cut-offs currently advocated for use in CRC screening generally do fall within the working ranges documented by the manufacturers of FIT as shown in *Table 1*.

Moreover, even if the f-Hb cut-offs are harmonised to a single value using the EWG advocated units of μg Hb/g faeces, the outcomes are not identical (4). This work has also been the subject of an editorial in this journal (48) which discusses, in detail, the comparison of quantitative FIT analytical systems. The editorial gives recommendations on reporting of f-Hb concentration data and, very importantly, advocates ubiquitous application of the FITTER guidelines proposed by the EWG (49) in all publications concerning comparison of FIT. Interestingly, it was shown that the apparent large differences in clinical outcomes almost entirely disappeared when f-Hb cut-offs were adjusted so that all FIT achieved defined specificities at which sensitivities were also similar. The final conclusion of Gies *et al.* (4) was that, instead of simply using f-Hb cut-offs recommended by the manufacturers, screening programmes should choose f-Hb based on intended levels of specificity and manageable positivity rates. Of course, it is much easier to manipulate positivity rates and, indeed, two recent very large comparisons of two commonly used FIT analytical systems demonstrated differences if the same f-Hb cut-off was used to enhance comparison, but such differences were much less apparent when comparisons were done at

Table 1 Overview of the nine quantitative FIT studies in Gies *et al.* (4)

Quantitative FIT brand	Manufacturer	Faecal sampling device (faecal mass/buffer volume)	Analytical instrument	Analytical range (μg Hb/g faeces)	Pre-set threshold (μg Hb/g faeces)
Laboratory-based					
CAREprime Hb	Alfresa Pharma, Tokyo, Japan	Specimen Collection Container A (10 mg/1.9 mL)	CAREprime	0.76–228.0	6.30
Hb ELISA	Immundiagnostik, Bensheim, Germany	IDK Extract (15 mg/1.5 mL)	Dynex System	0.086–50.0	2.00
OC Sensor	Eiken Chemical, Tokyo, Japan	OC Auto-Sampling Bottle3 (10 mg/2.0 mL)	OC Sensor io	10–200	10
Ridascreen Hb	R-Biopharm, Darmstadt, Germany	RIDA TUBE Hb (10 mg/2.5 mL)	Dynex System	0.65–50.0	8.00
SENTiFIT-FOB Gold	Sentinel Diagnostics, Milan, Italy	SENTiFIT pierceTube (10 mg/1.7 mL)	SENTiFIT 270 analyzer	1.70–129.88	17.0
Point of care					
Eurolyser FOB test	Eurolyser Diagnostica, Salzburg, Austria	Eurolyser FOB sample Collector (19.9 mg/1.6 mL)	Eurolyser CUBE	2.01–80.4	8.04
ImmoCARE-C	CARE diagnostica, Voerde, Germany	Sample Collection Tube (20 mg/2.5 mL)	CAREcube	3.75–250.0	6.25
QuantOn Hem	Immundiagnostik, Bensheim, Germany	QuantOn Hem TUBE (15 mg/1.5 mL)	Smartphone* with App/iOS	0.30–100.0	3.70
QuikRead go iFOBT	Orion Diagnostica, Espoo, Finland	QuikRead FOB Sampling Set (10 mg/2.0 mL)	QuikRead go	5–200	15

*, iPhone 6S was used for this study. FIT, faecal immunochemical test; Hb, haemoglobin; App, mobile application software; iOS, iPhone operating system.

equivalent positivity rate (50,51).

f-Hb cut-offs in assessment of the symptomatic

In contrast to the use of quantitative FIT in CRC screening, when used in the triage of patients presenting in primary care with lower gastrointestinal symptoms, a low f-Hb is required (2). The National Institute for Health and Care Excellence (NICE), whose evidence-based guidelines are widely adopted, recommend a f-Hb cut-off of 10 μg Hb/g faeces in this clinical setting (52). The evidence for the NICE DG 30 guidance on: quantitative faecal immunochemical tests to guide referral for CRC in primary care, is documented in depth in a systematic review (2), and the use of FIT in the timely diagnosis of colorectal disease has been reviewed in depth by Steele and Fraser (18). As stated above, in this clinical setting, quantitative FIT are used along with symptoms and results of other investigations as rule-out tests for SCD (53). A negative result implies that there is little likelihood of SCD. A positive result suggests the need for referral for further investigation. The major interest in this application is that, in part because of the success of screening programmes which give information on symptoms to those with negative results and urge consultation in primary care if these occur, together with local, regional and national efforts encouraging individuals with symptoms of lower gastrointestinal disease to seek early clinical care, the colonoscopy resource is overwhelmed. However, symptoms are very poor indicators of SCD (54) and FIT provide a means to triage patents with symptoms, saving a considerable number of referrals to secondary care.

