Population screening for colorectal cancer means getting FIT
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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. New Zealand, Europe, and North America have the highest CRC incidence, and Africa and Asia the lowest. 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.

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 incidence 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. Some in the United States are committed to a single step colonoscopy screening strategy.
because they argue that FIT has little effect on CRC incidence.\textsuperscript{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.\textsuperscript{16,17} 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 faecal occult blood test, immunohistochemical test, etc. as the only recommended term. The WEO EWP has chosen not to enforce the use of FIT\textsuperscript{19} or FIT\textsuperscript{20} 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 in quantitative FIT systems. 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.

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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. 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. 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. 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).

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. 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.

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. In the 1970s,
Barrows et al. and Songster et al. 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.

FIT were first used in the 1980s for screening in Japan. Research with FIT was subsequently described in Australia, England, Israel, and the United States 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. 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.

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. 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. 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. 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. 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. In a 2007 Kaiser Permanente study, FIT performance characteristics were compared with those of the
high sensitivity guaiac FOBT (gFOBT) 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 gFOBT (Table 1). In 2001 the Dutch published the first randomized comparison between gFOBT and FIT in an average risk screening population. 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. 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.

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 gFOBT and FS 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. As described earlier, FIT like gFOBT use detection of occult fecal blood in feces to identify those screened subjects most likely to harbor advanced neoplasm. 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 mortality and preliminary data from Italy 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, population screening program.

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

<table>
<thead>
<tr>
<th>Finding per test</th>
<th>No. of persons screened</th>
<th>No. of neoplasms detected</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Likelihood ratio (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./total % (95% CI)</td>
<td>No./total % (95% CI)</td>
<td>No./total % (95% CI)</td>
<td>No./total % (95% CI)</td>
<td>No./total % (95% CI)</td>
<td>No./total % (95% CI)</td>
</tr>
<tr>
<td>Distant cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitive guaiac test</td>
<td>5,799</td>
<td>14</td>
<td>9/14</td>
<td>64.3 (35.6-86.0)</td>
<td>9,210/5,785</td>
<td>90.1 (89.3-90.8)</td>
</tr>
<tr>
<td>FIT 1</td>
<td>5,356</td>
<td>11</td>
<td>9/11</td>
<td>81.8 (47.8-96.8)</td>
<td>5,181/5,245</td>
<td>96.9 (96.4-97.4)</td>
</tr>
<tr>
<td>Distant adenomas ≥ 1 cm</td>
<td>5,799</td>
<td>126</td>
<td>52/126</td>
<td>41.3 (27.7-50.4)</td>
<td>5,141/5,673</td>
<td>90.6 (89.8-91.4)</td>
</tr>
<tr>
<td>Sensitive guaiac test</td>
<td>5,799</td>
<td>112</td>
<td>23/112</td>
<td>29.5 (21.4-38.9)</td>
<td>5,104/5,244</td>
<td>97.3 (96.8-97.7)</td>
</tr>
<tr>
<td>FIT 1</td>
<td>5,356</td>
<td>112</td>
<td>23/112</td>
<td>29.5 (21.4-38.9)</td>
<td>5,104/5,244</td>
<td>97.3 (96.8-97.7)</td>
</tr>
<tr>
<td>Distant advanced neoplasms</td>
<td>5,799</td>
<td>137</td>
<td>59/137</td>
<td>43.1 (34.7-51.8)</td>
<td>5,137/5,662</td>
<td>90.7 (89.9-91.5)</td>
</tr>
<tr>
<td>Sensitive guaiac test</td>
<td>5,799</td>
<td>137</td>
<td>59/137</td>
<td>43.1 (34.7-51.8)</td>
<td>5,137/5,662</td>
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</tbody>
</table>


Likelihood ratio (+)=sensitivity/(1-specificity).
high sensitivity, occult blood screening programs are the same as with a high-quality colonoscopy screening program. 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.

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 2012 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 WBO 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 WBO EWP.

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 (μg 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. The solution is to standardize the units used and adopt hemoglobin concentration in feces (μg hemoglobin/g feces). Manufacturers have agreed to adopt these standardized units and the WBO 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 WBO 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 Austria 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. In The Netherlands the observation of a seasonal variation was confirmed by demonstration of an average fall in FIT positivity from 10% to 6% since 2011, companies have been actively enhancing the effectiveness of their preservative...
buffer, 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.19

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.46 and by Hol et al.44 illustrate the difference. They compared gFOBT/FIT uptake rates and showed 47% and 60% respectively in one46 and 49.5% and 61.5% respectively in the other.44

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 Scotland;49 thus, it is not surprising that similar uptake and adherence has been reported in FIT screening programs over two rounds. Two Dutch studies49,56 have shown uptake and adherence after two rounds of FIT testing to be from 63% to 66% (Table 2).56 An Italian study evaluating the outcome of four rounds of FIT screening over 7 years showed an adherence rate of 43% for all four rounds, with participation in each round ranging from 56% to 63%.70

Several investigators have examined ways to improve participation and a major national study in England is examining general practitioner endorsement, the use of narrative leaflets, and enhancing the information content of reminder letters. In the United States, Potter et al.72-75 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.50 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 2. Participation Rates and Advanced Neoplasia Detection Rates at Round 1 of Participants in a FIT Screening Program Screened at Intervals from 1-3 Years

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>After 1 yr</th>
<th>After 2 yr</th>
<th>After 3 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participation</td>
<td>63.2</td>
<td>62.5</td>
<td>64.0</td>
</tr>
<tr>
<td>Positivity</td>
<td>5.1</td>
<td>6.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Detection rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advanced aepae</td>
<td>1.6</td>
<td>2.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Positivity and advanced aepae 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.78 FIT, fecal immunochemical test for hemoglobin.

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

<table>
<thead>
<tr>
<th>Data for 6 SFDPH clinics participating in the FLU-FOBT RCT</th>
<th>No. of flu shot recipients</th>
<th>CRCS up to date among flu shot recipients, no (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008/9 (1 yr before)</td>
<td>2,260</td>
<td>1,385 (61.5)</td>
</tr>
<tr>
<td>2009/10 (intervention yr)</td>
<td>3,634</td>
<td>1,982 (56.5)</td>
</tr>
<tr>
<td>2010/11 (1 yr later)</td>
<td>4,223</td>
<td>2,440 (59.5)</td>
</tr>
</tbody>
</table>

Increased number of flu shot 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 test (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.79 FIT, fecal immunochemical test for hemoglobin; SFDPH, San Francisco Department of Public Health; FLU-FOBT, primary care intervention pair 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 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 companion 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. 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.

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 therefore adopt 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
collection 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
quantitative 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 quantitative 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

Allison JE, et al: FIT for Colorectal Cancer Screening 125
an editorial by Imperiale on a 2007 Israeli study 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, 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 discussed in a recent editorial, 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.

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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. However, 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 choice.
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

1. Potter MB. Strategies and resources to address colorectal cancer screening rates and disparities in the United States and globally. Annu Rev Public Health 2013;34:413-419.
16. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and


70. Deeters MJ, Dewet RM, Bozort FM, Stooban AK, Focke PA, Deiker 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.


