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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)

James E. Allison^{*†}, Callum G. Fraser[‡], Stephen P. Halloran[§], and Graeme P. Young^{||}

^{*}Division of Gastroenterology, University of California San Francisco School of Medicine, San Francisco, CA, [†]Division of Research, Kaiser Permanente Northern California, Oakland, CA, USA, [‡]Centre for Research into Cancer Prevention and Screening, University of Dundee School of Medicine, Dundee, Scotland, [§]NHS Bowel Cancer Screening Southern Programme Hub, Royal Surrey County Hospital and University of Surrey, Guildford, UK, and ^{||}Flinders Centre for Innovation in Cancer, Flinders University, Bedford Park SA, Australia

Fecal immunochemical tests for hemoglobin (FIT) are changing the manner in which colorectal cancer (CRC) is screened. Although these tests are being performed worldwide, why is this test different from its predecessors? What evidence supports its adoption? How can this evidence best be used? This review addresses these questions and provides an understanding of FIT theory and practices to expedite international efforts to implement the use of FIT in CRC screening. (*Gut Liver* 2014;8:117-130)

Key Words: Population screening; Colorectal cancer; Fecal immunochemical test for hemoglobin; Colorectal cancer screening

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second in women.¹ Australia, New Zealand, Europe, and North America have the highest CRC incidence, and Africa and Asia the lowest.^{2,3} Developing countries are witnessing an increasing incidence of CRC that is probably related to the adoption of high risk Western behavior with increased smoking, high alcohol consumption, physical inactivity, and less healthy diets.^{2,4}

CRC mortality rates have declined in many economically developed countries but only in the United States has a decrease in incidence been described. A large proportion of the observed decrease in CRC mortality and incidence is likely to be due to screening and so the case to screen is clear, although the choice of screening method is not. The design of a screening system

will be influenced by many important factors including the following:

- 1) Evidence (peer reviewed publications) that the selected approach will be effective in decreasing both CRC mortality and incidence.

- 2) Evidence of cost effectiveness to support the economic and political case for a sustainable screening program.

- 3) Evidence of an effective organizational model that works within available resources (financial and human) and provides the high volume and high quality organizational analytical and clinical services necessary for population-based screening.

- 4) Evidence of the effectiveness of a suitable primary screening test such as fecal immunochemical tests for hemoglobin (FIT) and reliable comparative information about the analytical and clinical performance of the test.

This review addresses the aspects of the last point: the suitability and effectiveness of FIT for population screening.

Recognition of the high burden of CRC has helped make the case for national screening programs. Randomized controlled trials using guaiac-based fecal occult blood tests (gFOBTs) or flexible sigmoidoscopy (FS) have provided convincing evidence that organized population-based screening can decrease CRC mortality and incidence⁵⁻¹² and therefore the case for CRC screening is very strong.

FIT has gained international acceptance as being the worthy successor to the proven gFOBT. It can provide the first step of a two-step strategy where a positive FIT precedes a diagnostic structural examination such as colonoscopy or computed tomographic colonography.¹³⁻¹⁵ Some in the United States are committed to a single step colonoscopy screening strategy

Correspondence to: James E. Allison

Division of Gastroenterology, University of California San Francisco School of Medicine, SFGH Campus 1001 Potrero Avenue, NH-3D San Francisco, CA 94110, USA

Tel: +1-415-206-8709, Fax: +1-415-641-0745, E-mail: jallison@medsfgh.ucsf.edu

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because they argue that FIT has little effect on CRC incidence.¹⁶ They claim that the principle of CRC detection using fecal tests (gFOBT and FIT) is inherently insensitive and nonspecific because the tests detect blood, a surrogate for advanced neoplasia, and depend on the relevant neoplasia (cancer and/or advanced adenomas) bleeding in sufficient quantities to enable detection in feces.^{17,18} This review provides the evidence that supports FIT population-based screening and should help inform clinicians, laboratory medicine service providers, health service funders, and health policy experts why FIT is the most effective noninvasive CRC screening test currently available. The review also provides guidance on how to identify suitable FIT systems.

FIT FACTS

The fecal immunochemical test for hemoglobin with the abbreviation FIT was recommended as the preferred name for this screening test by a World Endoscopy Organization (WEO) Expert Working Party (EWP) in 2012 to avoid confusion with gFOBTs and to emphasize the significant analytical and clinical differences. FIT should replace terms such as immunological fecal occult blood test, immunohistochemical test, etc. as the only recommended term. The WEO EWP has chosen not to endorse the use of FIT⁵⁰ or FIT¹⁰⁰ as a method of denoting both the test name and cutoff concentration because, until standardized reporting units have been widely adopted, the use of this nomenclature has the potential of misleading the reader. Constancy of terminology and reporting units aids understanding and transference of ideas and research observations across time and geography.

FIT use antibodies specific for human hemoglobin. They use the principle that a suitable monoclonal or polyclonal antibody can recognize and will bind to the intact globin component of human hemoglobin. By using labeled antibodies, the bound antibody-hemoglobin complex can be detected and measured by a variety of techniques. It can provide a positive or negative test result in qualitative FIT or a fecal hemoglobin concentration in quantitative FIT systems. Quantitative FIT are the test of choice for population screening since they can provide consistently high quality, automated measurements using high performance analytical systems that will enable selection of the preferred fecal hemoglobin cut-off concentration above which the risk of CRC merits colonoscopic investigation. Quantitative FIT thus allow the screening program the opportunity to select an acceptable sensitivity/specificity balance and the ability to ensure that colonoscopic referral rates can be met by local circumstances, particularly the available colonoscopic resources.

While gFOBT and FIT share the same clinical detection principle (bleeding) and biomarker (hemoglobin), the analytical methods are very different. gFOBT detects the presence of blood's heme component when hydrogen peroxide is added during analysis and oxidizes guaiac to form a blue colored dye.

Intact heme enables oxidation and is responsible for the clinical efficacy of gFOBT. Although gFOBT are cheap and simple tests, they require a significant quantity of heme to effect a visible change in color and are therefore not very sensitive analytically to the presence of blood. The method relies on simple oxidation, and therefore any dietary hemoglobin in red meat etc., has the potential to give a false positive result, as can drugs or foods that have peroxidase properties (i.e., some uncooked fruits and vegetables). Antioxidants in drugs or foods (vitamin C or E) also have the potential to give false negative results by interfering with the oxidation of guaiac. The gFOBT is therefore an inherently insensitive and nonspecific test.

FIT detect blood by immunoassay. An antibody is prepared that specifically recognizes the globin component of human hemoglobin. FIT analysis measures the quantity of antibody bound to hemoglobin using a variety of methods. The FIT technique is analytically sensitive to low concentrations of hemoglobin and is unlikely to be subject to significant interference from other constituents of feces.

Substantial clinical benefits are possible because of the superior analytical technique used in FIT. Sensitive and specific analysis for hemoglobin means that FIT can detect smaller levels of bleeding and thus smaller cancers and more adenomas and the number of false positives is reduced for a given hemoglobin concentration. As will be discussed later, the enhanced clinical sensitivity and specificity of FIT is maintained even when the performance of a single-sample FIT is compared with the traditional three-sample gFOBT. While the heme component of hemoglobin is a relatively robust molecule and it can survive with its oxidative properties intact even after traversing the full length of the gastrointestinal (GI) tract, the globin detected by FIT is prone to degradation from GI proteases. This property adds significant organ specificity to the list of clinical merits of FIT and makes it is less likely to present false positive results from upper gastrointestinal tract bleeding than might be rarely observed by gFOBT.

FIT products have two general designs that use different analytical techniques, lateral flow immunochromatographic analysis and immunoturbidimetric analysis. Most qualitative products are designed for use at the point-of-care, outside of a laboratory and by clinicians. These tests use the ubiquitous lateral flow immunochromatographic system adopted for most pregnancy tests and many point-of-care tests (POCTs) for drugs and hormones. This system separates soluble hemoglobin from feces using passive flow (laterally) along a separation material (chromatographic) for the hemoglobin to be captured by antibodies to human hemoglobin and made visible using various visualizing techniques (Fig. 1). The manufacturer can adjust the visibility of the line that shows the presence of hemoglobin, essentially changing the cutoff concentration that will lead to further investigation, but it requires skill and practice to obtain consistency in visual interpretation.

