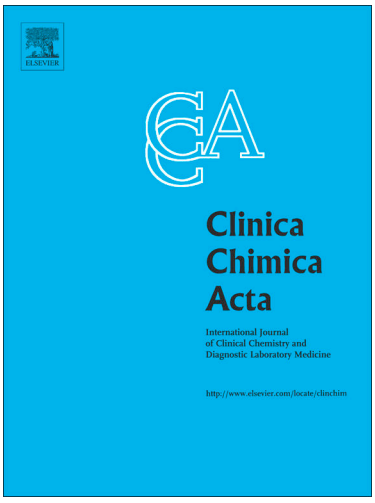

Faecal Immunochemical Tests for Haemoglobin: Analytical Challenges and Potential Solutions

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Faecal Immunochemical Tests for Haemoglobin: Analytical Challenges and Potential Solutions

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Abbreviations: Faecal immunochemical test for haemoglobin (FIT); International Federation of Clinical Chemistry faecal immunochemical test for haemoglobin working group (IFCC FIT-WG); faecal haemoglobin (f-Hb); external quality assessment (EQA); Internal quality control (IQC

Abstract

Quantitative faecal immunochemical tests for haemoglobin (FIT) are being used increasingly around the world in colorectal cancer screening programmes, and in patients presenting with lower bowel symptoms to determine who should proceed to further bowel visualisation investigations, usually colonoscopy. The clinical utility of FIT is well reported. There are a number of analytical challenges including pre-analytical variation, difficulty setting up external quality assessment schemes, access to third party internal quality control material and a lack of standardisation or harmonisation of FIT methods. Here we report the work of the International Federation of Clinical Chemistry FIT Working Group. We provide an overview of the main pre-analytical variables; discuss different approaches to external quality assurance of FIT; propose a solution to third party internal quality assurance materials and summarise the challenges of standardisation and harmonisation of FIT.

Background

Colorectal cancer (CRC) is one of the most prevalent cancers worldwide [1]. Early detection of neoplastic lesions can provide the best opportunity for treatment and prevention. CRC and adenomas (sometimes neoplastic precursors of CRC) can shed blood, and therefore examining faecal samples for occult blood can indicate an increased likelihood of CRC or adenoma being present. This can be achieved with faecal immunochemical tests for haemoglobin (FIT) which utilise antibodies that bind to the globin moiety of haemoglobin (Hb) to detect Hb in faeces.

Quantitative FIT is used in screening programmes around the world [2, 3] with a positive result, ie. a faecal Hb concentration (f-Hb) above a defined threshold, determining who should proceed to further bowel visualisation investigations, usually colonoscopy. Different thresholds are used to define a positive result, often decided by the available colonoscopy resource. Thresholds used in screening programmes usually range from 15 µg Hb/g faeces to 150 µg Hb/g faeces [2].

More recently, a growing body of evidence has supported the use of FIT, alongside clinical signs and symptoms and the full blood count, for triaging patients presenting in primary with care with lower bowel symptoms to bowel visualisation [4, 5]. England has incorporated the use of FIT into the NICE guidelines to guide referral for suspected CRC [6], at least for patients at low risk of CRC, with a low threshold for positivity at 10 µg Hb/g faeces.

Evidence also suggests that f-Hb can be used to assess future risk for neoplasia. Individuals with a f-Hb that is above the examinational limit of quantitation (but below

the threshold applied for follow-up), have a higher risk of future advanced neoplasia compared to those with a f-Hb below the limit of detection. This has been observed both in screening programmes and in FIT used in post-polypectomy surveillance [7-12].

Whilst the clinical utility of FIT is well recognised, there has been very little attention focused on the examination aspects of the investigation.

FIT are available in qualitative or quantitative formats. In 2012, the World Organization for Endoscopy (WEO), Colorectal Cancer Screening Committee (CRCSC), Expert Working Group (EWG) on FIT for Screening identified 47 different systems available on the market [13]. Those that are used in laboratories are generally quantitative examinations and there are four FIT systems widely available for laboratory use, and hence most commonly used across the world (HM-JACKarc, Hitachi Chemical Diagnostics Systems Co., Ltd, Tokyo, Japan; NS-Prime, Alfresa Pharma Corporation, Osaka, Japan; OC-Sensor PLEDIA, Eiken Chemical Co. Ltd, Tokyo, Japan and FOB Gold, Sentinel Diagnostics, Milan, Italy). The examination performance characteristics of these have recently been evaluated [14]. All four systems were demonstrated to perform well against the performance criteria documented by the manufacturers.

With the increasing use of FIT in a number of different clinical scenarios, the challenges and weaknesses of the examination methods need to be addressed.

