Practical Advances in Stool Screening for Colorectal Cancer

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Key Words
Colorectal cancer, fecal DNA test, fecal immunochemical test, FIT, screening, adenomas

Abstract
The rationale for screening for colorectal cancer (CRC) is well established, and several tests are currently recommended. Colonoscopy has become a popular modality in most of the United States and other countries. Despite colonoscopy being highly accurate and therapeutic, many patients prefer a noninvasive screening test. Testing stool for occult blood by the chemical guaiac reagent (gFOBT) has been available for decades and is effective at reducing mortality from CRC. However, because of limitations in sensitivity and specificity, newer fecal immunochemical tests (FITs) were developed that detect occult blood using enzyme immunoassays. Because of their improved sensitivity and specificity, FITs have replaced gFOBT for screening in many settings. Detecting neoplasia-associated genetic changes in stool has also become feasible; first-generation stool DNA tests showed greater sensitivity for CRC, with similar specificity to gFOBT. Improvements to stool DNA tests have made them more sensitive and less complex. As the performance characteristics for FIT and stool DNA tests continue to evolve, stool-based testing for CRC is expected to become a more reliable component in the armamentarium for CRC screening. (JNCCN 2010;8:81–92)

Colorectal cancer (CRC) is a very common and often fatal cancer in the United States and worldwide. Screening has been shown to be highly effective in preventing the incidence and subsequent mortality from CRC, and has been endorsed by all major medical societies, receiving a Grade A recommendation by the United States Preventive Services Task Force (USPSTF) in its most recent guidelines. The various screening methods available to patients and physicians include structural screening tests that provide images of the colon through either endoscopic (colonoscopy, flexible sigmoidoscopy) or radiographic (air contrast barium enema, CT colonography) means, or noninvasive stool-based tests. Over the past decade, colonoscopy has become a popular, and in some cases the preferred, screening test in the United States because it not only detects cancers and polyps with high accuracy but is simultaneously therapeutic because of its ability to remove polyps and even early cancers.

Despite its considerable strengths, colonoscopy is associated with organizational, logistical, and patient-related barriers that often limit its full acceptance as a screening method. These include the need for a bowel preparation; the use of sedation in most instances (which also requires the need for a patient escort); loss of time from work; risk of complications, such as perforation and bleeding; and the small chance that colonoscopy may miss polyps and even cancers. Patients sometimes express fear of the procedure and embarrassment.

For all these reasons, stool-based screening tests are an attractive alternative for many screen-eligible people because they do not require a cleansing bowel preparation or the need to miss work, are noninvasive, and can be performed in the privacy of the home. Moreover, in parts of the world where colonoscopy resources are limited, stool-based tests, which have been based on detecting occult blood, remain the foundation of CRC screening.

For decades, stool-based tests relied on detecting fecal occult blood with the chemical guaiac reagent (gFOBT; e.g., Hemoccult). Although this approach reduces CRC
mortality by one third when the test is performed annually,1 gFOBTs, including the more sensitive versions (e.g., Hemoccult SENSA), have limited sensitivity and specificity for CRC, are not very good at detecting adenomas, and rely on patients complying with annual if not biennial testing to show efficacy. Newer stool tests include fecal immunochemical tests (FIT) and stool DNA tests. This article summarizes the current status of FIT and stool DNA testing for CRC screening.

FIT

Technological Aspects

gFOBT is based on a peroxidase reaction with the heme component of hemoglobin. gFOBTs have been shown to decrease CRC mortality, but this technology has multiple limitations. Sensitivity and specificity are somewhat limited, and the test is not specific for human heme, thereby necessitating dietary restrictions before the test. Ingestion of vitamin C can inactivate the peroxidase reaction, leading to a false-negative result. Currently the 2 most commonly used and tested gFOBTs are Hemoccult II and the more sensitive Hemoccult SENSA (Beckmann Coulter, Fullerton, California).

To improve on these limitations, immunoassays were developed to detect occult blood in the stool. FITs, sometimes referred to as immunochemical fecal occult blood tests (iFOBT), use an ELISA directed against human globin. As such, FIT does not require any dietary restrictions. Furthermore, because globin is degraded as it travels through the gastrointestinal tract, FIT is more specific for colorectal bleeding than gFOBT. Some FITs use a hemoglobin-stabilizing buffer to avoid degradation during transport of the specimen to the laboratory. However, despite this, prolonged delays in returning the test can result in more false-negative tests, particularly when detecting adenomas.4 A potential advantage for FIT is that many of these assays can be used in a quantitative manner to modify the performance characteristics to various populations.5 Although gFOBT is fairly standardized, many FIT kits are marketed, each with its own unique test characteristics. Significant variability exists between antigen target stability and stool sampling techniques among FIT assays. These differences make comparison of FIT studies difficult.