Quantitation requires laboratory technology and immunoturbidimetry is one of several immunoassay techniques well established in clinical laboratories. It introduces the challenge of collecting a small fecal sample and transporting it to the laboratory. Different FIT use different approaches to controlling sample size and none is particularly good at doing it accurately. Accurate results also require FIT manufacturers to ensure sample stability and they go about it in different ways with varying success.

FIT technology provides the opportunity to address several important practical screening issues. A screening test only has merit if the uptake by the invited population is acceptable. Screening participation with FIT has been found to be consistently higher than with gFOBT.^{19,20} The evidence was recently described in a systematic review and meta-analysis and, in addition to pointing out the merits of FIT, it concluded that more research examining FIT from a participant perspective was warranted.²¹ The relative merits of using FIT rather than gFOBT include improved participation rates because FIT typically use only a single fecal sample instead of the three required for gFOBT. The FIT specimen collection system described earlier is simple to use and is less messy than gFOBT cards and, once the sample probe has been returned to the collection device, the feces is safe and out of sight. Unlike the 3-sample gFOBT card system, once the single FIT sample collection is complete it can be returned immediately for testing and the participant

has no need to store and handle a gFOBT card laden with feces. From an aesthetic perspective the FIT device appears modern, scientific, and clinical, unlike the simple gFOBT card.

A challenge for all screening programs is to reduce the socioeconomic gradient seen in the uptake of CRC screening. A recent study in Scotland described the impact on participation of changing from gFOBT to FIT and showed the greatest increase in FIT uptake was in men, younger participants, and individuals in the lower socioeconomic strata, all groups for which uptake is generally poor.²² In the United States, the introduction of screening using FIT in clinics and hospitals that care for all patients regardless of their socioeconomic and insurance status (e.g., San Francisco General Hospital and the Alameda County Medical Center) has greatly increased screening rates (personal communication).

FIT that are suitable for population screening are best measured on automated analytical systems. These enable fast and reliable analysis and remove the subjective visual assessment that is a severe limitation to consistent and reliable screening with gFOBT and qualitative FIT (Fig. 2).²³⁻²⁵

Quantitative FIT provide the concentration of hemoglobin in feces and studies have consistently shown that this concentration is related to both detection rate and to the severity of the lesion. By adjusting the cutoff concentration upwards positivity decreases, fewer colonoscopies are performed and fewer lesions (but proportionally more advanced lesions) are detected. This approach has been trialed in Scotland using a very high cutoff chosen to provide the same positivity as their gFOBT and, while its positive predictive value for cancer was not improved significantly, it did improve participation rates.²⁶ Conversely, where colonoscopy resource is not a limitation, the sensitivity of FIT can be increased by adjusting the cutoff concentration to a lower fecal hemoglobin concentration.

FIT brings both practical and clinical advantages to population screening and some of those advantages have yet to be fully exploited. No longer do we determine the risk of a cardiac event just from cholesterol concentration measurement—we include other measured variables including age, sex, body mass index (BMI), smoking, family history, etc., and the same applies to staging the severity of kidney disease. With a quantitative FIT we can do the same and bring together, in a multivariate risk score, other accessible risk factors such as age, sex, family history, screening history, and perhaps BMI and smoking etc. Quantitative FIT opens opportunities for major enhancement to the current binary risk (positive or negative) outcome offered by gFOBT and is an approach that has been recently described by Stegeman *et al.*²⁷

FIT Performance Is Superior to gFOBT: The Evidence

Simon, in an early review of fecal occult blood testing, states that research on FIT began in the late 1970s.²⁸ In the 1970s,

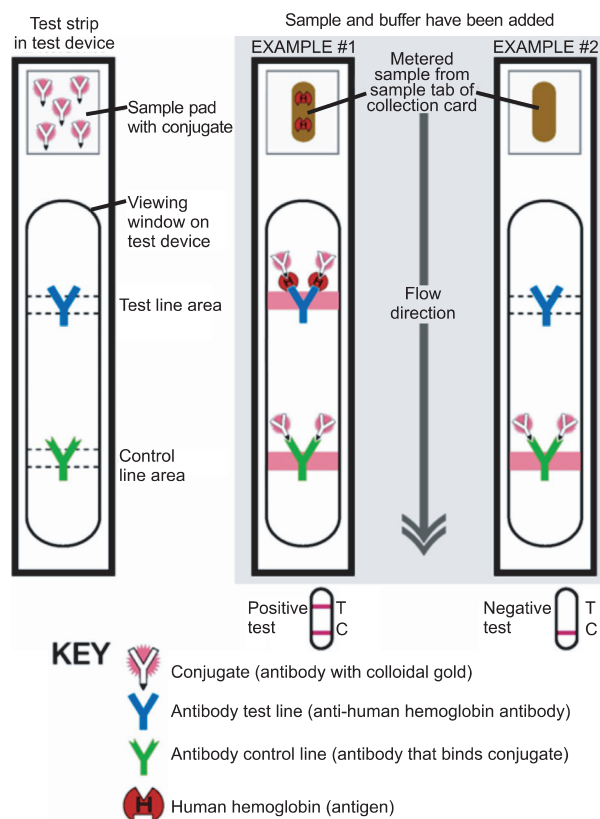


Fig. 1. Cartoon illustrating lateral flow immunochromatographic analysis principle of a fecal immunochemical test for hemoglobin.

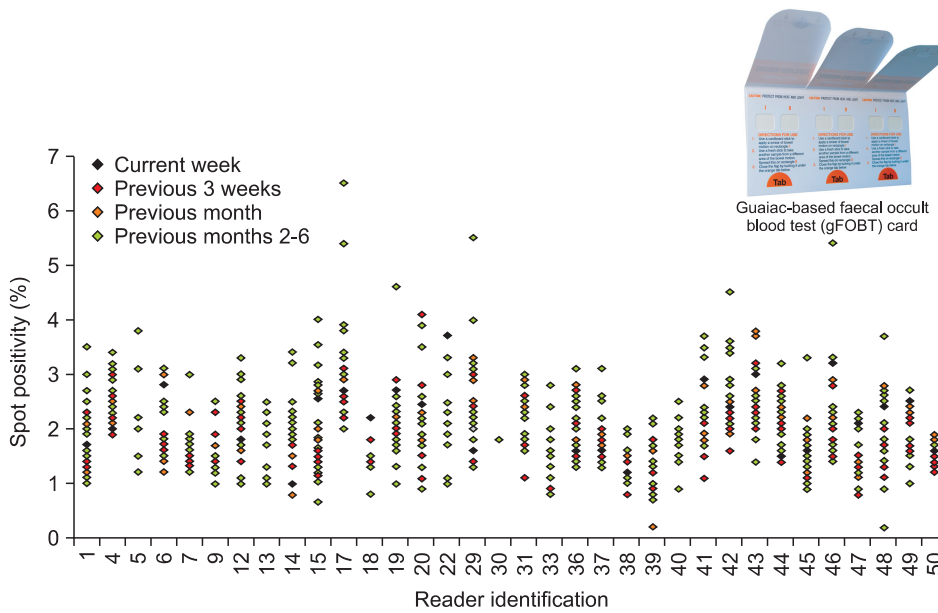


Fig. 2. Variable positivity rates of well-trained, well-supervised, and well-monitored analysts based on their guaiac fecal occult blood test results.

Barrows *et al.*²⁹ and Songster *et al.*^{30,31} and their coworkers developed an immunochemical test for occult blood that, unlike gFOBT, detected human hemoglobin free of cross-reactivity with animal hemoglobin and other dietary constituents. They then adapted the test for CRC screening by using a simple punched-out filter paper disc on which the fecal specimen was placed for development. In 1981, an immunofluorescent test for fecal occult blood was described by Vellacott *et al.*³²

FIT were first used in the 1980s for screening in Japan. Research with FIT was subsequently described in Australia,^{33,34} England,³⁵ Israel,³⁶ and the United States^{37,38} in the 1990s and was followed from 2005 by a progressively large body of literature that described the clinical benefits of FIT relative to gFOBT.³⁹⁻⁴³ These studies showed that FIT provided a superior sensitivity/specificity for advanced colorectal neoplasia (cancer and advanced adenomas) over that provided by gFOBT and, because they identified a significant proportion of advanced adenomas as well as CRC, it became clear that they offered the potential for decreasing both mortality from and incidence of CRC.