The International Federation of Clinical Chemistry and Laboratory Medicine, Scientific Division, Working Group on FIT (IFCC FIT-WG) was set up in 2017 to address these crucial issues [15]. The terms of reference that were set when the group initially formed are described in table 1. Here we attempt to describe the

challenges associated with each term of reference and any progress made by the IFCC FIT-WG.

Comparability of measurement results

As described above, the ability to set different f-Hb thresholds for referral is important for FIT to be used in screening programmes, as well as for assessment of symptomatic patients and in post-polypectomy surveillance programmes. There are many different FIT systems available [16]; as well as the four laboratory analysers already documented, and there are large numbers of qualitative and quantitative point of care systems available. Currently, these FIT systems do not use calibration techniques that are traceable to an international reference preparation with concentration assigned by a high order method. In screening programmes, different systems give the same clinical outcomes but only related to the positivity, not the f-Hb threshold [17-19]. The clinical impact of the differences in numerical values obtained on the different systems has not been well studied, however, evidence has demonstrated that different f-Hb are obtained when both homogenous and patient samples are analyzed on the different FIT systems [19-22], as would be expected with examinations that are not harmonised.

The current lack of result comparability among the different FIT systems limits the transfer of a threshold recommended by an expert group or clinical research studies to screening programmes or to symptomatic services because the threshold is only valid for a specific FIT system. This is especially important as new FIT systems are introduced into clinical programmes. Enhancement of the result comparability would facilitate the use of common thresholds, reduce health-care costs, improve clinical management and lowers the risk of clinical error. The following aspects are essential to achieve that goal: universal measurement units, acceptable measurement

uncertainties, suitable internal quality control (IQC) and external quality assessment (EQA) materials and traceability of measured values.[23].

The need for universal measurement units of FIT was recognized by the WEO, CRCSC, EWG on FIT for Screening [24]. This Group recommended standardising reporting units for expressing the result from ng/ml corresponding to the concentration of Hb in the buffer of the specimen collection device, to µg Hb/g faeces eliminating the variability of the mass of faeces collected into the volume of buffer [25]. This is now accepted international practice, although, pedantically, the proper units to use are also argued to be µg Hb/ml faeces since the devices collect a consistent volume dependent on the geometry of the grooves or dimples of the collection device into a constant volume of buffer. Possibly only the use of µg Hb/ml faeces as units might enable f-Hb information to be used to reduce sampling related variability [26]. Starting from data reported in literature on faecal sampling [27] the uncertainty related to the pre-analytical phase ranges between 16 to 31 % for the different FIT systems using commercial sampling devices. This component of uncertainty will significantly affect the expanded uncertainty (U) of the f-Hb methods and should be reduced in order to minimize variation between results.

The universal measurement units were just a first step. Big challenges remain to obtain comparable results among the different FIT systems. Because of the inherent difficulties associated with measuring Hb in a faecal matrix, the ability to harmonise any aspect of the method will prove challenging.

Pre-examination (pre-analytical) variables

When considering how to harmonise FIT systems, it is important to consider not only the examination variables, but also the pre-examination variables. FIT are susceptible to a large number of pre-examination variables. Each manufacturer of FIT systems has designed a different sampling probe [27]. The probe surface, as well as number and depth of grooves or dimples in the probe, and tightness of the collar through which the probe is inserted back into the device after specimen collection have all been shown to affect the mass of faeces collected [28, 29]. Different manufacturers provide different guidance on how to collect a sample (dip in to the sample or scrape along the surface). The volume of buffer within the collection device is also specific to each device. It has been demonstrated that the faecal sample itself will affect results [30]. Faecal samples are not homogeneous, and any blood shed from colorectal lesions is unlikely to be evenly distributed throughout the passed faeces. In addition, the consistency of the sample itself, as per the Bristol Stool Scale [31] will influence the ease with which the specimen is collected and the instructions provided to patients on how to collect the sample differ between manufacturer, for example collection of material across a large surface area versus single point sampling [32, 33]. A recent study has demonstrated the wide variability that exists in the quantity of specimen collected in a surveillance programme [34].

The stability of the Hb in the specimen collected into the device is affected by both storage and transit temperatures [35, 36]. This is supposedly mitigated by the buffers used by manufacturers, but their composition, including preservatives and stabilisers, vary [30, 37] [38] and the exact composition of each buffer is not publicly available. At 5 °C, almost all FIT showed fairly stable Hb results throughout a 7 day period. At 20 °C, most FIT still showed fairly stable results over 4 days, whereas

positivity rates significantly declined from day 4 onwards for most FIT at 35 °C. The Hb degradation due to higher temperatures can result in the measured f-Hb being below the positivity threshold and colorectal disease missed [39]. With the focus being on continual improvement and evolution of FIT systems, manufacturers also change formulation of some of their reagents with the impact being that results from a single system might not be transferable over time [40, 41].