Performance Characteristics and Efficacy

FIT has been used clinically worldwide for almost 25 years. However, until recently no randomized evidence supported its efficacy. The bulk of evidence supporting FIT is from observational trials. Initial data on FIT came from case-control studies in Japan. Saito et al.6 compared 193 patients who died of CRC to 3 age- and gender-matched controls, and examined previous exposure to FIT testing. The odds ratio of dying of CRC in screened versus non-screened patients was 0.4 if screened within 1 year of diagnosis, and 0.48 if screened within 3 years.

A second case-control study performed by the same group looked at patients with advanced cancers defined as lesions invading the muscularis propria.7 The odds ratio of developing an advanced cancer for patients screened with FIT within 3 years of diagnosis versus non-screened patients was 0.54. When the case patients were divided into colon and rectal cancer, FIT seemed to provide a higher degree of protection for rectal cancer.

FIT is the accepted CRC screening strategy in Japan. Lee et al.8 evaluated a large population-based cohort of more than 42,000 Japanese individuals. Over a 13-year follow-up period, 597 incident cases of CRC and 132 deaths occurred. They reported a 70% decrease in CRC mortality in patients who underwent screening compared with those who did not, and a 59% decrease in incident cases of advanced CRC.

Morikawa et al.9 conducted the largest screening trial of FIT in which all patients also underwent colonoscopy. In a cohort of 21,805 patients, they reported 65.8% FIT sensitivity for invasive cancer; sensitivity increased with more advanced stages. The reported sensitivity for Dukes’ A cancer was 50%, which increased to 78.3% for Dukes’ C/D lesions. Overall 5.6% of patients had a positive FIT.

Because annual or biennial gFOBT has been shown in several large randomized trials to reduce CRC mortality,1 several studies have compared FIT performance to conventional gFOBT. Wong et al.10 compared the performance of a high-sensitivity gFOBT (Hemoccult SENSA) with the Flexsure immunochemical test. They evaluated 135 average-risk patients using both tests and all patients underwent colonoscopy. They reported sensitivity, specificity, and positive predictive values of 91%, 70%, and 18% for Hemoccult SENSA and 82%, 94%, and 47% for...
FIT, respectively. Combining the tests did not lead to improved performance.

Levi et al. 11 compared 151 patients who underwent both gFOBT and FIT with all patients undergoing colonoscopy. The sensitivity of both tests for advanced neoplasia (defined as carcinoma or an advanced adenoma) was 75%. However, FIT showed a specificity of 94% compared with 34% for gFOBT, which led to positive predictive values of 60% and 12%, respectively. In addition, Cole et al. 12 studied 460 patients who completed Hemoccult SENSA and FIT testing with the Insure test kit. They reported an 84.6% sensitivity for cancer using FIT versus 38.5% for Hemoccult SENSA. Sensitivity for significant neoplasia was 77.3% and 50%, respectively. Unlike the study by Levi et al., 11 specificities and positive predictive values between the 2 tests did not differ.

Finally, Allison et al. 13 examined the performance characteristic of Hemoccult SENSA and Flexsure FIT in a population of 5841 average-risk patients undergoing routine screening. Colonoscopy was offered to patients with a positive stool test, and sigmoidoscopy was performed in 80% of patients with a negative stool test. The sensitivities of FIT for CRC and advanced adenomas were 81.8% and 29%, respectively, compared with 64.3% and 41.3% for Hemoccult SENSA. Specificities for CRC and advanced adenomas were 96.9% and 97.3%, respectively, for FIT, compared with 90.1% and 90.6%, respectively, for Hemoccult SENSA. Overall FIT seems to have higher sensitivity and specificity for CRC than high-sensitivity gFOBT.

In a recent report of a large prospective randomized trial comparing gFOBT and FIT, van Rossum et al. 14 randomized more than 20,000 patients to be screened with Hemoccult II or the OC-SENSOR FIT. Patients with a positive stool test were offered colonoscopy. The rates of FIT participation (59%) and test positivity (5.5%) were higher than with Hemoccult II. Detection rates for advanced adenomas and cancer were higher in the FIT group, albeit at the expense of a slightly worse specificity. Nonetheless, the study was the first to show, in a randomized fashion, that intention-to-screen detection rates for cancers and adenomas are higher with FIT than with gFOBT.