The use of a single low f-Hb cut-off is advocated, but, as for CRC screening, it might be that using other important variables such as age, sex, body mass index, smoking habits, diet, previous family history, alcohol intake, exercise and other relevant variables could improve the diagnostic accuracy through risk-scoring. A recent review assesses the value of the 15 models published to date on risk prediction

for CRC in symptomatic patients (55). The models have high sensitivity for CRC (90–98%), areas under the receiver operating characteristic (ROC) curves were >0.85 and there was better discrimination when compared with referral guidelines such as those of NICE CG67 (56) and of the Scottish Intercollegiate Guidelines Network (SIGN) 126 (57). It is important to note that the NICE guideline on diagnosis and management of CRC has been recently updated to NG12 (58). This recent guideline has been compared to the use of f-Hb in three Scottish studies: it was concluded that f-Hb provides a good rule-out test for SCD and has significantly higher overall diagnostic accuracy than NG12 (59). However, only four of the 15 models included a positive test for the presence of occult blood in faeces as a risk factor. More recently, Cubiella *et al.* have published a complex risk-scoring model called COLONOPREDICT (60) and a simpler model, termed the FAST Score, the faecal Hb, age and sex test score, with these three variables (61), but it remains to be seen whether these off any advantage in practice over simpler use of f-Hb alone.

Reporting low f-Hb

FIT are used in asymptomatic population screening and in the assessment of the symptomatic. Although, as outlined above and in more detail elsewhere, these are very different applications (62), there is now significant interest in "low" f-Hb in both clinical settings. The reason "analytical detection capability" is important for f-Hb estimations is mainly because the detection limits of current FIT are near the decision limits in both clinical settings. Indeed, it seems now very common for f-Hb data below that defined by manufacturers as the lower limit of the analytical working range to be documented, as detailed in a recent editorial (40): for example, Brenner and Werner (28) used a broad range of possible f-Hb cut-offs between 1 and 50 µg Hb/g faeces, Grobbee *et al.* (46) explored f-Hb below the cut-off of 10 µg Hb/g faeces in categories; 0, >0–2, ≥2–4, ≥4–6, ≥6–8 and ≥8–10 µg Hb/g faeces, Aniwan *et al.* (63) used different f-Hb cut-offs, including 5 µg Hb/g faeces, clearly below the lower working limit of 10 µg Hb/g faeces and Mowat *et al.* (64) termed any result that was reported as a positive numerical result greater than 0 µg Hb/g faeces as a "detectable" f-Hb.

The current discrepancies in reporting low f-Hb may be due to misunderstanding caused by the plethora of terms used for the detection capability of FIT and variation in the recommendations and guidelines from different professional and official groups (39). It has been proposed (40) that the recommendations of the Clinical and Laboratory Standards Institute (CLSI), supported by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (65), should be widely applied. A simple summary prepared by a well-known manufacturer of laboratory equipment and reagents is available on the Internet (66).

The terms recommended to be used to describe the detection capability are Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ). These terms, which should be used by all, follow:

- LoB: LoB is the highest measured result likely to be observed (typically at 95% certainty) for a sample containing no f-Hb (a blank sample);
- LoD: LoD is the lowest concentration where f-Hb can be detected 95% of the time;
- LoQ: LoQ is the lowest f-Hb that can be determined when some predefined analytical performance specifications (APS) for the characteristics of bias and imprecision (or total error) are satisfied. The APS should be established using an accepted and well-documented strategy.

Delineation of desirable analytical performance through setting of objective APS is a necessary prerequisite to documentation of the LoQ. The setting of APS has been the subject of much research for many years but, recently, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) published a consensus statement on defining APS (67). The consensus agreed three different models to set APS:

- Model 1: strategies based on the effect of analytical performance on clinical outcomes;
- Model 2: strategies based on components of biological variation of the measurand;
- Model 3: strategies based on state of the art.