An additional variable relates to the Hb variant present in the faeces and that manufacturers use different polyclonal Hb antibodies. It is not known exactly what each manufacturer's antibody system is detecting in terms of the globin moiety. Whether these products are all measuring human Hb, all its variants and significant degradation products is not clear. Carroll et al [42] investigated the impact of 20 different Hb variants on f-Hb results. All of the variants with mutations in the globin molecule were adequately detected by the polyclonal antibody systems in the four laboratory analysers tested. However, the assessment of variants with missing alpha or beta globin chains resulted in a low recovery. As seen with the consequences of high temperature, lack of detection of certain variants of Hb can also have clinical implications as these variants can lead to low f-Hb, and consequently missed colorectal disease. However, these variants are not largely expressed in the population with approximately 5 percent of the world population having a globin variant, but only 1.7 percent have an alpha or beta trait [43] and these participants or patients are likely to have regular transfusions and so any f-Hb may be reflective of donor blood. In terms of other potential assay interferences, the use of antibodies specific to the globin component of human Hb make cross reactivity with dietary Hb, such as from red meat products, extremely unlikely and the antibody detection methods should not be impacted by other dietary

components.[44] There is a risk that if patients sample from the toilet bowl the sample will be diluted or contaminated with residues of previous defaecations and urinations, chemical toilet cleaners, disinfectants and fragrances. As such the recommendation should be to collect faecal sample into a clean collection bowl or on to a piece of toilet tissue. In summary, there are a large number of pre-examination variables that exist that can affect measured f-Hb. Some of these are patient variables and many are specific differences between manufacturers. The IFCC FIT-WG made a decision to document all these pre-examination variables (Figure 1) but that addressing them immediately would prove extremely challenging for manufacturers and so it would not become a primary focus of the group.

Quality assurance of FIT examinations

To ensure consistency in the FIT examinations between the different systems, laboratory quality assurance is critical. The International Organization for Standardization (ISO) 15189:2012 has specific requirements to assure quality in medical laboratories [45]. These encompass, amongst many other aspects, the ability to use independent third party IQC material and to participate in an inter-laboratory comparison programme, such as an EQA or proficiency testing programme.

Internal Quality Control (IQC) for FIT

Despite the ISO requirements, availability of third party IQC material is lacking [46]. Each individual manufacturer provides its own IQC material for use on its own system. A recent study, facilitated by discussion amongst manufacturers at meetings of the IFCC FIT-WG, demonstrated that the IQC material from each of four manufacturers is compatible on the other three FIT systems [20]. The assigned

values of each of these materials was different when examined on a FIT system that wasn't that of the providing manufacturer. This is to be expected because of the lack of standardisation as described previously however it does at least provide the potential for third party IQC materials for FIT to be widely available if manufacturers were willing to market their IQC materials independently of their FIT systems.

External Quality Assessment (EQA) for FIT

With the increasing use of FIT, EQA schemes are being established worldwide to support the quality requirements. Whilst all programmes have clear objectives and target participants, each EQA scheme design is different and programmes vary in terms of sample type, frequency and in the number of samples and Hb concentrations sent for each distribution.

A recent study [21] investigated the suitability of a wide number of EQA materials provided by different programmes. The pre-examination factors described above and summarised in Figure 1 make designing an EQA scheme particularly challenging.

The 'ideal' EQA material would be real patient samples or samples that closely resemble patient materials, i.e., faecal like matrix. In this format, samples can be received and assayed in the same way as patient samples. However, for FIT, homogeneity may be difficult to guarantee and there are also stability issues to consider with this type of matrix. Hb in faeces can begin to degrade within a matter of days, or even hours at room temperature, and there is little evidence that storage of faeces at 4^{o}C reduces the rate of f-Hb degradation [47]. EQA providers, therefore, need to consider how they can ensure homogeneity of material and how Hb can be stabilised following material preparation.

Stability of faecal Hb in patient samples, and hence faecal like matrices, can be improved by immediate collection into manufacturer specific specimen collection devices [47, 48]. The challenge with this approach is assuring uniform collection techniques across the batch of EQA material for each collection device.

Utilising lyophilised faecal material spiked with Hb could potentially overcome the issue of stability. However, this does not mimic patient material and introduces pre-analytical steps, which would not normally be present when assaying patient samples.