The performance characteristics of FIT vary according to how it is used. Just as gFOBT depends on submitting samples from 3 (usually consecutive) bowel movements, large differences in FIT sensitivity and specificity are reported based on how many tests a patient completes. For example, Nakama et al. 15 examined test performance in 4611 asymptomatic adult patients completing a FIT on 1, 2, or 3 consecutive days, all of whom underwent colonoscopy as the gold standard examination. For 1, 2, or 3 sample collections, the sensitivities for CRC were 56%, 83%, and 89%, respectively, with specificities of 97%, 96%, and 94%, respectively. They concluded that optimal results were obtained when patients obtained stool specimens over 2 consecutive days. Although no consensus exists as to the optimum number of stool samples needed for FIT, the balance of data concur that repeated collections enhance sensitivity.

The use of nonsteroidal anti-inflammatory drugs (NSAIDs), including low-dose aspirin or anticoagulants, may increase gastrointestinal tract blood loss, thereby potentially affecting the results of stool occult blood testing (gFOBT or FIT). With increasing numbers of patients on these medications, the ramifications of their use on occult blood testing is important to understand. Levi et al. 16 conducted a cross-sectional study of 1221 patients who underwent colonoscopy after completing 3 FIT examinations, of which 227 patients were on either low-dose aspirin or NSAIDs. They reported a statistically insignificant trend toward enhanced sensitivity in aspirin/NSAID users but, more importantly, no decrease in specificity. The number of patients taking anticoagulants was too small to make any conclusions. Although this area deserves further study, it appears that patients may remain on low-dose aspirin/NSAIDs while undergoing FIT without adversely affecting test outcomes.

It is important to note that not all studies have shown FIT to have better performance characteristics than gFOBT. Ko et al. 17 studied more than 5900 patients in a CRC screening program; half were screened with Hemoccult SENSA and half with Flexure OBT. Approximately half of the patients in each group returned the cards and 9% of tests in each group were positive. Of the patients with a positive test who underwent colonoscopy, no significant difference was seen in numbers of total or advanced adenomas.

The fact that several FIT assays are quantitative allows for adjustments in cutoff values that can maximize sensitivity and specificity. For example, Levi et al. 18 showed that the amount of fecal hemoglobin...
detected with FIT increased from patients without neoplasia to those with advanced adenomas to those with cancer. Furthermore, by varying the cutoff hemoglobin value for a positive test, the performance characteristics of the test could be maximized in a particular population. In this study, a hemoglobin cutoff value of 75 ng/mL provided optimal test characteristics, with sensitivity and specificity for cancer of 94.1% and 87.5%, respectively. The reported sensitivity and specificity for clinically significant neoplasia (defined as cancer or advanced adenoma) was 67% and 91.4%, respectively. These investigators more recently reported that, through altering the threshold of the test, the resulting changes in sensitivity and specificity for adenomas would change the number of colonoscopies needed, a factor that might be useful for planners of public health screening policy.19 This might have important implications for the “cascade” concept of CRC screening promoted by the World Gastroenterological Organization (WGO), which tailors CRC tests in different countries according to their particular resources, CRC incidence, and competing public health concerns.20

Several other studies have reported on the performance and test characteristics of various other FIT assays. However, some studies performed colonoscopy only when FIT was positive, and therefore sensitivity cannot be calculated for all of the studies. These are summarized in Table 1.

**Cost Effectiveness**

One advantage of gFOBT is its extremely low test cost. FIT kits are more expensive, but many are considered cost-effective because of enhanced performance characteristics compared with gFOBT. Nakama et al.21 studied various cutoff hemoglobin values to attempt to determine the most cost-effective value of FIT. They studied 4260 patients undergoing FIT with the OC Hemodia test. All patients received a subsequent colonoscopy and 27 cancers were found. The investigators reported a hemoglobin cutoff of 150 ng/mL yielded a sensitivity and specificity for cancer of 81% and 96%, respectively. This was the most cost-effective cutoff value with a reported cost of $2492.98 per cancer detected. More recent cost-effectiveness analyses suggest that FIT with high adherence to yearly testing is highly cost-effective and may even be comparable to colonoscopy every 10 years.22

**Patient Preference**

A key component of any screening program is patient acceptance and participation. The need for dietary restriction may hinder participation in gFOBT screening. Cole and Young23 studied 1203 patients in Aus-

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**Table 1 Summary of FIT Studies**