In the United States, the federal U.S. Food and Drug Administration (FDA) approved the use of a qualitative FIT system in 1988, and another in 1996, but it did not and has still not approved the use of quantitative FIT. Outside of the United States a plethora of qualitative and several quantitative FIT are now available and several quantitative FIT are now used in national screening programs.

Despite the fact that FIT have been available since the late 1970s, it was not until the late 1990s and the early 2000s, that the English language scientific literature on FIT performance characteristics became robust. Much of the later research has been conducted in Japan, Italy, Israel, Australia, The Netherlands, Germany, Korea, and the UK where population-

based screening programs have or are being developed. The FDA restrictions in the United States and the promotion of colonoscopy as the “best” and “preferred” CRC screening test modality has continued and has stifled both FIT research and the implementation of United States-based population screening programs.⁴⁴

Acceptance of FIT as an important screening test for CRC began in the 1990s in Japan, Australia, the United States, Israel, and Scotland where studies demonstrating their performance characteristics and cost effectiveness were first published.^{33,35-38,45-47} Though the studies varied in design, numbers, population screened, and FIT product used, all demonstrated the superior performance characteristics of FIT to the commonly used low sensitivity gFOBT. A convincing demonstration of FIT superiority to both the standard and sensitive guaiac fecal occult blood test (sFOBT) was performed in a Kaiser Permanente facility in northern California and published in 1996.³⁷ Test sensitivity (result of testing a screening population once only) was estimated by performing long-term follow-up (2 to 4 years) of test-negative participants as suggested by Cole and Morrison.⁴⁸ Test-negative subjects who developed CRC or advanced adenomas were identified through a search of the computer databases at the Kaiser Permanente Northern California Regional Cancer Registry Project and the pathology departments at the participating medical centers.

By 2010, the evidence of FIT superiority over the standard gFOBT was clear and sufficiently convincing for the *European guidelines for quality assurance in CRC screening and diagnosis* to recommend adoption of FIT in preference to gFOBT.^{13,14} In a 2006 Israeli study, FIT sensitivity for advanced neoplasms was similar to that for gFOBT but FIT had better specificity and positive predictive value.⁴⁹ In a 2007 Kaiser Permanente study, FIT performance characteristics were compared with those of the

high sensitivity guaiac FOBT (sFOBT) for identifying left-sided colorectal advanced neoplasms in a large group of average risk individuals. All participants with negative tests were encouraged to have a flexible sigmoidoscopy (FS). Sensitivity for left-sided CRC was 82% for FIT and 64% for the sFOBT (Table 1).³⁹ In 2008 the Dutch published the first randomized comparison between gFOBT and FIT in an average risk screening population.⁴³ In this study the number needed to scope to find one CRC was not different between gFOBT and FIT but participation and detection rates for advanced adenomas and cancer were significantly higher in the group tested with FIT: 2.5 times more advanced adenomas and cancer and 2.2 times more cancers were detected with FIT compared with gFOBT. The authors concluded that gFOBT significantly underestimated the prevalence of advanced adenomas and cancer compared with FIT in a screening population (Fig. 3). A study published in 2010 demonstrated similar findings in a Korean population.⁴² Based on this accumulated knowledge of population screening with FIT, an editorial published in *Gut* in 2012 suggested that the use of gFOBT for CRC screening was a less effective and obsolete strategy.⁵⁰

The Preferred Worldwide Screening Test Options for CRC

Of the many CRC screening tests available only four are

used in national, regional, and local screening programs and they are gFOBT, FIT, FS, and optical colonoscopy. Only traditional gFOBT⁵⁻⁷ and FS⁸⁻¹¹ have been shown to decrease CRC mortality and/or incidence in randomized controlled trials. Fletcher reviewing a FIT study for the ACP Journal Club in 1996 stated that although randomized controlled trials of FIT have not been undertaken to show that FIT decreases CRC mortality and incidence, they are unnecessary since FIT has demonstrated superior performance characteristics to gFOBT.⁵¹ As described earlier, FIT like gFOBT use detection of occult fecal blood in feces to identify those screened subjects most likely to harbor advanced neoplasia. FIT provide a significant analytical enhancement to the same biomarker. In the same way that we did not reappraise the use of electrolyte measurements when clinical laboratories moved from flame photometry to ion specific electrodes or glucose when we moved from colorimetric to enzymatic measurement, we have no need to reappraise the use of FIT; clinical effectiveness has already been demonstrated.

Studies like those recently published in Australia, show down-staging of CRC in a population study, a surrogate measure for effect of screening on mortality⁵² and preliminary data from Italy⁵³ and Taiwan, suggest a reduction in incidence. In the absence of randomized controlled trial evidence for a screening test's effectiveness in decreasing CRC mortality and incidence, investigators frequently turn to modeling studies. A 2008 study showed years of life saved through a high-quality,

Table 1. Performance Characteristics of Fecal Immunochemical Test for Hemoglobin versus Guaiac-Based Fecal Occult Blood Test for Left Sided Advanced Neoplasms

Finding per test	No. of persons screened	No. of neoplasms detected	Sensitivity		Specificity		Positive predictive value		Likelihood ratio (+) ^a	
			No./total	% (95% CI)	No./total	% (95% CI)	No./total	% (95% CI)	Ratio	95% CI
Distal cancer										
Sensitive guaiac test	5,799	14	9/14	64.3 (35.6-86.0)	5,210/5,785	90.1 (89.3-90.8)	9/584	1.5 (0.8-3.0)	6.5	4.3-9.6
FIT 1	5,356	11	9/11	81.8 (47.8-96.8)	5,181/5,345	96.9 (96.4-97.4)	9/173	5.2 (2.6-10.0)	26.7	19.4-36.6
Distal adenomas ≥1 cm										
Sensitive guaiac test	5,799	126	52/126	41.3 (32.7-50.4)	5,141/5,673	90.6 (89.8-91.4)	52/584	8.9 (6.8-11.6)	4.4	3.5-5.5
FIT 1	5,356	112	33/112	29.5 (21.4-38.9)	5,104/5,244	97.3 (96.8-97.7)	33/173	19.1 (13.7-25.9)	11.0	7.9-15.3
Distal advanced neoplasms										
Sensitive guaiac test	5,799	137	59/137	43.1 (34.7-51.8)	5,137/5,662	90.7 (89.9-91.5)	59/584	10.1 (7.8-12.9)	4.6	3.8-5.7
FIT 1	5,356	121	40/121	33.1 (24.9-42.3)	5,102/5,235	97.5 (97.0-97.9)	40/173	23.1 (17.2-30.3)	13.0	9.6-17.6

Adapted from Allison JE, *et al.* J Natl Cancer Inst 2007;99:1462-1470, with permission from Oxford University Press.³⁹

CI, confidence interval; FIT, fecal immunochemical tests for hemoglobin.

^aLikelihood ratio (+)=sensitivity/(1-specificity).