Homogeneity is easier to achieve with liquid samples (Hb spiked buffer) but again these do not mimic patient materials and are not presented to the FIT system in the same way a patient sample would be. These samples may, however, be less prone to pre-analytical variation [21]. As such they enable the analytical part of the examination procedure to be analysed, independently of any pre-analytical interfering factors.

A question considered by the IFCC FIT-WG was whether the group should be asking EQA providers to provide information on the entire measurement process, including pre-examination as well as examination performance and not just the examination phase? The answer is yes, ideally, but the IFCC FIT-WG would suggest that EQA providers concentrate on assessment of the examination performance of the FIT systems as a priority.

Based on the work done by the South of England Bowel Cancer Screening Hub research team in Guildford [21] and the advances made by EQA providers since this study, the IFCC FIT-WG plan to provide expert advice to EQA providers on what type of FIT EQA would be most beneficial to users of FIT systems.

A recent editorial describes the on-going challenges of quality assurance of FIT as well as progress made [49].

Further efforts to enhance comparability of measurement results

A major focus of the IFCC FIT-WG has been to work on the comparability of the results generated using FIT systems and the terms of reference of the group include, to harmonise examination of haemoglobin in faecal samples using FIT and to determine the feasibility of developing reference materials and/ or commutable calibrators (Table 1). The aim is to harmonise the f-Hb results obtained on the different systems rather than to attempt to harmonise the procedures.

Achieving common traceability for measurement results from different FIT systems require the set-up of calibration hierarchy as described in the international standard ISO 17511:2020. Ideally, the definition of the SI unit is the starting point of the calibration hierarchy and this is transferred to the results the FIT systems through a documented unbroken chain of calibrations. Primary and secondary reference measurement procedures are essential to transfer the SI unit to the suitable calibrators.

Due to the challenges of the faecal matrix the required reference measurement procedures are currently not available. Therefore the IFCC FIT-WG is now working towards comparability of results from the FIT methods using a calibration hierarchies of lower metrological order. When the different FIT systems use the same commutable conventional calibrator, the comparability of the obtained results may also be improved. If the comparability is statistically and clinically acceptable, the results of these FIT systems are considered to be harmonized [50]. The first step in the process to identify a suitable calibrator is a commutability assessment. A study is

currently ongoing in which the commutability of three different types of candidate reference material is investigated. Two consist of a frozen buffer solution containing human Hbwhile the third type is a lyophilized extract from human faecal samples spiked with human Hb. The recommendations of the IFCC Working Group on Commutability are applied in this study [51, 52]. Successful implementation of a common calibration hierarchy will help to generate more comparable f-Hb results.

Since the IFCC FIT-WG was established in 2017, the global use of FIT has increased rapidly, especially with the COVID pandemic where it has been used to help triage patients for endoscopic examination [53-56]. Good progress has also been made in understanding and trying to improve some of the examination challenges.