<table>
<thead>
<tr>
<th>Author</th>
<th>Test Name</th>
<th>Subjects (N)</th>
<th>FIT Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castiglione et al.48</td>
<td>OC Hemodia (OCH)</td>
<td>786</td>
<td>NR</td>
<td>NR</td>
<td>94% OCH</td>
</tr>
<tr>
<td></td>
<td>HemeSelect (HS)</td>
<td></td>
<td></td>
<td></td>
<td>91% HS</td>
</tr>
<tr>
<td>Weller et al.49</td>
<td>Variable</td>
<td>6208</td>
<td>NR</td>
<td>83% CRC</td>
<td>96% CRC</td>
</tr>
<tr>
<td>Castiglione et al.10</td>
<td>HemeSelect</td>
<td>1725</td>
<td>NR</td>
<td>NR</td>
<td>97% CRC</td>
</tr>
<tr>
<td>Rozen et al.32</td>
<td>BM Test Colon Albumin</td>
<td>527</td>
<td>NR</td>
<td>35% AA/CRC</td>
<td>90% AA/CRC</td>
</tr>
<tr>
<td>Robinson et al.52</td>
<td>HemeSelect</td>
<td>808</td>
<td>NR</td>
<td>70% CRC/44% AA</td>
<td>88%</td>
</tr>
<tr>
<td>Castiglione et al.53</td>
<td>HemeSelect</td>
<td>8008</td>
<td>NR</td>
<td>NR</td>
<td>92%–97% CRC</td>
</tr>
<tr>
<td>Rozen et al.54</td>
<td>HemeSelect</td>
<td>403</td>
<td>NR</td>
<td>86% HS AA/CRC</td>
<td>98% HS AA/CRC</td>
</tr>
<tr>
<td></td>
<td>Flexsure (FS)</td>
<td>1410</td>
<td>NR</td>
<td>35% AA/CRC</td>
<td>99% AA/CRC</td>
</tr>
<tr>
<td>Rozen et al.55</td>
<td>Flexsure</td>
<td>250</td>
<td>70 SU</td>
<td>62% AA/CRC</td>
<td>93% AA/CRC</td>
</tr>
<tr>
<td>Wong et al.56</td>
<td>Magstream</td>
<td>500</td>
<td>100 ng/mL</td>
<td>77% AA/CRC</td>
<td>95% AA/CRC</td>
</tr>
<tr>
<td>Vilkin et al.57</td>
<td>OC Sensor</td>
<td>164</td>
<td>NR</td>
<td>91% CRC</td>
<td>94% CRC</td>
</tr>
<tr>
<td>Lohsiriwat et al.58</td>
<td>OC Light</td>
<td>330</td>
<td>50 ng/dL</td>
<td>69% AA</td>
<td>92% AA/CRC</td>
</tr>
<tr>
<td>Rozen et al.59</td>
<td>OC Micro</td>
<td>2975</td>
<td>50–200 ng/dL</td>
<td>NR</td>
<td>95%–98% CRC</td>
</tr>
</tbody>
</table>

Abbreviations: AA, advanced adenoma; BM, Boehringer Mannheim; CRC, colorectal cancer; NR, not reported; SU, standard units.
Advances in Stool Screening

Emerging Applications of FIT
FIT has been examined extensively in many countries where it is the primary screening modality for CRC. It has also been examined as a possible adjunctive test to colonoscopy. This is particularly relevant in countries, like the United States, where colonoscopy is the dominant screening modality. Bampton et al. examined interval use of FIT in a cohort deemed high risk because of a personal or family history of colorectal neoplasia and were enrolled in a colonoscopic screening program. These investigators reported that 7.3% of patients who completed a FIT had a positive result. Of these patients, 27% had a significant finding of cancer, advanced adenoma, or more than 2 adenomas. Overall, 11.5% of patients with a positive-interval FIT were found to have cancer.

Although these data suggest that an interval FIT may be a useful adjunctive test in detecting additional lesions in high-risk patients undergoing colonoscopy, several questions remain. How many of these lesions were missed at colonoscopy rather than new lesions is unclear. Thus, the optimal timing of FIT in patients undergoing colonoscopy is uncertain. Furthermore, no data are available on appropriate intervals of FIT use between colonoscopic examinations. Lastly, the overall numbers of cancers detected in this high-risk cohort were still low (0.8%) and not very different from interval cancer detection rates with gFOBT, for which use has fallen out of favor in this setting. Nonetheless, with additional research, emerging FIT may prove to be useful as an interval test in high-risk cohorts.

The low specificity of gFOBT results in a high false-positive rate and many normal follow-up colonoscopies. Experts have proposed that FIT can serve as a second-tier screening test after a positive gFOBT to reduce unnecessary colonoscopies and costs in a screening program. Fraser et al. examined this hypothesis, asking 1124 subjects with a positive gFOBT to perform an FIT test, with 558 participating. Of that group, FIT was negative in 302 and positive in 256 subjects. All patients underwent colonoscopy. In the FIT-negative group, the rate of cancer was 0.7% and the rate of large or multiple polyps was 4%. In the FIT-positive group, the rates for cancer or large/multiple polyps were 18.5% and 21.3%, respectively. This 2-tiered approach may be reasonable when keeping overall costs of screening low is a premium, because it potentially can reduce the number of colonoscopies needed after a positive gFOBT.