For f-Hb concentrations, there are no studies on the effect of analytical performance on clinical outcomes. There are no data on the biological variation of f-Hb. Thus, it seems that interim APS should be based upon the state of the art. Interim APS for f-Hb analyses have been proposed by the author to the Working Group on FIT of the Scientific Division of the IFCC (68) as follows:

- Analytical bias should be <2%;
- Analytical imprecision should be coefficient of variation (CV) <5%;
- Total analytical error (bias + 1.65 × CV) should be <10%.

A proposal documented previously in this journal (40) is

that the LoD could be used in the academic research setting in order to obtain data that could impact the future use of f-Hb in real clinical practice, while the LoQ is the lowest f-Hb that can be reported in routine clinical practice. However, a variety of more sophisticated reporting options are possible (65,66) and further work is required on how numerical data on f-Hb < LoD, f-Hb between LoD and LoQ and f-Hb ≥ LoQ should be reported for academic and routine clinical uses.

Conclusions and future needs

Although FIT are used in asymptomatic CRC screening and in assessment of patients presenting in primary care with lower gastrointestinal symptoms, some problems remain. Many qualitative and quantitative FIT are available. Qualitative FIT give dichotomous results and the most important characteristic for interpretation of test results is the C_{50}, often described as the cut-off: the determination of the C_{50} and the ubiquitous use of this terminology is required. The performance and the interpretation of results of qualitative FIT are fraught with difficulties and more objective studies on their use in CRC screening are required, as are comparison of different FIT in real practice. Their application in assessment of patients presenting with lower abdominal symptoms requires further study. Quantitative FIT have many advantages: in screening, the f-Hb used as cut-off can be decided for each screening effort. Different FIT give different outcomes and, although there are a variety of strategies available for the comparison of FIT (69), comparisons of clinical outcomes are best done using a consistent positivity rate. Standardisation of the assignment of f-Hb to calibrators with good traceability through the hierarchy of materials and methods would assist in harmonisation of results across different FIT. Many factors affect f-Hb and incorporation of the important variables in risk-scoring approaches warrants further attention as do applications of the currently popular concepts of precision medicine. FIT must have a low f-Hb for assessment of patients with symptoms. The terminology concerning the detection capability of FIT requires harmonisation and determination and application of these concepts is required urgently in both academic research and routine clinical practice. The limit of quantitation is the lowest f-Hb that can be determined when some predefined APS for the characteristics of bias and imprecision (or total error) are satisfied. APS for f-Hb analyses require investigation, agreement, dissemination and application. Finally, the reporting of numerical data on f-Hb requires considerable further consideration and then promulgation of consensus guidelines from international professional bodies.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Hemant Goyal) for the series "Role of biomarkers in gastrointestinal disorders" published in *Journal of Laboratory and Precision Medicine*. The article has undergone external peer review.

Conflicts of Interest: The series "Role of biomarkers in gastrointestinal disorders" was commissioned by the editorial office without any funding or sponsorship. The author has undertaken paid consultancy with Immunostics Inc., Ocean, NJ, USA, and Kyowa-Medex Co. Ltd, Tokyo, Japan, and has received support for attendance at conferences from Alpha Labs Ltd, Eastleigh, Hants, UK.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Young GP, Symonds EL, Allison JE, et al. Advances in fecal occult blood tests: the FIT revolution. Dig Dis Sci 2015;60:609-22.
2. Westwood M, Lang S, Armstrong N, et al. Faecal immunochemical tests (FIT) can help to rule out colorectal cancer in patients presenting in primary care with lower abdominal symptoms: a systematic review conducted to