In conclusion, improving the comparability of the result from different FIT examination methods will be challenging, but is essential in ensuring that this apparently simple investigation used around the world within CRC screening programmes, in triaging symptomatic patients and in surveillance programmes fulfils its optimal use for early detection of significant colorectal disease.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 68 (2018) 394-424.
[2] E.H. Schreuders, A. Ruco, L. Rabeneck, R.E. Schoen, J.J. Sung, G.P. Young, E.J. Kuipers. Colorectal cancer screening: a global overview of existing programmes. Gut. 64 (2015) 1637-1649.
[3] H.-M. Chiu, S.L.-S. Chen, A.M.-F. Yen, S.Y.-H. Chiu, J.C.-Y. Fann, Y.-C. Lee, S.-L. Pan, M.-S. Wu, C.-S. Liao, H.-H. Chen, S.-L. Koong, S.-T. Chiou. Effectiveness of fecal immunochemical testing in reducing colorectal cancer mortality from the One Million Taiwanese Screening Program. Cancer. 121 (2015) 3221-3229.
[4] M. Westwood, S. Lang, N. Armstrong, S. van Turenhout, J. Cubiella, L. Stirk, I.C. Ramos, M. Luyendijk, R. Zaim, J. Kleijnen, C.G. Fraser. 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 Medicine. 15 (2017) 189.
[5] C. MacLeod, P. Wilson, A.J.M. Watson. Colon capsule endoscopy: an innovative method for detecting colorectal pathology during the COVID-19 pandemic? Colorectal Dis. 22 (2020) 621-624.
[6] National Institute for Health and Care Excellence. Quantitative faecal immunochemical tests to guide referral for colorectal cancer in primary care DG30. 2017.
[7] A. Buron, F. Macia, M. Andreu, M. Pellise, X. Castells, J. Grau. Population-based colorectal cancer screening: Interval cancers and relationship with the quantitative faecal immunological for hemoglobin. Med Clin (Barc). 152 (2019) 303-306.
[8] E.J. Grobbee, E.H. Schreuders, B.E. Hansen, M.J. Bruno, I. Lansdorp-Vogelaar, M.C.W. Spaander, E.J. Kuipers. Association between concentrations of hemoglobin determined by fecal immunochemical Tests and long-term development of advanced colorectal neoplasia. Gastroenterology. 153 (2017) 1251-1259.
[9] L.S. Chen, A.M. Yen, S.Y. Chiu, C.S. Liao, H.H. Chen. 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. 12 (2011) 551-558.
[10] E. Symonds, C. Cock, P. Bampton, R. Fraser, G. Young. Are Negative Fecal Immunochemical Test Hemoglobin Levels Predictive of Future Surveillance Colonoscopy Outcomes? Gastroenterology. 156 (2019) S-182.
[11] E.L. Symonds, K. Cornthwaite, R.J.L. Fraser, P. Bampton, C. Cock, G.P. Young. Reducing the number of surveillance colonoscopies with faecal immunochemical tests. Gut. 69 (2020) 784-785.
[12] J. Digby, S. Cleary, L. Gray, P. Datt, D.R. Goudie, R.J.C. Steele, J.A. Strachan, A. Humphries, C.G. Fraser, C. Mowat. Faecal haemoglobin can define risk of colorectal neoplasia at surveillance colonoscopy in patients at increased risk of colorectal cancer. United European Gastroenterol J. 8 (2020) 559-566.
[13] H. Seaman. 5th Meeting of the Expert Working Group (EWG) – 'FIT for Screening' DDW, Chicago 2014, Meeting Report: World Endoscopy Organization, Colorectal Cancer Screening Committee, http://www.worldendo.org/fit-for-screening-meeting-reports.html, 2014 (Accessed 05 November 2020).
[14] C. Piggott, M.R.R. Carroll, C. John, S. O'Driscoll, S.C. Benton. Analytical evaluation of four faecal immunochemistry tests for haemoglobin. Clin Chem Lab Med. (2020). doi: 10.1515/cclm-2020-0251.
[15] International Federation of Clinical Chemistry and Laboratory Medicine. Fecal Immunochemical Testing (WG-FIT), http://www.ifcc.org/ifcc-scientific-division/sd-working-groups/wg-fit/, 2018 (Accessed 05 November 2020).