Stool DNA Tests
Technological Aspects
Because so much is known about the molecular pathogenesis of colorectal neoplasia, DNA is a highly relevant and promising analytic in stool. Human DNA represents only 0.01% of total stool DNA; the other 99.99% coming from nonhuman sources, such as the microflora and diet. Therefore, until 15 years ago, the notion that specific gene mutations could be detected among such a tiny fraction of human DNA, seemed an insurmountable challenge. However, using sensitive polymerase chain reaction (PCR) techniques with PCR inhibitors, methods to detect mutated human DNA from stool were developed and continue to improve. The latest tests seem to be limited more by the choice of markers and their method of analysis. Unlike gFOBT and some FIT tests, which require 2 to 3 stool samples for a single test, stool DNA testing requires only 1 sample.

The earliest stool DNA studies investigated single markers as proof-of-principle that gene mutations such as KRAS and P53 could be detected in stool. These studies showed that the same mutation that occurred in cancer tissue could be found in the stool of patients with an approximately 80% concordance. However, concordance ranged from 53% to 100%, and thus the sensitivity for detecting CRC in these...
early single-marker stool DNA studies was usually less than 40%.

This soon led to the development of multimarker panels, representing what are now considered first-generation stool DNA tests (Table 2). These tests analyzed a rather complex marker panel of multiple mutations in genes known to be involved in microsatellite-stable CRCs (APC, TP53, KRAS), a marker of microsatellite instability (BAT-26), and an assay that detected long DNA as a surrogate measure of aberrant colonocyte apoptosis. As expected for the early development of any new screening test, preliminary studies mainly used case-control designs to investigate patients with CRC and those with normal colonoscopies to establish the approximate sensitivity and specificity. Several independent studies using the multimarker panel PreGen-Plus (Exact Sciences Corporation, Marlborough, Massachusetts) showed a sensitivity for CRC of approximately 62% to 69%, and a 97% to 98% specificity (Table 2). The first study from the Mayo Clinic had the highest sensitivity (91%), which in hindsight was probably related to the rapid freezing of the stool samples by the central laboratory shortly after evacuation. In most other studies, stool samples were chilled by patients placing frozen packs around the sample container (or freezing the container with the sample), and the specimens were shipped by overnight courier.

In the past 5 years, technical advancements in stool DNA tests have been rapidly emerging, with a goal toward improving performance characteristics. Techniques have been developed to preserve stool DNA with a stabilizing buffer, and for better extraction of DNA from stool. These 2 improvements alone resulted in an increase in sensitivity for CRC from 52% to 73% with the PreGen-Plus assay. In addition, highly sensitive techniques such as BEAMing and digital melt curve analysis (DMC) can increase the ability to detect very low-abundance mutations in stool. For example, in a recent pilot study of 26 adenomas with tissue-proven KRAS mutations, the DMC assay detected 59% of adenomas, which was superior to Hemoccult (7%), Hemoccult SENSA (15%), and PreGen-Plus (26%), without compromising specificity.

**Performance Characteristics and Efficacy**

Building on the promising results of the preliminary series using first-generation stool DNA tests, 2 large prospective multicenter studies of asymptomatic, average-risk individuals were performed (Table 2). In the first study, Imperiale et al. compared stool DNA (PreGen-Plus) with Hemoccult II, with all subjects undergoing colonoscopy. The results showed that the sensitivity for detecting CRC was 52% with stool DNA compared with 13% with Hemoccult. The 2 tests had comparable 94% to 95% specificity. The sensitivity of stool DNA for adenomas with high-grade dysplasia and for villous adenomas was 33% and 18%, respectively, representing double the sensitivity rates of Hemoccult. This study firmly established that in an average-risk screening setting, stool DNA was better than gFOBT for detecting CRC. However, the sensitivity of 52% for CRC was lower than expected, which in hindsight was mainly caused by poor performance of the long DNA component of the assay from DNA degradation in transit. Methods are now established for better DNA preservation and extraction, and these are now used in newer versions of the stool DNA test.

A second large-scale prospective average-risk study was performed by Ahlquist et al. during 2001 to 2007. Initially, the same PreGen-Plus assay used in the Imperiale study was used, and was compared with both Hemoccult and Hemoccult SENSA, with all patients undergoing colonoscopy. However, during the study, the manufacturer altered the stool DNA test, prompting an unplanned interim analysis. In addition, a new stool DNA test (SDT-2) was applied to all patients in the study who had CRC, high-grade dysplasia, adenomas larger than 2 cm, a sample of 50 subjects with adenomas between 1 and 2 cm, and 75 subjects with normal colonoscopy. The SDT-2 assay consisted of 3 markers considered to be more broadly informative of both cancers and adenomas, KRAS mutations, vimentin gene methylation, and scanning of APC mutation cluster region. For detecting screen-relevant neoplasms (defined as CRC, high-grade dysplasia, adenomas larger than 2 cm, a sample of 50 subjects with adenomas between 1 and 2 cm, and 75 subjects with normal colonoscopy. The SDT-2 assay had a sensitivity of 20%, which was better than Hemoccult (11%) but similar to Hemoccult SENSA (21%). However, the sensitivity of SDT-2 for screen-relevant neoplasms was better (46%) and compared more favorably with that of Hemoccult (16%) and Hemoccult SENSA (24%). SDT-2 detected substantially more adenomas 1 cm or larger than the 2 FOBTs.