inform new NICE DG30 diagnostic guidance. BMC Med 2017;15:189.
3. Allison JE, Fraser CG, Halloran SP, et al. Population screening for colorectal cancer means getting FIT: the past, present, and future of colorectal cancer screening using the fecal immunochemical test for hemoglobin (FIT). Gut Liver 2014;8:117-30.
4. Gies A, Cuk K, Schrotz-King P, et al. Direct comparison of diagnostic performance of 9 quantitative fecal immunochemical tests for colorectal cancer screening. Gastroenterology 2017. [Epub ahead of print].
5. Rapi S, Berardi M, Cellai F, et al. Effects of fecal sampling on preanalytical and analytical phases in quantitative fecal immunochemical tests for hemoglobin. Int J Biol Markers 2017;32:e261-6.
6. Fraser CG, Allison JE, Young GP, et al. Quantitation of hemoglobin improves fecal immunochemical tests for non-invasive screening. Clin Gastroenterol Hepatol 2013;11:839-40.
7. Fraser CG, Allison JE, Halloran SP, et al. A proposal to standardize reporting units for fecal immunochemical tests for hemoglobin. J Natl Cancer Inst 2012;104:810-4.
8. Fraser CG, Rapi S, Rubeca T. RE: A proposal to standardize reporting units for fecal immunochemical tests for hemoglobin. J Natl Cancer Inst 2015;108. pii: djv312.
9. CLSI. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
10. Clinical Laboratory Improvement Amendments of 1988 (CLIA); Fecal Occult Blood (FOB) Testing. Federal Register 2017;82:No. 202.488770-3. Available online: https://www.gpo.gov/fdsys/pkg/FR-2017-10-20/pdf/2017-22813
11. Tao S, Seiler CM, Ronellenfitsch U, et al. Comparative evaluation of nine faecal immunochemical tests for the detection of colorectal cancer. Acta Oncol 2013;52:1667-75.
12. Daly JM, Bay CP, Levy BT. Evaluation of fecal immunochemical tests for colorectal cancer screening. J Prim Care Community Health 2013;4:245-50.
13. Daly JM, Xu Y, Levy BT. Which fecal immunochemical test should I choose? J Prim Care Community Health 2017. [Epub ahead of print].
14. Tao S, Brenner H. Well adjusted qualitative immunochemical faecal occult blood tests could be a promising alternative for inexpensive, high-quality colorectal cancer screening. Eur J Cancer Prev 2013;22:305-10.
15. McDonald PJ, Anderson CM, Fraser CG. Acceptance quality checks for qualitative fecal immunochemical tests ensure screening program consistency. Int J Cancer 2011;128:247-8.
16. Elias SG, Kok L, de Wit NJ, et al. Is there an added value of faecal calprotectin and haemoglobin in the diagnostic work-up for primary care patients suspected of significant colorectal disease? A cross-sectional diagnostic study. BMC Med 2016;14:141.
17. Högberg C, Karling P, Rutegård J, et al. Diagnosing colorectal cancer and inflammatory bowel disease in primary care: The usefulness of tests for faecal haemoglobin, faecal calprotectin, anaemia and iron deficiency. A prospective study. Scand J Gastroenterol 2017;52:69-75.
18. Steele RJ, Fraser CG. Faecal Immunochemical Tests (FIT) for Haemoglobin for Timely Assessment of Patients with Symptoms of Colorectal Disease. In: Olsson L. editor. Timely Diagnosis of Colorectal Cancer. Springer, Cham, 2018.
19. Brown LF, Fraser CG. Effect of delay in sampling on haemoglobin determined by faecal immunochemical tests. Ann Clin Biochem 2008;45:604-5.
20. National Screening Unit. International comparisons with New Zealand's bowel screening programme. Available online: https://www.nsu.govt.nz/health-professionals/national-bowel-screening-programme/international-comparisons-new-zealand%E2%80%99s
21. Toes-Zoutendijk E, van Leerdam ME, Dekker E, et al. Real-time monitoring of results during first year of Dutch colorectal cancer screening program and optimization by altering fecal immunochemical test cut-off levels. Gastroenterology 2017;152:767-75.e2
22. Steele RJ, McDonald PJ, Digby J, et al. Clinical outcomes using a faecal immunochemical test for haemoglobin as a first-line test in a national programme constrained by colonoscopy capacity. United European Gastroenterol J 2013;1:198-205.
23. Canadian Partnership Against Cancer. Colorectal cancer screening in Canada: Monitoring & evaluation of quality indicators. Results report: January 2013 — December 2014. Available online: https://content.cancerview.ca/download/cv/prevention_and_screening/screening_and_early_diagnosis/documents/colorectal_cancer_screening_canada_monitoring_evaluating_report_2013?attachment=0
24. Levi Z, Rozen P, Hazazi R, et al. A quantitative