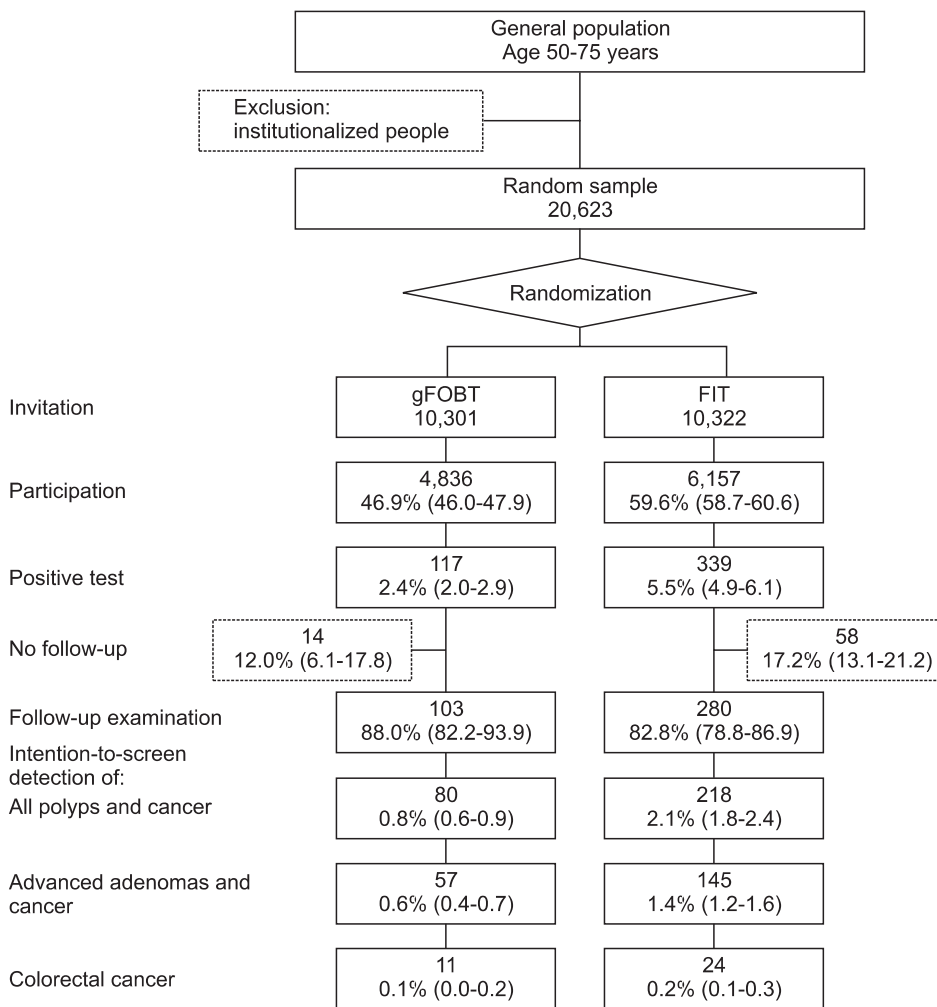


Fig. 3. Flow chart from invitation to detection with numbers, percentages, and 95% confidence intervals between brackets. Adapted from van Rossum LG, *et al.* Gastroenterology 2008;135:82-90, with permission from Elsevier.⁴³ gFOBT, guaiac-based fecal occult blood test; FIT, fecal immunochemical test for hemoglobin.

high sensitivity, occult blood screening program are the same as with a high-quality colonoscopy screening program.⁵⁴ Based on this modeling study and the accumulated evidence of FIT screening being effective for identifying patients with advanced adenomas, the American College of Physicians, the National Colorectal Cancer Roundtable, the American Cancer Society, and the *Journal of the American Medical Association* have all issued statements that evidence does not yet support any one screening test over another and that the currently available CRC screening tests mentioned above are believed to be similarly efficacious.⁵⁵⁻⁵⁷

Individual countries and/or health organizations need to consider the four currently recommended screening modalities for feasibility and public acceptability and should undertake well designed pilot studies before committing to implementation. The United States has no national CRC screening program but many screening thought leaders and the media have promoted the use of colonoscopy since 2000. Poland and Germany, however, are the only countries to have formally embraced colonoscopy screening but with mixed results. The evidence supporting colonoscopy is expert based and it will be years before results are available from two randomized controlled

trials (United States and Spain) that are comparing annual or biennial FIT with optical colonoscopy every 10 years. Results from the first round of screening in the Spanish study were published in 2012⁵⁸ and had some very interesting findings. Subjects in the FIT group were more likely to participate in screening than were those in the colonoscopy group and the numbers of subjects in whom CRC was detected were similar in the two study groups. Although more adenomas were detected in the colonoscopy group, this is no surprise. It is well known that structural examinations identify more adenomas at initial screen but, since adenomas are not malignant and FIT screening is advised annually or biennially, there should be confidence that a program of repeated screens will allow for identification of most of those few adenomas likely to become fatal cancers.

FIT Important Issues: Present and Future

What issues should influence the choice of a FIT and how reliable and safe are these devices in practice? While many FIT are available on the market, most are designed for use at POC and only a few currently meet criteria that we consider important for population-based screening and even fewer have

been used in large-scale screening.

FIT is a developing test and the WEO EWP, FIT for screening, has and is contributing to this development. The issues described below illustrate how FIT has evolved into a mature screening test. It highlights aspects of FIT technology and clinical application that are worthy of consideration prior to designing a FIT-based screening program or issuing tender documents for FIT procurement. These issues have been the subject of several recent editorials, many describing the recommendations of the WEO EWP.⁵⁹⁻⁶³

1) FIT terminology and the units used for reporting are inconsistent and need to be standardized so that published FIT studies are amenable to comparison.

2) The stability of hemoglobin in the specimen collection devices needs to be characterized in a standardized way so that products can be compared and judgments can be made on their suitability for screening in different environments and under different climatic conditions.

3) FIT need to be easy to use if they are to be accessible to the target population. We need to be conscious of poor uptake amongst disadvantaged groups and of the effects that aging, learning and physical disabilities and poor eye sight might have upon participation rates. For FIT and FIT screening programs to be clinically effective participation in multiple FIT screening rounds is essential.

4) The evidence for clinically effective single FIT biennial screening is good, but the option for multiple samples and/or alternative screening frequency remains.

5) FIT encourage a choice between local surgery or clinic-based POCT and the use of centralized, automated accredited laboratories using qualitative or quantitative FIT.

6) More well designed and comparative, analytical and clinical effectiveness studies will help identify FIT products eligible for consideration for population screening.

7) Quantitative FIT results provide opportunities to enhance risk stratification, better exploit endoscopy resources, and maximize clinical effectiveness.

The following sections discuss each of the above issues.

1. FIT terminology and reporting units

Manufacturers of quantitative FIT have, until recently, reported the concentration of hemoglobin dissolved in the devices' collection buffer (ng hemoglobin/mL buffer). This concentration is dependent upon the mass of feces added to the buffer and the volume of buffer in which it is dissolved. While this enables comparison in studies with a single FIT, the data cannot be compared with data from other FIT.⁵⁹ The solution is to standardize the units used and adopt hemoglobin concentration in feces (μg hemoglobin/g feces).^{62,64} Manufacturers have agreed to adopt these standardized units and the WEO EWP is currently preparing a recommended method to standardize how manufacturers and others should determine the average fecal

mass collected by a device and added to the buffer. Clearly, a standardized process and material is necessary because the term currently used, average fecal sample, is a description without practical meaning.

By using a quantitative FIT, the screening program can select a cutoff fecal hemoglobin concentration appropriate to its resources and target clinical outcomes. Qualitative FIT do not provide this option since such FIT use lateral immunochromatographic devices on which the positivity marker becomes visible. The concentration at which the marker becomes positive is determined by the manufacturer and is affected by the visual skills of the device reader. The cutoff of each qualitative FIT needs to be stated by manufacturers and provided in μg hemoglobin/g feces.

While the adoption of a common reporting unit will help comparison, it will not make FIT products the same. The antibodies are likely to show marginal differences between manufacturer and the conditions of analysis will also contribute to reporting differences, although the adoption of a calibration preparation which has itself been calibrated against an internationally agreed hemoglobin standard will reduce reporting variation and begin to harmonize results. The WEO EWP has recommended that all calibrants should be traceable to an internationally recognized standard (e.g., the World Health Organization or the International Committee for Standardization in Haematology), a standard that has already been adopted by most manufacturers.

2. Fecal hemoglobin stability

The heme and the globin components of hemoglobin degrade at different rates in the intestines. The iron containing heme degrades slowly but the protein globin is subject to more rapid enzymatic proteases action. Since FIT analysis depends on antibodies binding to globin, it is more susceptible to false negative results if samples are not adequately preserved. In 2009, the government of Australia had to temporarily suspend invitations to participation in the National Bowel Cancer Screening Program after problems were found in the buffer of the FIT kits that were distributed between December 2008 and May 2009. The buffer in the FIT specimen collection device was not sufficiently effective at minimizing hemoglobin degradation at high temperatures. As a result, the collection devices returned for analysis yielded a lower than expected proportion of positive results, which increased the likelihood that some participants would receive false negative results. The potential for inaccurate FIT results during hot weather was investigated in Italian retrospective studies and showed hemoglobin concentrations 17% higher in the winter when 13% more cancers were detected.⁶⁵ In The Netherlands the observation of a seasonal variation was confirmed by demonstration of an average fall in FIT positivity from 10% to 6%.⁶⁶ Since 2011, companies have been actively enhancing the effectiveness of their preservative

buffers, using antibacterial and stabilizing agents and a 2013 UK National Health Service evaluation report has shown minimal deterioration in samples stored at temperatures up to 25°C in four quantitative FIT products.⁶⁷

Dry sampling in FIT systems reduces the rate of microbial and enzymatic degradation but it is not consistent across all samples and requires significant additional processing of the FIT on arrival in the laboratory. As the UK study has shown, FIT sample stability is less likely to be a problem as the new more effective stabilizing buffers are introduced.