In aggregate, the data from these stool DNA trials, and the preliminary studies leading up to them, formed the basis for including stool DNA among
CRC screening options by the American Cancer Society, U.S. Multi-Society Task Force, and American College of Radiology, thereby serving to acknowledge the potential for stool DNA screening tests. In contrast, however, the USPSTF found insufficient evidence to recommend fecal DNA testing for rou-

<table>
<thead>
<tr>
<th>Author</th>
<th>Marker Panel</th>
<th>Sensitivity Cancer (%)</th>
<th>Adenomas (%)</th>
<th>Specificity Negative/Normals (%)</th>
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<tr>
<td>Dong et al.</td>
<td>$P53; KRAS; MSI$</td>
<td>36/51 (71)</td>
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<td>$P53; KRAS; MSI$</td>
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<td>—</td>
<td>18/18 (100)</td>
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<td>Koshiji et al.</td>
<td>LOH; MSI</td>
<td>29/30 (97)</td>
<td>—</td>
<td>30/30 (100)</td>
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<td>Ahlquist et al.</td>
<td>PreGen-Plus*</td>
<td>20/22 (91)</td>
<td>9/11 (82)</td>
<td>26/28 (93)</td>
</tr>
<tr>
<td>Tagore et al.</td>
<td>PreGen-Plus</td>
<td>33/52 (63)</td>
<td>16/28 (57)</td>
<td>111/113 (98.2)</td>
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<td>Syngal et al.</td>
<td>PreGen-Plus</td>
<td>40/65 (62)</td>
<td>6/22 (27)</td>
<td>—</td>
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<td>Brand et al.</td>
<td>PreGen-Plus</td>
<td>11/16 (69)</td>
<td>—</td>
<td>—</td>
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<td>Calistri et al.</td>
<td>PreGen-Plus</td>
<td>33/53 (62)</td>
<td>—</td>
<td>37/38 (97)</td>
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<td>Imperiale et al.</td>
<td>PreGen-Plus</td>
<td>16/31 (51.6)</td>
<td>84/689 (12)</td>
<td>1344/1423 (94.4)</td>
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<td>Ahlquist et al.</td>
<td>PreGen-Plus</td>
<td>3/12 (25)</td>
<td>47/614 (7.7)</td>
<td>2246/2340 (96)</td>
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<td>SDT-2†</td>
<td></td>
<td>11/19 (58)</td>
<td>55/123 (45)</td>
<td>63/75 (84)</td>
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<td>Lenhard et al.</td>
<td>HIC1 methylation</td>
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<td>4/13 (31)</td>
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<td>Petko et al.</td>
<td>CDKN2A; MGMT; MLH1 methylation</td>
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<td>7/19 (37)</td>
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<td>Chen et al.</td>
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<td>—</td>
<td>178/198 (90)</td>
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<td>Itzkowitz et al.</td>
<td>PreGen-Plus version 2‡</td>
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<td>6/7 (86)</td>
<td>298/363 (82)</td>
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<td>3/13 (77)</td>
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<td>SFRP2 methylation</td>
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<td>11/21 (52)</td>
<td>23/24 (96)</td>
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<td>15/21 (71)</td>
<td>23/24 (96)</td>
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<td>3/25 (12)</td>
<td>26/30 (87)</td>
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<td>Wang and Tang</td>
<td>SFRP2 methylation</td>
<td>60/69 (87)</td>
<td>21/34 (62)</td>
<td>28/30 (93)</td>
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<td>Oberwalder et al.</td>
<td>SFRP2 methylation</td>
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<td>6/13 (46)</td>
<td>6/6 (100)</td>
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<td>Nagasaka et al.</td>
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<td>18/56 (32)</td>
<td>103/113 (91)</td>
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<td>Zhang et al.</td>
<td>SFRP1 methylation</td>
<td>16/19 (84)</td>
<td>7/7 (100)</td>
<td>12/14 (86)</td>
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<td>Glöckner et al.</td>
<td>TFPI2 methylation</td>
<td>36/47 (76)</td>
<td>4/19 (21)</td>
<td>(79–93)</td>
</tr>
<tr>
<td>Melotte et al.</td>
<td>NDRG4 methylation (test set)</td>
<td>17/28 (61)</td>
<td>—</td>
<td>42/45 (93)</td>
</tr>
<tr>
<td>Baek et al.</td>
<td>Vimentin; MGMT; MLH1 methylation</td>
<td>45/60 (75)</td>
<td>31/52 (60)</td>
<td>32/37 (87)</td>
</tr>
<tr>
<td>Mayor et al.</td>
<td>Chromosome 2q14.2 methylation</td>
<td>8/30 (27)</td>
<td>—</td>
<td>29/30 (97)</td>
</tr>
</tbody>
</table>