immunochemical fecal occult blood test for colorectal neoplasia. Ann Intern Med 2007;146:244-55.
25. Digby J, Fraser CG, Carey FA, et al. Faecal haemoglobin concentration is related to severity of colorectal neoplasia. J Clin Pathol 2013;66:415-9.
26. Hol L, Wilschut JA, van Ballegooijen M, et al. Screening for colorectal cancer: random comparison of guaiac and immunochemical faecal occult blood testing at different cut-off levels. Br J Cancer 2009;100:1103-10.
27. Moss S, Mathews C, Day TJ, et al. Increased uptake and improved outcomes of bowel cancer screening with a faecal immunochemical test: results from a pilot study within the national screening programme in England. Gut 2017;66:1631-44.
28. Brenner H, Werner S. Selecting a cut-off for colorectal cancer screening with a fecal immunochemical test. Clin Transl Gastroenterol 2017;8:e111.
29. Digby J, Fraser CG, Carey FA, et al. Interval cancers using a quantitative faecal immunochemical test (FIT) for haemoglobin when colonoscopy capacity is limited. J Med Screen 2016;23:130-4.
30. Massat NJ, Moss SM, Halloran SP, et al. Screening and primary prevention of colorectal cancer: a review of sex-specific and site-specific differences. J Med Screen 2013;20:125-48.
31. Arana-Arri E, Idigoras I, Uranga B, et al. Population-based colorectal cancer screening programmes using a faecal immunochemical test: should faecal haemoglobin cut-offs differ by age and sex? BMC Cancer 2017;17:577.
32. van Turenhout ST, Oort FA, van der Hulst RW, et al. Prospective cross-sectional study on faecal immunochemical tests: sex specific cut-off values to obtain equal sensitivity for colorectal cancer? BMC Gastroenterol 2014;14:217.
33. Alvarez-Urturi C, Andreu M, Hernandez C, et al. Impact of age- and gender-specific cut-off values for the fecal immunochemical test for hemoglobin in colorectal cancer screening. Dig Liver Dis 2016;48:542-51.
34. McDonald PJ, Strachan JA, Digby J, et al. Faecal haemoglobin concentrations by gender and age: implications for population-based screening for colorectal cancer. Clin Chem Lab Med 2011;50:935-40.
35. Symonds EL, Osborne JM, Cole SR, et al. Factors affecting faecal immunochemical test positive rates: demographic, pathological, behavioural and environmental variables. J Med Screen 2015;22:187-93.
36. Fraser CG, Rubeca T, Rapi S, et al. Faecal haemoglobin concentrations vary with sex and age, but data are not transferable across geography for colorectal cancer screening. Clin Chem Lab Med 2014;52:1211-6.
37. Digby J, McDonald PJ, Strachan JA, et al. Deprivation and faecal haemoglobin: implications for bowel cancer screening. J Med Screen 2014;21:95-7.
38. Kapidzic A, van de Meulen P, Hol L, et al. Gender differences in fecal immunochemical test performance for early detection of colorectal neoplasia. Clin Gastroenterol Hepatol 2015;13:1464-71.e4.
39. Fraser CG. Assessment of faecal haemoglobin concentration distributions is vital for faecal immunochemical test (FIT)-based colorectal cancer screening programmes. J Med Screen 2016;23:52-3.
40. Fraser CG. Faecal haemoglobin concentration and personalised assessment of the risk of colorectal neoplasia. J Lab Precis Med 2017;2:71.
41. Chen LS, Yen AM, Chiu SY, et al. Baseline faecal occult blood concentration as a predictor of incident colorectal neoplasia: longitudinal follow-up of a Taiwanese population-based colorectal cancer screening cohort. Lancet Oncol 2011;12:551-8.
42. Chen LS, Yen AM, Fraser CG, et al. Impact of faecal haemoglobin concentration on colorectal cancer mortality and all-cause death. BMJ Open 2013;3:e003740.
43. Digby J, Fraser CG, Carey FA, et al. Faecal haemoglobin concentration is related to detection of advanced colorectal neoplasia in the next screening round. J Med Screen 2017;24:62-8.
44. Cooper JA, Moss SM, Smith S, et al. FIT for the future: a case for risk-based colorectal cancer screening using the faecal immunochemical test. Colorectal Dis 2016;18:650-3.
45. Ma GK, Ladabaum U. Personalizing colorectal cancer screening: a systematic review of models to predict risk of colorectal neoplasia. Clin Gastroenterol Hepatol 2014;12:1624-34.e1.
46. Grobbee EJ, Schreuders EH, Hansen BE, et al. Association between concentrations of hemoglobin determined by fecal immunochemical tests and long-term development of advanced colorectal neoplasia. Gastroenterology 2017;153:1251-9.e2
47. Magnusson B, Örnemark U. editors. Eurachem Guide: The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics. 2nd edition. 2014. Available online: https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf
48. Allison JE, Fraser CG. Comparison of quantitative faecal