3. Maximizing FIT uptake and adherence

Many studies have demonstrated that the uptake and adherence rates in screening programs using FIT are better than in screening programs using gFOBT. Two Dutch cohort studies reported by van Rossum *et al.*⁴³ and by Hol *et al.*⁶⁸ illustrate the difference. They compared gFOBT/FIT uptake rates and showed 47% and 60% respectively in one⁴³ and 49.5% and 61.5% respectively in the other.⁶⁸

A problem for all fecal-based (gFOBT or FIT) screening is that maximal clinical benefit requires good initial uptake rates that are maintained over multiple screening rounds, annually or biennially. In 2009, good adherence (85%) over three rounds using gFOBT every 2 years was reported in Scotland⁶⁹ thus, it is not surprising that similar uptake and adherence has been reported in FIT screening programs over two rounds. Two Dutch

Table 2. Participation Rates and Advanced Neoplasia Detection Rates at Round 2 of Participants in a FIT Screening Program Screened at Intervals from 1-3 Years

Characteristic	After 1 yr	After 2 yr	After 3 yr
Participation	63.2	62.5	64.0
Positivity	5.1	6.8	5.6
Detection rate			
Advanced neoplasia	1.6	2.1	1.6

Positivity and advanced neoplasia is at the second screening visit (R2) for those who participated in the first screening visit (R1). Data are presented as percentage. Adapted from van Roon AH, *et al.* Gut 2013;62:409-415, with permission from BMJ Publishing Group Ltd.⁷¹ FIT, fecal immunochemical test for hemoglobin.

studies^{70,71} have shown uptake and adherence after two rounds of FIT testing to be from 63% to 86% (Table 2).⁷¹ An Italian study evaluating the outcome of four rounds of FIT screening over 7 years showed an adherence rate of 48% for all four rounds, with participation in each round ranging from 56% to 63%.⁷²

Several investigators have examined ways to improve participation and a major national study in England is examining general physician endorsement, the use of narrative leaflets, and enhancing the information content of reminder letters. In the United States, Potter *et al.*⁷³⁻⁷⁶ have developed and promoted a unique and successful way to increase FIT uptake and participation by using trained clinical teams to offer FIT at the same time as patients receive their annual influenza vaccinations (Table 3).⁷⁷ It has been known for a long time that endorsement by the primary care practitioner consistently improves participation in screening for CRC.^{78,79}

4. Determining the number of FIT samples necessary for best results and participant acceptance

There are only a few studies that can help resolve this challenging issue. A study reported in 2011 by van Roon *et al.*⁸⁰ is the most comprehensive study yet undertaken to examine the value of more than one sample FIT testing at a range of cutoff fecal hemoglobin concentrations. It examined participation and clinical outcomes with one or two FIT after changing the FIT cutoff and by using the two FIT results individually and together. It observed no difference in participation rate when requesting completion of one or two kits and concluded that, for the detection of advanced adenomas, a single FIT performed as well as two, unless colonoscopy capacity was particularly high or low when the use of two FIT might prove beneficial.

5. FIT measurement and interpretation: POCT or central laboratory?

In the United States, FIT manufacturers mostly market FIT as POCT devices but there is little or no training, quality control monitoring, or oversight of procedures in place to give confidence in the reliability of the result and in the interpretation of the test result. Although qualitative FIT might

Table 3. Increased, Updated Screenings from Individuals Who Were Offered FIT Concurrent with Their Annual Flu Shots

Data for 6 SFPDPH clinics participating in the FLU-FOBT RCT	No. of flu shot recipients	CRCS up to date among flu shot recipients, no (%)
2008/9 (1 yr before)	3,260	1,385 (42.5)
2009/10 (intervention yr)	3,634	1,982 (54.5)
2010/11 (1 yr later)	4,333	2,440 (55.8)

Increased number of flu shots given and the number and proportion of flu shot recipients becoming up to date with colorectal cancer screening (CRCS). CRCS up to date defined as having fecal occult blood tests (FOBT) within 12 months, flexible sigmoidoscopy within 5 years or colonoscopy within 10 years. Adapted from Walsh JM, *et al.* Health Educ Res 2012;27:886-894, with permission from Oxford University Press.⁷⁷ FIT, fecal immunochemical test for hemoglobin; SFPDPH, San Francisco Department of Public Health; FLU-FOBT, primary care intervention pairs in the offering of FOBT with yearly influenza vaccine activities; RCT, randomized controlled trial.

be considered simple to use, and they all contain some form of integral control, the color development is dynamic and reading time is critical because delayed reading may give false positive or false negative results. The skill and visual acuity of a trained operator is important if detection of trace positive test lines on the immunochromatographic FIT devices is to be consistent. External quality assessment and performance monitoring forms an important part of good laboratory practice and this must be extended to POCT if a POCT-based screening program is to be reliable and effective.

6. Analytical and clinical effectiveness studies of different FIT

It is normally possible to determine clinical effectiveness of a new device or method by a thorough analytical assessment followed by comparison with an assessment of a similar device of known analytical characteristics and established clinical performance. A new FIT would therefore require an in-depth assessment of the product's analytical performance, an assessment of the presentation of the device to the invited subject, a review of the product design, ease of use and associated literature, and then a comparison of the data with that of a similar device with known analytical and clinical performance characteristics. This principle is well established for introducing new analytical devices and new test methods into clinical laboratories. The principle is less robust when innovative products are introduced and comparison data are not available. Innovative products or products that are significantly different from those already in use require thorough analytical assessment followed by clinical trials or pilots. FIT is such a technology; it uses a well-established biomarker (hemoglobin) which has been subject to at least four randomized controlled trials and it improves the detection system in a way that not only enables it to be more sensitive and/or specific but introduces the opportunity to detect advanced adenomas as well as cancers.

When choosing a quantitative FIT for a screening program we recommend those which have been well characterized both analytically and clinically and are suitable for use in comparative assessment of other similar devices. Decisions should be made with the knowledge of potential deficiencies such as sample stability reported in the Dutch and Italian studies cited above. More studies comparing FIT performance in large average risk populations are needed. One such study from France was published recently.⁸¹ The recent evaluation of the four quantitative FIT products with potential for population screening in the NHS Cancer Screening Program will help to select a FIT for national adoption.⁶⁷

The selection of FIT products is only the beginning and close monitoring with quality control and assessment programs are necessary if FIT results are going to meet the quality standards required for population screening. While accredited laboratories

with appropriate ISO standards will provide confidence for quantitative FIT, maintaining high quality for POCT qualitative FIT is a challenge, particularly if the test is being performed on many sites across a large geographical area. Consideration needs to be given, prior to the development of a new screening program, to systems for external quality monitoring of the analytical performance of POCT FIT and how poor performance will be managed. Real time monitoring of test outcomes in screening programs is important and was what alerted the Australian program to a problem with sample stability.

7. Which FIT (qualitative or quantitative) are best for population screening?

The issue of qualitative versus quantitative FIT has been visited throughout this review. Qualitative FIT are not well suited to organized population-based screening programs which have at their core, common, and consistent processes, close monitoring of performance data, audit and a general emphasis on high performance quality, economies of scale, and cost effectiveness. Activities are generally centralized and the opportunity for individual preference is minimal. Qualitative FIT cannot take advantage of the ability of quantitative FIT to select and adjust fecal hemoglobin cutoff concentrations; it cannot therefore adjust positivity rates to meet endoscopic resource or select target clinical sensitivity or positive predictive value. It cannot fully exploit the relationship between hemoglobin concentration and severity of the lesion to maximize detection nor can it easily adopt a sophisticated multivariate risk stratification model which is likely to demand ready access to participant clinical and screening history for risk computation.