*PreGen-Plus marker panel: APC; KRAS; P53; MSI; long DNA.
†SDT2: KRAS; APC scan; methylated vimentin.
‡PreGen-Plus Version 2: methylated vimentin; long DNA.

Table 2 Summary of Stool DNA Studies

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tine screening.

This is because first-generation stool DNA tests, despite their superiority over guaiac tests, still showed modest sensitivity for cancer (in the 55% range), were complex assays (thereby making them expensive), and were not good at detecting adenomas. However, the original molecular markers were purposefully chosen to detect cancers more than adenomas.

**New Markers**

In the past 5 years, attention has been paid to identifying new markers of colorectal neoplasia. A major focus has been the recognition that gene hypermethylation is a more common pathway in CRC than previously believed. Chen et al. made the intriguing observation that hypermethylation of the vimentin gene, a gene not considered part of the usual molecular alterations associated with the adenoma–carcinoma sequence, was only rarely found in normal colonic tissue but was common in colonic adenomas and carcinomas. When analyzed in stool, vimentin methylation was present in 43% of patients with CRC but only 10% of normal controls (Table 2). This prompted other studies analyzing vimentin methylation and long DNA. Using improved methods of DNA preservation and purification from stool, Itzkowitz et al. reported that vimentin methylation as a single marker resulted in 73% sensitivity for CRC with 87% specificity. A simpler version of the long DNA assay that analyzed 2 instead of 4 DNA loci resulted in sensitivity and specificity of 65% and 93%, respectively. The combination of the 2 markers gave optimal sensitivity (88%) and specificity (82%). Importantly, cancers were detected regardless of location in the colon or stage. Nearly identical findings were observed in an independent validation set of patients.

What emerged from these studies, which have enrolled the most number of subjects with cancer and controls with normal colonoscopies of all the most recent stool DNA studies, was the observation that a single methylation marker 1) can have a greater than 75% sensitivity for CRC, 2) can detect cancer of the proximal colon just as well as the distal colon, 3) can identify CRC regardless of stage, and 4) can make stool DNA testing easier to perform, thereby enabling distribution to local clinical laboratories.

Many other studies in the past few years have looked at other methylated genes as markers of CRC and adenomas using stool DNA. One of the most commonly studied genes is SFRP2, a gene involved in inhibiting the Wnt signaling pathway. Epigenetic silencing of SFRP2 by methylation is frequently found in the stool of patients with CRC, with sensitivities ranging from 63% to 94% and specificity ranging from 77% to 96% in several small series (Table 2). The frequency of SFRP2 methylation is lower in stool DNA of patients with adenomas, but seems to occur in approximately half of them (Table 2). A related gene, SFRP1 was found to be methylated in 84% of patients with CRC compared with 14% of controls with normal colonoscopies.

Other promising methylated gene targets for stool DNA testing include tissue factor pathway inhibitor 2 (TFPI2), a potential tumor suppressor gene. This gene was aberrantly methylated in the tissue of almost all colorectal adenomas (97%) and cancers (99%). When analyzed in the stool of patients with stage I to III CRC and controls with normal colonoscopies, results of training and validation sets showed that TFPI2 methylation showed sensitivities ranging from 76% to 89% for cancer and 21% for adenomas, and a specificity of approximately 93%. Methylation of the promoter region of another tumor suppressor gene, N-myc downstream-regulated gene 4 (NDRG4), occurred in 70% to 86% of CRC tissues compared with 4% in noncancerous colonic mucosa. In fecal DNA, methylated NDRG4 had a sensitivity of 61% and 53% for detecting CRC in training and validation sets, respectively, with corresponding specificities of 93% and 100%.

As shown in Table 2, the sensitivity for detecting adenomas with many of the newer markers generally ranges from 45% to 65%. Larger studies of patients with adenomas will be required before the value of any given marker or set of markers can be established.