[16] A. Gies, K. Cuk, P. Schrotz-King, H. Brenner. Direct Comparison of Diagnostic Performance of 9 Quantitative Fecal Immunochemical Tests for Colorectal Cancer Screening. Gastroenterology. 154 (2018) 93-104.
[17] B. Passamonti, M. Malaspina, C.G. Fraser, B. Tintori, A. Carlani, V. D'Angelo, P. Galeazzi, E. Di Dato, L. Mariotti, S. Bulletti, M.R. D'Amico, D. Gustinucci, N. Martinelli, N. Spita, E. Cesarini, T. Rubeca, M. Giaimo, N. Segnan, C. Senore. 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. 67 (2016) 485-496.
[18] E.J. Grobbee, M. van der Vlugt, A.J. van Vuuren, A.K. Stroobants, M.W. Mundt, W.J. Spijker, E.J. Bongers, E.J. Kuipers, I. Lansdorp-Vogelaar, P.M. Bossuyt, E. Dekker, M.C. Spaander. A randomised comparison of two faecal immunochemical tests in population-based colorectal cancer screening. Gut. 66 (2016) 1975-1982.
[19] A. Gies, K. Cuk, P. Schrotz-King, H. Brenner. Direct Comparison of Diagnostic Performance of 9 Quantitative Fecal Immunochemical Tests for Colorectal Cancer Screening. Gastroenterology. 154 (2017) 93-104.
[20] C. Piggott, Z. Shugaa, S.C. Benton. Independent internal quality control (IQC) for faecal immunochemical tests (FIT) for haemoglobin: use of FIT manufacturers' IQC for other FIT systems. Clin Chem Lab Med. (2020). doi: 10.1515/cclm-2020-0286.
[21] S. O'Driscoll, C. Piggott, H. Bruce, S.C. Benton. An evaluation of ten external quality assurance scheme (EQAS) materials for the faecal immunochemical test (FIT) for haemoglobin. Clin Chem Lab Med. (2020). doi: 10.1515/cclm-2020-0210.
[22] C.J. Chapman, A. Banerjea, D.J. Humes, J. Allen, S. Oliver, A. Ford, K. Hardy, N. Djedovic, R.F. Logan, J.R. Morling. Choice of faecal immunochemical test matters: comparison of OC-Sensor and HM-JACKarc, in the assessment of patients at high risk of colorectal cancer. Clin Chem Lab Med. (2020). doi: 10.1515/cclm-2020-1170.
[23] M. Plebani. Harmonization in laboratory medicine: the complete picture. Clin Chem Lab Med. 51 (2013) 741-751.
[24] J.E. Allison, C.G. Fraser, S.P. Halloran, G.P. Young. Comparing fecal immunochemical tests: improved standardization is needed. Gastroenterology. 142 (2012) 422-424.
[25] C.G. Fraser, J.E. Allison, S.P. Halloran, G.P. Young. A proposal to standardize reporting units for fecal immunochemical tests for hemoglobin. J Natl Cancer Inst. 104 (2012) 810-814.
[26] S. Rapi, F. Cellai, T. Rubeca. Is it possible to correctly assess the pre-analytical characteristics of faecal tests? Journal of Laboratory and Precision Medicine. 3 (2018).
[27] S. Rapi, T. Rubeca, C.G. Fraser. How to improve the performances of Fecal Immunological Tests (FIT): Need for standardization of the sampling and pre-analytical phases and revision of the procedures for comparison of methods. Int J Biol Markers. 30 (2015) e127-131.
[28] S. Rapi, M. Berardi, F. Cellai, S. Ciattini, L. Chelazzi, A. Ognibene, T. Rubeca. Effects of fecal sampling on preanalytical and analytical phases in quantitative fecal immunochemical tests for hemoglobin. Int J Biol Markers. 32 (2017) e261-e266.
[29] C. Piggott, C. John, H. Bruce, S.C. Benton. Does the mass of sample loaded affect faecal haemoglobin concentration using the faecal immunochemical test? Ann Clin Biochem. 55 (2018) 702-705.
[30] J.E. Allison, C.G. Fraser, S.P. Halloran, G.P. Young. Comparing fecal immunochemical tests: improved standardization is needed. Gastroenterology. 142 (2012) 422-424.
[31] S.J. Lewis, K.W. Heaton. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 32 (1997) 920-924.
[32] L. von Karsa, J. Patnick, N. Segnan. European guidelines for quality assurance in colorectal cancer screening and diagnosis. First Edition--Executive summary. Endoscopy. 44 (2012) Se1-8.
[33] E.L. Amitay, A. Gies, K. Weigl, H. Brenner. Fecal Immunochemical Tests for Colorectal Cancer Screening: Is Fecal Sampling from Multiple Sites Necessary? Cancers (Basel). 11 (2019) 400.