immunochemical tests for haemoglobin (FIT) analytical systems. J Lab Precis Med 2017. [in press].
49. Fraser CG, Allison JE, Young GP, et al. Improving the reporting of evaluations of faecal immunochemical tests for haemoglobin: the FITTER standard and checklist. Eur J Cancer Prev 2015;24:24-6.
50. Grobbee EJ, van der Vlugt M, van Vuuren AJ, et al. A randomised comparison of two faecal immunochemical tests in population-based colorectal cancer screening. Gut 2017;66:1975-82.
51. Passamonti B, Malaspina M, Fraser CG, et al. A comparative effectiveness trial of two faecal immunochemical tests for haemoglobin (FIT). Assessment of test performance and adherence in a single round of a population-based screening programme for colorectal cancer. Gut 2016. [Epub ahead of print].
52. National Institute for Health and Care Excellence. Quantitative faecal immunochemical tests to guide referral for colorectal cancer in primary care. 2017. Available online: https://www.nice.org.uk/guidance/dg30
53. Fraser CG. Diagnostic work-up of patients presenting in primary care with lower abdominal symptoms: which faecal test and triage strategy should be used? BMC Med 2016;14:139.
54. Vega P, Valentín F, Cubiella J. Colorectal cancer diagnosis: Pitfalls and opportunities. World J Gastrointest Oncol 2015;7:422-33.
55. Williams TG, Cubiella J, Griffin SJ, et al. Risk prediction models for colorectal cancer in people with symptoms: a systematic review. BMC Gastroenterol 2016;16:63.
56. National Institute for Health and Care Excellence. Referral guidelines for suspected cancer. 2005. Available online: https://www.nice.org.uk/guidance/cg27
57. Scottish Intercollegiate Guidelines Network. Diagnosis and management of colorectal cancer. Guideline 126. Edinburgh, SIGN, Revised 2016. Available online: http://www.sign.ac.uk/assets/sign126.pdf
58. National Institute for Health and Care Excellence. Suspected cancer: recognition and referral. NICE guideline [NG12]. Manchester, NICE, Last updated 2017. Available online: https://www.nice.org.uk/guidance/ng12
59. Quyn AJ, Steele RJ, Digby J, et al. Application of NICE guideline NG12 to the initial assessment of patients with lower gastrointestinal symptoms: not FIT for purpose. Ann Clin Biochem 2017. [Epub ahead of print].
60. Cubiella J, Vega P, Salve M, et al. Development and external validation of a faecal immunochemical test-based prediction model for colorectal cancer detection in symptomatic patients. BMC Med 2016;14:128.
61. Cubiella J, Digby J, Rodríguez-Alonso L, et al. The fecal hemoglobin concentration, age and sex test score: Development and external validation of a simple prediction tool for colorectal cancer detection in symptomatic patients. Int J Cancer 2017;140:2201-11.
62. Fraser CG. FIT: A tale of two settings. Gastroenterology Today 2017;27(2):38-9. Available online: http://www.gastroenterologytoday.com/
63. Aniwan S, Ratanachu-Ek T, Pongprasobchai S, et al. impact of fecal Hb levels on advanced neoplasia detection and the diagnostic miss rate for colorectal cancer screening in high-risk vs. average-risk subjects: a multi-center study. Clin Transl Gastroenterol 2017;8:e113.
64. Mowat C, Digby J, Strachan JA, et al. Faecal haemoglobin and faecal calprotectin as indicators of bowel disease in patients presenting to primary care with bowel symptoms. Gut 2016;65:1463-9.
65. CLSI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition. CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
66. Beckman Coulter. Understanding detection capability: LoB, LoD and LoQ in the clinical laboratory. Available online: https://www.beckmancoulter.com/ucm/idc/groups/validatedcustomer/@wsr/@literature/documents/document/glb_bci_155969.pdf
67. Sandberg S, Fraser CG, Horvath AR, et al. Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem Lab Med 2015;53:833-5.
68. Benton SC. IFCC - FIT Working Group (FIT-WG). IFCC e-news 2017:16-7. Available online: http://www.ifcc.org/media/461890/IFCCeNewsJune2017.pdf
69. Fraser CG. Comparison of quantitative faecal immunochemical tests for haemoglobin (FIT) for asymptomatic population screening. Transl Cancer Res 2016;5:S916-9.

doi: 10.21037/jlpm.2017.11.01
Cite this article as: Fraser CG. Interpretation of faecal haemoglobin concentration data in colorectal cancer screening and in assessment of symptomatic patients. J Lab Precis Med 2017;2:96.