**Cost-Effectiveness**

An earlier cost-effectiveness analysis placed stool DNA testing in context with gFOBT, sigmoidoscopy, and colonoscopy, and estimated the cost at $695. This analysis showed that stool DNA testing every 5 years would cost $47,700 per life year gained, and that to make stool DNA comparable to colonoscopy, it would have to achieve 65% sensitivity for CRC and 40% for large adenomas, 95% specificity, a screening interval of 2 years, and a cost of $195.

The same authors recently updated their cost-effectiveness analysis, this time including FIT, which was modeled to have 76% sensitivity for CRC and 40% for large adenomas, specificity of 91%, and a cost of $22. Newer versions of the stool DNA test
were modeled at a cost of $300 and a base case interval of every 3 years. In this analysis, FIT dominated over colonoscopy and all versions of stool DNA test, assuming perfect adherence. However, stool DNA testing with complete adherence became more effective than FIT with adherence below 50% and, assuming a per-cycle adherence of 50% for stool DNA test, was more effective than FIT with adherence below 19%. Needless to say, adherence with stool testing is a crucial factor contributing to the success of a screening program. Just how much impact the frequency of a stool test (e.g., annual vs. every 3–5 years) or the number of stools per cycle will have on patient adherence remains to be studied.

**Patient Preferences**

A prospective survey of 4042 (84%) subjects participating in the Imperiale study, who all underwent stool DNA testing, Hemoccult II, and colonoscopy, showed that stool DNA testing received the same or higher mean ratings than gFOBT for most prep- and test-related features and, except for perceived accuracy, also received higher ratings than colonoscopy. Thus, a higher proportion of patients preferred stool DNA (45%) to both FOBT (42%) and colonoscopy (15%). A more recent study by the same group administered a computerized decision aid to ambulatory care patients in a primary care setting who had not previously undergone CRC screening (or only FOBT testing). Theoretically, colonoscopy (51%), stool DNA (28%), and FOBT (18%) were preferred over other screening options. The findings suggested that there are 2 groups of patients: those who prefer the most accurate test (colonoscopy) and those who prefer the least invasive test (stool DNA and FOBT). Preferences for stool DNA compared with FIT need to be explored, especially using more recent estimates of sensitivity, specificity, and costs.

**Emerging Applications of Stool DNA Tests**

Although not yet tested in a clinical trial, experts have suggested that stool DNA testing be considered during the interval between colonoscopies to detect neoplasms, particularly those in the proximal colon sometimes missed by colonoscopy. Also, because stool may contain DNA from any organ that communicates with the luminal GI tract, it is conceivable that stool DNA testing might be useful for detecting upper gastrointestinal neoplasms. Nagasaka et al. recently reported that SFRP2 and RASSF2 methylation can detect gastric and CRC in stool DNA.

Ahlquist also reported that fecal DNA can be used to detect cancers of not only the colorectum and stomach, but also the oropharynx, esophagus, pancreas, and bile duct and/or gallbladder. Collectively, these cancers result in more than 130,000 deaths each year in the United States alone. Because some of these cancers (e.g., pancreatic and biliary) are hard to screen with current imaging techniques, further development of stool DNA as a pan-detection assay for gastrointestinal tract cancers represents an intriguing possibility.

**Conclusions**

FIT represents a significant advance over gFOBT and has generally been shown to have at least the same if not better sensitivity for CRC as high-sensitivity gFOBT. It has improved specificity and thus better positive predictive value. Furthermore, it can be performed without dietary restrictions, which may lead to enhanced patient acceptance and may have a future role in interval screening between colonoscopic examinations. In several countries it is used as a first-line screening test for CRC. Recent guidelines in the United States designate it as a test primarily for detecting cancer, and state it should be used on an annual basis for screening.

Stool DNA testing also has better sensitivity for CRC than gFOBT, with comparable specificity in large-scale prospective studies of average-risk patients. Stool DNA testing, interval uncertain, has been endorsed in the most recent guidelines of the U.S. Multi-Society Task Force (USMSTF) but not the USPSTF. Advances in technology, including the use of newer and simpler marker panels, many of which are hypermethylated genes, have shown much higher sensitivities than first-generation tests, albeit with some compromise in specificity. This has resulted in reduced test costs. Studies suggest that patient preference is high for stool DNA testing.

Future studies are needed to directly compare FIT with stool DNA tests with respect to performance characteristics, adenoma detection, patient preferences, and cost-effectiveness. Advances in analytical methods and the identification of new molecular markers for CRC should make stool DNA testing more sensitive and specific. Both FIT and stool DNA tests remain suboptimal for detecting ad-
enomas, including advanced adenomas. Future studies should also track the adherence and acceptability of stool-based tests by patients. Currently, results of stool-based CRC testing with current technology suggest that over the next decade, greater strides will be made in significantly diminishing the incidence and mortality of this highly preventable disease.

References


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