[34] E.L. Symonds, C.G. Fraser, D. Bastin, G. Berwald, G.P. Young. The effect of the variability in fecal immunochemical test sample collection technique on clinical performance. Cancer Epidemiol Biomarkers Prev. 2020. doi: 10.1158/1055-9965.EPI-20-0984.
[35] C.G. Fraser, S.P. Halloran, J.E. Allison, G.P. Young. Making colorectal cancer screening FITTER for purpose with quantitative faecal immunochemical tests for haemoglobin (FIT). Clin Chem Lab Med. 51 (2013) 2065-2067.
[36] J. Tinmouth, I. Lansdorp-Vogelaar, J.E. Allison. Faecal immunochemical tests versus guaiac faecal occult blood tests: what clinicians and colorectal cancer screening programme organisers need to know. Gut. 64 (2015) 1327-1337.
[37] T. Rubeca, F. Cellai, M. Confortini, C.G. Fraser, S. Rapi. Impact of preanalytical factors on fecal immunochemical tests: need for new strategies in comparison of methods. Int J Biol Markers. 30 (2015) e269-274.
[38] E. Gnatta, M. Zaninotto, M.G. Epifani, A. Padoan, R. Gjini, M. Plebani. A new sampling device for faecal immunochemical testing: haemoglobin stability is still an open issue. Clin Chem Lab Med. (2014).
[39] A. Gies, K. Cuk, P. Schrotz-King, H. Brenner. Direct comparison of ten quantitative fecal immunochemical tests for hemoglobin stability in colorectal cancer screening. Clin Transl Gastroenterol. 9 (2018) 168.
[40] E.L. Symonds, S.R. Cole, D. Bastin, R.J. Fraser, G.P. Young. Effect of sample storage temperature and buffer formulation on faecal immunochemical test haemoglobin measurements. J Med Screen. 24 (2017) 176-81.
[41] G. Grazzini, L. Ventura, T. Rubeca, S. Rapi, F. Cellai, P.P. Di Dia, B. Mallardi, P. Mantellini, M. Zappa, G. Castiglione. Impact of a new sampling buffer on faecal haemoglobin stability in a colorectal cancer screening programme by the faecal immunochemical test. Eur J Cancer Prev. 26 (2016) 285-291.
[42] M.R. Carroll, C. John, D. Mantio, N.K. Djedovic, S.C. Benton. An assessment of the effect of haemoglobin variants on detection by faecal immunochemical tests. Ann Clin Biochem. 55 (2018) 706-709.
[43] D. Rund, E. Rachmilewitz. Beta-thalassemia. N Engl J Med. 353 (2005) 1135-1146.
[44] S.P. Halloran, G. Launoy, M. Zappa. European guidelines for quality assurance in colorectal cancer screening and diagnosis. First Edition Faecal occult blood testing. Endoscopy. 44 (2012) SE65-SE87.
[45] International Organization for Standardization. ISO 15189:2007 Medical laboratories – Particular requirements for quality and competence. Geneva: ISO Publications; 1997.
[464] I.M. Godber, S.C. Benton, C.G. Fraser. Setting up a service for a faecal immunochemical test for haemoglobin (FIT): a review of considerations, challenges and constraints. J Clin Pathol. 71 (2018) 1041-1045.
[47] S. Mellen, M. de Ferrars, C. Chapman, S. Bevan, J. Turvill, D. Turnock. Evaluation of sample stability for a quantitative faecal immunochemical test and comparison of two sample collection approaches. Ann Clin Biochem. 55 (2018) 657-664.
[48] L.F. Brown, C.G. Fraser. Effect of delay in sampling on haemoglobin determined by faecal immunochemical tests. Ann Clin Biochem. 45 (2008) 604-605.
[49] C.G. Fraser. Assuring the quality of examinations using faecal immunochemical tests for haemoglobin (FIT). Clin Chem Lab Med. (2020). doi: 10.1515/cclm-2020-1509
[50] G.H. White. Metrological traceability in clinical biochemistry. Ann Clin Biochem. 48 (2011) 393-409.
[51] W.G. Miller, H. Schimmel, R. Rej, N. Greenberg, F. Ceriotti, C. Burns, J.R. Budd, C. Weykamp, V. Delatour, G. Nilsson, F. MacKenzie, M. Panteghini, T. Keller, J.E. Camara, I. Zegers, H.W. Vesper. IFCC Working Group Recommendations for Assessing Commutability Part 1: General Experimental Design. Clin Chem. 64 (2018) 447-454.

[52] G. Nilsson, J.R. Budd, N. Greenberg, V. Delatour, R. Rej, M. Panteghini, F. Ceriotti, H. Schimmel, C. Weykamp, T. Keller, J.E. Camara, C. Burns, H.W. Vesper, F. MacKenzie, W.G. Miller. IFCC Working Group Recommendations for Assessing Commutability Part 2: Using the Difference in Bias between a Reference Material and Clinical Samples. Clin Chem. 64 (2018) 455-464.
[53] Scottish Government. Guidance for the use of FIT in the prioritization of patients with colorectal symptoms now and in the recovery period after COVID, https://www.nhsggc.org.uk/media/261915/clinical-guidance-quantitative-faecal-immunochemical-testing-004.pdf, 2020 (Accessed 15 October 2020)
[54] NHS England and NHS Improvement. Specialty guides for patient management during the coronavirus pandemic. Clinical guide for triaging patients with suspected colorectal cancer, https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/06/C0551-triaging-patients-with-lower-gi-symptoms-16-june.pdf, 2020 (Accessed 14 October 2020)
[55] W. Maclean, R. Singh, P. Mackenzie, D. White, S. Benton, J. Stebbing, T. Rockall, I. Jourdan. The two-week rule colorectal cancer pathway: an update on recent practice, the unsustainable burden on diagnostics and the role of faecal immunochemical testing. Ann R Coll Surg Engl. 102 (2020) 308-11.
[56] British Society of Gastroenterology. Advice regarding working in endoscopy for vulnerable clinical staff during the COVID-19 pandemic 2020, https://www.bsg.org.uk/covid-19-advice/advice-regarding-working-in-endoscopy-for-vulnerable-clinical-staff-during-the-covid-pandemic/, 2020 (Accessed 06 November 2020)

Terms of Reference of IFCC FIT -WG
• To harmonise and/or standardise analysis of haemoglobin in faecal samples by immunochemistry (FIT)
• To standardise the pre-analytical phase
• To establish EQA and 3rd party IQC programmes
• To determine impact of assay interference of Hb variants and other factors
• To determine the feasibility of developing reference materials and/or commutable calibrators

Table 1: Terms of Reference of IFCC FIT-WG (2017)