The advantage of qualitative FIT is that, in the absence of an organized screening program and at minimal cost to an individual, a simple noninvasive screen for CRC can be performed. It is potentially, and perhaps superficially, a more personal and appealing method of screening and evidence shows that a motivated primary care physician (PCP) is an effective advocate for screening and will increase screening participation. Effective replication across a population with less motivated PCPs presents a challenge.

The likely total cost of population-based screening using qualitative FIT will be greater than that of organized screening using an automated high volume quantitative FIT system if full-cost analysis is undertaken. For most health systems, the cost of the test will be small alongside the cost of clinical services. Quantitative FIT provide the tools to control colonoscopy referrals. For qualitative tests, the referral rates are dictated by the manufacturer's embedded fecal hemoglobin cutoff concentration.

We advocate the use of quantitative, automated FIT over qualitative FIT (especially those without automation). The evidence to support the opportunities for more effective screening strategies using quantitative FIT is still growing and

Table 4. The Quantitative FIT for Hemoglobin Results Showed a Direct Correlation between the Amount of Blood in the Stool and the Occurrence of Advanced Neoplasms (Cancer and Advanced Adenomas)

Characteristic	Patients, no (%)	Lesion size, mm		Mean FIT result, ng/mL	
		Mean±SD	95% CI	Mean±SD	95% CI
Normal	739 (73.9)			35±143	25-45
Advanced adenoma	74 (7.4)	12.6±6.4	11.2-14.1	485±744	315-654
Colon site					
Proximal	31 (12.7)	12.4±6.8	10.1-14.7	499±774	227-772
Distal	42 (17.2)	12.9±6.2	11.0-14.7	501±737	279-724
Cancer stages					
Dukes A and B	15 (88.2)	30.7±9.3	26.0-35.4	1,045±777	652-1,439
Dukes C and D	2 (11.8)	50.0±7.1	40.2-59.8	1,399±1,452	614-3,411
Colon site					
Proximal	10 (58.8)	33.8±10.3	27.4-40.2	701±672	285-1,118
Distal	7 (41.2)	31.7±12.5	22.4-41.0	1,637±720	1,104-2,171

Adapted from Levi Z, *et al.* Ann Intern Med 2007;146:244-255, with permission from American College of Physicians.⁴⁰
FIT, fecal immunochemical test for hemoglobin; CI, confidence interval.

an editorial by Imperiale⁸² on a 2007 Israeli study⁴⁰ showed that the mean fecal hemoglobin value increases in a clinically important and statistically significant way as the neoplastic finding advanced from normal to nonadvanced adenoma to advanced adenoma to cancer (Table 4, Fig. 4). As discussed in a recent editorial,⁸³ these FIT have the advantage of being able to be modified for use in countries with nationally organized screening programs where cutoff hemoglobin concentration(s) can be determined to meet preset objectives and available financial and endoscopy resources. In the United States, the U.S. Food and Drug Administration has yet to approve quantitative FIT products.

8. Setting cutoff concentrations

In current practice, a single cutoff fecal hemoglobin concentration is used with quantitative FIT to divide the population into those who require follow-up, usually by colonoscopy, and those who do not. This practice does not exploit the full potential of quantitative FIT that can enable individualized tuning of the screening strategy applied. Other factors like age, sex, screening and family history, BMI, alcohol consumption, smoking, etc. also contribute to the likelihood of a significant neoplasm and, with quantitative FIT, these parameters can be explored to find a multivariate risk model that could provide enhanced risk stratification enabling more clinically and cost effective endoscopy referrals. As mentioned in FIT facts above, a recent publication from The Netherlands demonstrated that by combining risk factors with FIT in a multivariate risk score and referring higher risk patients for colonoscopy (a total of 102) rather than just those with a positive FIT, five more cases of advanced neoplasia would be identified.²⁷ The Dutch study showed that while FIT

concentration was the major contributor to their risk model, age, calcium intake, family history of CRC, and past or current smoking were all contributors but that BMI, menstrual status, fiber intake, aspirin/nonsteroidal anti-inflammatory drug use, and red meat intake were not significant.

Screening programs using FIT and gFOBT have shown major age and sex differences in fecal hemoglobin concentrations. In a randomized trial of gFOBT versus FIT, the positivity rate and the detection rate for all colorectal neoplasia was higher for men than women and higher for those aged 60 years and above.⁴³ In a study on the effects of sex on FIT, it was found that at any cutoff for hemoglobin concentration, sensitivity, and positive predictive value were substantially higher, and the specificity and negative predictive value were substantially lower among men than women.⁸⁴ The authors of the study raise the issue of a program having a different cutoff concentration for men and women, an arrangement that is commonplace for reference ranges used in clinical laboratories. It has been suggested that similar findings on sex and age mean that there is a need for more tailored screening strategies.⁸⁵

SUMMARY AND CONCLUSIONS

In 2014, it can no longer be argued that FIT is incapable of decreasing both CRC mortality and incidence in a screening population nor that fecal hemoglobin is an unsuitable marker for screen-relevant neoplasia. FIT is recognized by most countries with CRC population-based screening programs as the best screening test.¹⁴ FIT bring many advantages to CRC screening, presents new challenges and offers opportunities for further enhancement to screening programs. While new noninvasive tests will be developed, FIT is currently the test of

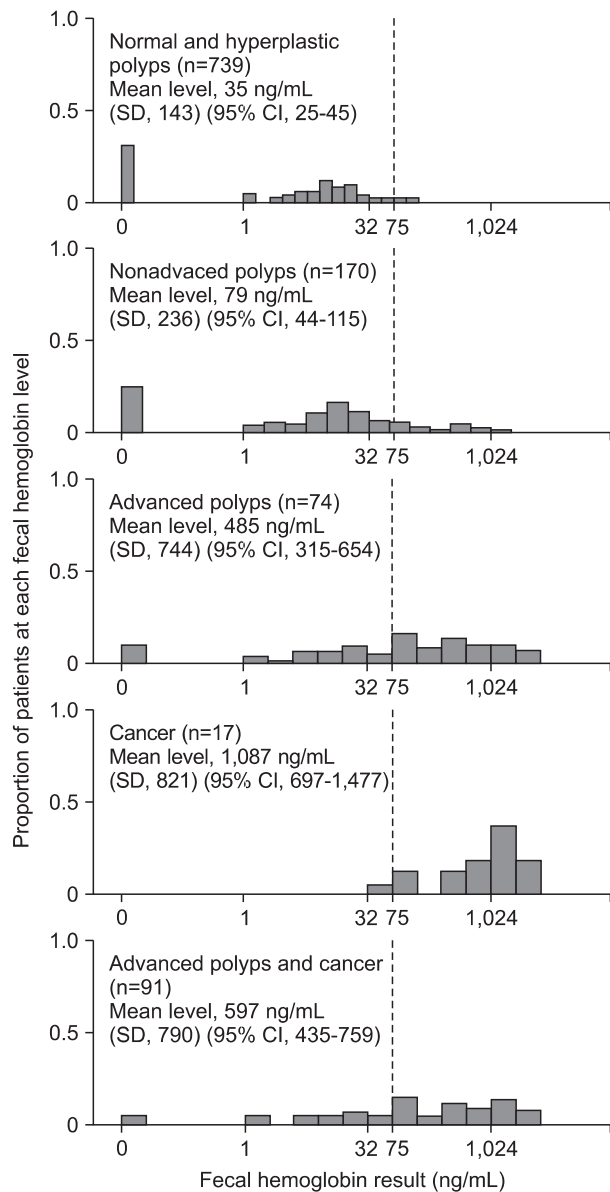


Fig. 4. Fecal hemoglobin concentrations in 1,000 consecutive ambulatory patients with increased risk of colorectal neoplasia or symptomatic. Adapted from Levi Z, *et al.* *Ann Intern Med* 2007;146:244-255, with permission from American College of Physicians.⁴⁰ SD, standard deviation; CI, confidence interval.

choice and is the biomarker test against which new tests must be compared and assessed.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