EQA: external quality assessment; Hb: haemoglobin; IQC: internal quality control

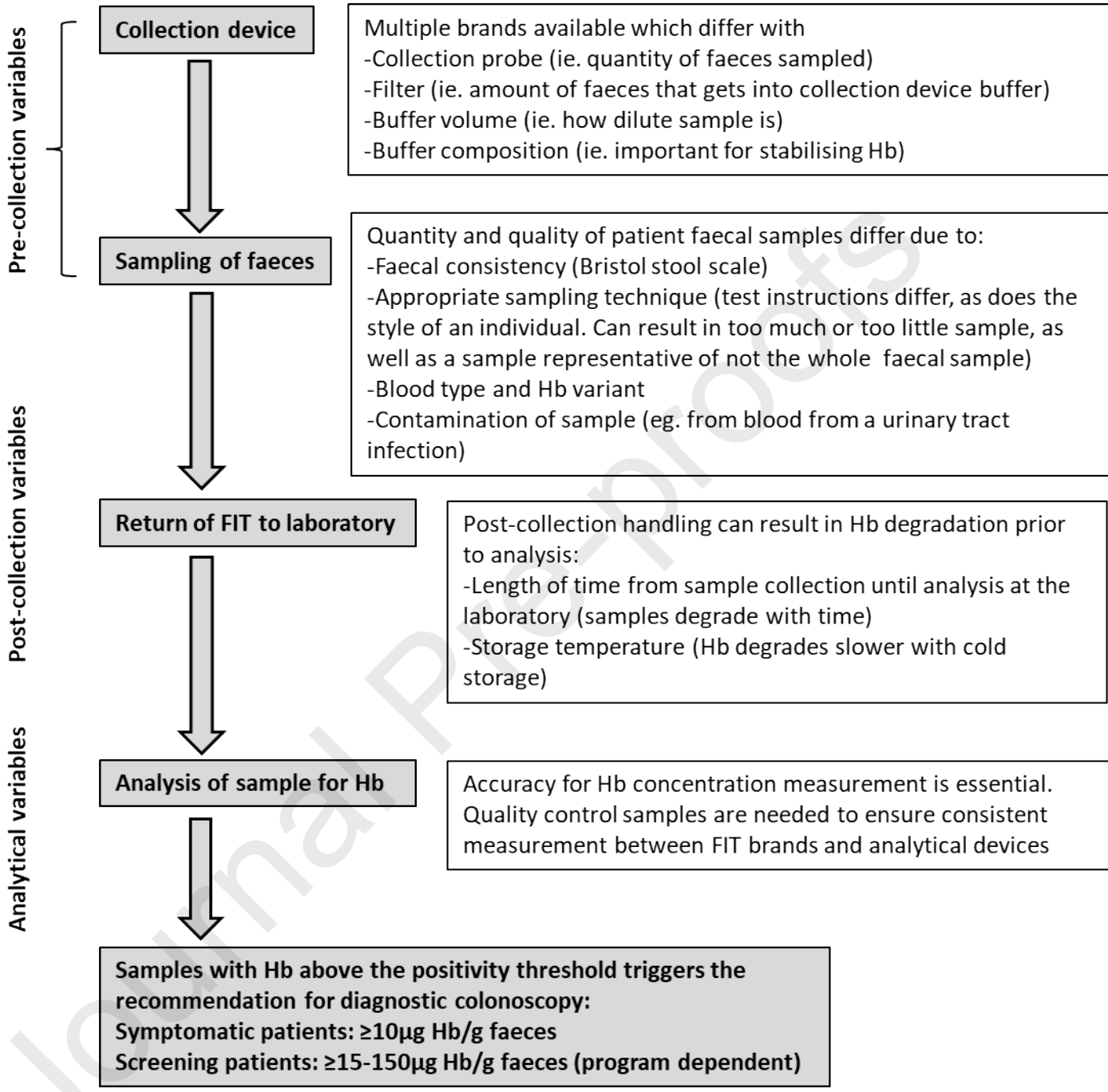

Figure 1. Overview of pre-analytical, post collection and analytical variables associated with the Faecal Immunochemical test

Sally C Benton drafted the manuscript with direct input from all co-authors. All co-authors reviewed and refined the manuscript. Before submission the manuscript was reviewed by all non-corporate members of the IFCC FIT-WG

Faecal Immunochemical Tests for Haemoglobin: Analytical Challenges and Potential Solutions – Highlights

- International Federation of Clinical Chemistry working group set up to address analytical challenges of the faecal immunochemical test for haemoglobin (FIT)
- Harmonisation study underway
- Summary included of key pre-analytical variables
- Analytical evaluation of external quality assurance schemes complete
- Four potential third party internal quality assurance materials available