- Potter MB. Strategies and resources to address colorectal cancer screening rates and disparities in the United States and globally. *Annu Rev Public Health* 2013;34:413-429.
- Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 2010;19:1893-1907.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011;61:69-90.
- Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. *CA Cancer J Clin* 2009;59:366-378.
- Hardcastle JD, Chamberlain JO, Robinson MH, *et al.* Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 1996;348:1472-1477.
- Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996;348:1467-1471.
- Mandel JS, Church TR, Bond JH, *et al.* The effect of fecal occult-blood screening on the incidence of colorectal cancer. *N Engl J Med* 2000;343:1603-1607.
- Hoff G, Grotmol T, Skovlund E, Bretthauer M; Norwegian Colorectal Cancer Prevention Study Group. Risk of colorectal cancer seven years after flexible sigmoidoscopy screening: randomised controlled trial. *BMJ* 2009;338:b1846.
- Atkin WS, Edwards R, Kralj-Hans I, *et al.* Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: a multicentre randomised controlled trial. *Lancet* 2010;375:1624-1633.
- Segnan N, Armaroli P, Bonelli L, *et al.* Once-only sigmoidoscopy in colorectal cancer screening: follow-up findings of the Italian Randomized Controlled Trial: SCORE. *J Natl Cancer Inst* 2011;103:1310-1322.
- Schoen RE, Pinsky PF, Weissfeld JL, *et al.* Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy. *N Engl J Med* 2012;366:2345-2357.
- Shaukat A, Mongin SJ, Geisser MS, *et al.* Long-term mortality after screening for colorectal cancer. *N Engl J Med* 2013;369:1106-1114.
- Segnan N, Patnick J, von Karsa L; European Commission Directorate General for Health & Consumers; International Agency for Research on Cancer. European guidelines for quality assurance in colorectal cancer screening and diagnosis. 1st ed. Luxembourg: Publications Office of the European Union, 2010.
- Halloran SP, Launoy G, Zappa M; International Agency for Research on Cancer. European guidelines for quality assurance in colorectal cancer screening and diagnosis: first Edition: faecal occult blood testing. *Endoscopy* 2012;44 Suppl 3:SE65-SE87.
- Imperiale TF. Noninvasive screening tests for colorectal cancer. *Dig Dis* 2012;30 Suppl 2:16-26.
- Levin B, Lieberman DA, McFarland B, *et al.* Screening and surveillance for the early detection of colorectal cancer and

- adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 2008;134:1570-1595.
17. Ahlquist DA, Skoletsky JE, Boynton KA, et al. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* 2000;119:1219-1227.
 18. Osborn NK, Ahlquist DA. Stool screening for colorectal cancer: molecular approaches. *Gastroenterology* 2005;128:192-206.
 19. Cole SR, Young GP. Effect of dietary restriction on participation in faecal occult blood test screening for colorectal cancer. *Med J Aust* 2001;175:195-198.
 20. Cole SR, Young GP, Esterman A, Cadd B, Morcom J. A randomised trial of the impact of new faecal haemoglobin test technologies on population participation in screening for colorectal cancer. *J Med Screen* 2003;10:117-122.
 21. Vart G, Banzi R, Minozzi S. Comparing participation rates between immunochemical and guaiac faecal occult blood tests: a systematic review and meta-analysis. *Prev Med* 2012;55:87-92.
 22. Digby J, McDonald PJ, Strachan JA, Libby G, Steele RJ, Fraser CG. Use of a faecal immunochemical test narrows current gaps in uptake for sex, age and deprivation in a bowel cancer screening programme. *J Med Screen* 2013;20:80-85.
 23. Fleisher M, Winawer SJ, Zauber AG, Smith C, Schwartz MK. Accuracy of fecal occult blood test interpretation. National Polyp Study Work Group. *Ann Intern Med* 1991;114:875-6.
 24. Niv Y. Fecal occult blood test: the importance of proper evaluation. *J Clin Gastroenterol* 1990;12:393-395.
 25. Burtonwood C, Butler P, Young M, Halloran S. Monitoring faecal occult blood test positivity in the NHS bowel cancer screening programme. *Gut* 2012;61:A334.
 26. 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 Eur Gastroenterol J* 2013;1:198-205.
 27. Stegeman I, de Wijkerslooth TR, Stoop EM, et al. Combining risk factors with faecal immunochemical test outcome for selecting CRC screenees for colonoscopy. *Gut* 2014;63:466-471.
 28. Simon JB. Occult blood screening for colorectal carcinoma: a critical review. *Gastroenterology* 1985;88:820-837.
 29. Barrows GH, Burton RM, Jarrett DD, Russell GG, Alford MD, Songster CL. Immunochemical detection of human blood in feces. *Am J Clin Pathol* 1978;69:342-346.
 30. Songster CL, Barrows GH, Jarrett DD. Immunochemical detection of human fecal occult blood. In: Schottenfeld D, Sherlock P, Winawer SJ, eds. *Colorectal cancer, prevention, epidemiology, and screening*. Volume 13. New York: Raven Press, 1980:193-204.
 31. Songster CL, Barrows GH, Jarrett DD. Immunochemical detection of fecal occult blood: the fecal smear punch-disc test: a new non-invasive screening test for colorectal cancer. *Cancer* 1980;45(Suppl):1099-1102.
 32. Vellacott KD, Baldwin RW, Hardcastle JD. An immunofluorescent test for faecal occult blood. *Lancet* 1981;1:18-19.
 33. St John DJ, Young GP, Alexeyeff MA, et al. Evaluation of new occult blood tests for detection of colorectal neoplasia. *Gastroenterology* 1993;104:1661-1668.
 34. Williams JA, Hunter R, Smith M, Coles ME, Hubert TW, Thomas DW. Evaluation of an immunological test for occult bleeding from colorectal neoplasia. *Aust N Z J Surg* 1982;52:617-621.
 35. Robinson MH, Marks CG, Farrands PA, Thomas WM, Hardcastle JD. Population screening for colorectal cancer: comparison between guaiac and immunological faecal occult blood tests. *Br J Surg* 1994;81:448-451.
 36. Rozen P, Knaani J, Papo N. Evaluation and comparison of an immunochemical and a guaiac faecal occult blood screening test for colorectal neoplasia. *Eur J Cancer Prev* 1995;4:475-481.
 37. Allison JE, Tekawa IS, Ransom LJ, Adrain AL. A comparison of fecal occult-blood tests for colorectal-cancer screening. *N Engl J Med* 1996;334:155-159.
 38. Petrelli N, Michalek AM, Freedman A, Baroni M, Mink I, Rodriguez-Bigas M. Immunochemical versus guaiac occult blood stool tests: results of a community-based screening program. *Surg Oncol* 1994;3:27-36.
 39. Allison JE, Sakoda LC, Levin TR, et al. Screening for colorectal neoplasms with new fecal occult blood tests: update on performance characteristics. *J Natl Cancer Inst* 2007;99:1462-1470.
 40. Levi Z, Rozen P, Hazazi R, et al. A quantitative immunochemical fecal occult blood test for colorectal neoplasia. *Ann Intern Med* 2007;146:244-255.
 41. Morikawa T, Kato J, Yamaji Y, Wada R, Mitsushima T, Shiratori Y. A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. *Gastroenterology* 2005;129:422-428.
 42. Park DI, Ryu S, Kim YH, et al. Comparison of guaiac-based and quantitative immunochemical fecal occult blood testing in a population at average risk undergoing colorectal cancer screening. *Am J Gastroenterol* 2010;105:2017-2025.
 43. van Rossum LG, van Rijn AF, Laheij RJ, et al. Random comparison of guaiac and immunochemical fecal occult blood tests for colorectal cancer in a screening population. *Gastroenterology* 2008;135:82-90.
 44. Allison JE. The imperative of equal funding for studies that evaluate any of the evidence-based, guideline-recommended colorectal cancer screening tests. *Clin Gastroenterol Hepatol* 2009;7:1269-1271.
 45. Saito H. Screening for colorectal cancer by immunochemical fecal occult blood testing. *Jpn J Cancer Res* 1996;87:1011-1124.
 46. Saito H, Soma Y, Koeda J, et al. Reduction in risk of mortality from colorectal cancer by fecal occult blood screening with immunochemical hemagglutination test. A case-control study. *Int J Cancer* 1995;61:465-469.
 47. Shimbo T, Glick HA, Eisenberg JM. Cost-effectiveness analysis of

- strategies for colorectal cancer screening in Japan. *Int J Technol Assess Health Care* 1994;10:359-375.
48. Cole P, Morrison AS. Basic issues in population screening for cancer. *J Natl Cancer Inst* 1980;64:1263-1272.
 49. Levi Z, Hazazi R, Rozen P, Vilkin A, Waked A, Niv Y. A quantitative immunochemical faecal occult blood test is more efficient for detecting significant colorectal neoplasia than a sensitive guaiac test. *Aliment Pharmacol Ther* 2006;23:1359-1364.
 50. Young GP, Fraser CG, Halloran SP, Cole S. Guaiac based faecal occult blood testing for colorectal cancer screening: an obsolete strategy? *Gut* 2012;61:959-960.
 51. Fletcher RH. Commentary. *ACP J Club* 1996;124:74.
 52. Cole SR, Tucker GR, Osborne JM, et al. Shift to earlier stage at diagnosis as a consequence of the National Bowel Cancer Screening Program. *Med J Aust* 2013;198:327-330.
 53. Ventura L, Mantellini P, Grazzini G, et al. The impact of immunochemical faecal occult blood testing on colorectal cancer incidence. *Dig Liver Dis* 2014;46:82-86.
 54. Zuber AG, Lansdorp-Vogelaar I, Knudsen AB, Wilschut J, van Ballegooijen M, Kuntz KM. Evaluating test strategies for colorectal cancer screening: a decision analysis for the U.S. Preventive Services Task Force. *Ann Intern Med* 2008;149:659-669.
 55. National Colorectal Cancer Roundtable. Tools & resources: the new FOBT clinician's reference resource [Internet]. [place unknown]: National Colorectal Cancer Roundtable, c2002 [cited 2013 Sep 27]. Available from: <http://nccr.org/about/provider-education/fobt-clinicians-reference-resources>.
 56. Qaseem A, Denberg TD, Hopkins RH Jr, et al. Screening for colorectal cancer: a guidance statement from the American College of Physicians. *Ann Intern Med* 2012;156:378-386.
 57. Sugeran DT. JAMA Patient Page: options for colorectal cancer screening. *JAMA* 2013;310:658.
 58. Quintero E, Castells A, Bujanda L, et al. Colonoscopy versus fecal immunochemical testing in colorectal-cancer screening. *N Engl J Med* 2012;366:697-706.
 59. Allison JE, Fraser CG, Halloran SP, Young GP. Comparing fecal immunochemical tests: improved standardization is needed. *Gastroenterology* 2012;142:422-424.
 60. Fraser CG, Allison JE, Young GP, Halloran SP. Newer fecal tests: opportunities for professionals in laboratory medicine. *Clin Chem* 2012;58:963-965.
 61. Fraser CG, Halloran SP, Allison JE, Young GP. Making colorectal cancer screening FITTER for purpose with quantitative faecal immunochemical tests for haemoglobin (FIT). *Clin Chem Lab Med* 2013;1-3.
 62. Fraser CG, Allison JE, Halloran SP, Young GP; Expert Working Group on Fecal Immunochemical Tests for Hemoglobin, Colorectal Cancer Screening Committee, World Endoscopy Organization. A proposal to standardize reporting units for fecal immunochemical tests for hemoglobin. *J Natl Cancer Inst* 2012;104:810-814.
 63. Fraser CG, Allison JE, Young GP, Halloran SP. Quantitation of hemoglobin improves fecal immunochemical tests for noninvasive screening. *Clin Gastroenterol Hepatol* 2013;11:839-840.
 64. Tao S, Seiler CM, Ronellenfitsch U, Brenner H. Comparative evaluation of nine faecal immunochemical tests for the detection of colorectal cancer. *Acta Oncol* 2013;52:1667-1675.
 65. Grazzini G, Ventura L, Zappa M, et al. Influence of seasonal variations in ambient temperatures on performance of immunochemical faecal occult blood test for colorectal cancer screening: observational study from the Florence district. *Gut* 2010;59:1511-1515.
 66. van Roon AH, Hol L, van Vuuren AJ, et al. Are fecal immunochemical test characteristics influenced by sample return time? A population-based colorectal cancer screening trial. *Am J Gastroenterol* 2012;107:99-107.
 67. NHS Bowel Cancer Screening Southern Programme Hub (BCSP). Evaluation of quantitative faecal immunochemical tests for haemoglobin [Internet]. Surrey: NHS BCSP; 2013 [cited 2014 Feb 3]. Available from: http://www.worldendo.org/assets/downloads/pdf/activities/fit_reports/gmec_fit_evaluation_report.pdf.
 68. Hol L, van Leerdam ME, van Ballegooijen M, et al. Screening for colorectal cancer: randomised trial comparing guaiac-based and immunochemical faecal occult blood testing and flexible sigmoidoscopy. *Gut* 2010;59:62-68.
 69. Steele RJ, McClements PL, Libby G, et al. Results from the first three rounds of the Scottish demonstration pilot of FOBT screening for colorectal cancer. *Gut* 2009;58:530-535.
 70. Denters MJ, Deutekom M, Bossuyt PM, Stroobants AK, Fockens P, Dekker E. Lower risk of advanced neoplasia among patients with a previous negative result from a fecal test for colorectal cancer. *Gastroenterology* 2012;142:497-504.
 71. van Roon AH, Goede SL, van Ballegooijen M, et al. Random comparison of repeated faecal immunochemical testing at different intervals for population-based colorectal cancer screening. *Gut* 2013;62:409-415.
 72. Crotta S, Segnan N, Paganin S, Dagnes B, Rosset R, Senore C. High rate of advanced adenoma detection in 4 rounds of colorectal cancer screening with the fecal immunochemical test. *Clin Gastroenterol Hepatol* 2012;10:633-638.
 73. Potter MB, Ackerson LM, Gomez V, et al. Effectiveness and reach of the FLU-FIT program in an integrated health care system: a multisite randomized trial. *Am J Public Health* 2013;103:1128-1133.
 74. Potter MB, Gildengorin G, Wang Y, Wu M, Kroon L. Comparative effectiveness of two pharmacy-based colorectal cancer screening interventions during an annual influenza vaccination campaign. *J Am Pharm Assoc (2003)* 2010;50:181-187.
 75. Potter MB, Phengrasamy L, Hudes ES, McPhee SJ, Walsh JM. Offering annual fecal occult blood tests at annual flu shot clinics increases colorectal cancer screening rates. *Ann Fam Med* 2009;7:17-23.
 76. Potter MB, Somkin CP, Ackerson LM, et al. The FLU-FIT program: an effective colorectal cancer screening program for high volume flu shot clinics. *Am J Manag Care* 2011;17:577-583.

77. Walsh JM, Gildengorin G, Green LW, Jenkins J, Potter MB. The FLU-FOBT Program in community clinics: durable benefits of a randomized controlled trial. *Health Educ Res* 2012;27:886-894.
78. Zajac IT, Whibley AH, Cole SR, et al. Endorsement by the primary care practitioner consistently improves participation in screening for colorectal cancer: a longitudinal analysis. *J Med Screen* 2010;17:19-24.
79. Hewitson P, Ward AM, Heneghan C, Halloran SP, Mant D. Primary care endorsement letter and a patient leaflet to improve participation in colorectal cancer screening: results of a factorial randomised trial. *Br J Cancer* 2011;105:475-480.
80. van Roon AH, Wilschut JA, Hol L, et al. Diagnostic yield improves with collection of 2 samples in fecal immunochemical test screening without affecting attendance. *Clin Gastroenterol Hepatol* 2011;9:333-339.
81. Raginel T, Puvinel J, Ferrand O, et al. A population-based comparison of immunochemical fecal occult blood tests for colorectal cancer screening. *Gastroenterology* 2013;144:918-925.
82. Imperiale TF. Quantitative immunochemical fecal occult blood tests: is it time to go back to the future? *Ann Intern Med* 2007;146:309-311.
83. Fraser CG. A future for faecal haemoglobin measurements in the medical laboratory. *Ann Clin Biochem* 2012;49(Pt 6):518-526.
84. Brenner H, Haug U, Hundt S. Sex differences in performance of fecal occult blood testing. *Am J Gastroenterol* 2010;105:2457-2464.
85. Khalid-de Bakker CA, Jonkers DM, Sanduleanu S, et al. Test performance of immunologic fecal occult blood testing and sigmoidoscopy compared with primary colonoscopy screening for colorectal advanced adenomas. *Cancer Prev Res (Phila)* 2011;4:1563-1571.