Digestion

Digestion 2007;76:26–33
DOI: 10.1159/000108391

Published online: October 19, 2007

New Stool Screening Tests for Colorectal Cancer

Graeme P. Young[a] Stephen Cole[b]

[a]Flinders Cancer Control Alliance, Flinders University, and [b]Bowel Health Service, Repatriation General Hospital, Adelaide, Australia

Key Words
Colorectal neoplasia · Fecal occult blood test · Guaiac-based fecal occult blood test · Fecal immunochemical test

Abstract
Background/Aims: The purpose of this review is to clarify the place of new-technology stool tests in screening for colorectal neoplasia. ***Findings:*** New technologies have been based on blood and cellular products of neoplasia. Fecal occult blood tests (FOBTs) based on guaiac (i.e. GFOBTs) have been proved to be effective, but their impact on mortality is modest. When GFOBTs are reconfigured to provide improved sensitivity for cancer, their specificity often becomes unacceptable. Fecal immunochemical tests (FITs) targeting the human hemoglobin molecule have been shown to have better sensitivity for neoplasia without an unacceptable deterioration in specificity. The new stool-sampling technologies for FITs also improve population participation rates in screening. Most recently, quantitative FITs have become available; these provide flexibility for the end-user as a desired sensitivity: specificity ratio can be selected that is feasible in the context of available colonoscopic resources. A multi-target fecal DNA test, comprising a test for undegraded DNA and certain common mutations, was found more sensitive for cancer, but not for adenoma, than the early GFOBTs. A more recent version including an epigenetic marker for the vimentin gene has further improved sensitivity for cancer, but performance relative to GFOBT or FIT is not clear. These 'fecal DNA tests' have not proved to be more specific for neoplasia than tests that detect blood. ***Conclusions:*** FIT should replace GFOBT as the first test in two-step screening of large populations. It is not yet clear that tests targeting nonhemoglobin molecular events provide a clear advantage over FIT.

Role of Stool-Based Screening Tests in the Screening Algorithm

As the goal of screening for colorectal cancer is to reduce population mortality from and/or morbidity due to colorectal cancer, potential screening tests require a rigorous evaluation that goes beyond what is required for diagnostic tests being used in situations where disease prevalence is high [1, 2]. Screening tests are applied to healthy people where the risk of disease is relatively low.

Screening tests may be applied in several contexts: ranging from population-based strategies where the approach is impersonal to the personalized setting where screening is recommended by a doctor. No matter what the context, screening is by its nature a process that aims to increase the likelihood that affected people, while at a curable and usually unsuspecting stage, receive effective diagnosis and treatment. Screening is, therefore, a process with multiple phases [3]:

- Invite and engage the person in screening
- Perform the screening test

KARGER

0012–2823/07/0761–0026$23.50/0

Prof. G. Young
Department of Gastroenterology, Flinders Medical Centre

Table 1. Available fecal screening tests – basis for detection of neoplasia, strength of evidence and determinants of performance

Detection goal	Technology	Strongest evidence for benefit	Sensitivity determinants	Specificity determinants
Fecal blood	GFOBT	population RCT – reduced incidence and mortality	amount of heme in feces	dietary heme; bleeding nonneoplastic lesions
	FIT	comparative cohort – better sensitivity and/or specificity	amount of globin in feces	bleeding nonneoplastic lesions
Fecal neoplasm-derived DNA	multitarget fecal DNA test	comparative cohort – assessing sensitivity and specificity	spectrum of DNA changes shed into feces	unclear

Modified from Young and Allison [1] with permission.

- Follow up result with colonoscopy if indicated
- Treat any lesions found
- Repeat screening or implement follow-up surveillance if neoplasia found.

There are two main choices at the point where a test is offered: (1) One-step testing. The diagnostic test, colonoscopy, is the screening test. Selection for colonoscopy is based on age, and many people screened will not have neoplasia. (2) Two-step testing. Here, a simpler test is offered first, e.g. a fecal occult blood test (FOBT), then those with a positive result proceed to colonoscopy. A simple screening test calls attention to the likelihood of disease being present and serves to direct resources to those most likely to benefit from diagnostic and therapeutic procedures [3].

Stool Screening Tests Act by Refining the Likelihood that Neoplasia Is Present

In two-step testing, the stool screening test filters out from the broader population those who are most likely to have colorectal neoplasia. This concept is embodied in the pretest:posttest likelihood ratio and is mathematically expressed as test sensitivity divided by the false-positive rate (1 – specificity) [3]. Depending on the test type used, various FOBT return a ratio in the range of 8–40 [1] meaning that those with a positive test are that much more likely to have colorectal cancer than those with a negative test.

The Biological Basis of Fecal Screening Tests

The usefulness of such tests depends on whether a colorectal neoplasm gives rise to changes in the constituents in feces. Such constituents might derive directly from the tumor itself or be secondary to its presence. The processes giving rise to such products can be classified [4] as: leakage, secretion, or exfoliation.

Hemoglobin, and indeed other blood-derived proteins such as haptoglobin and albumin, represent examples of leaked products. Tests have been developed based on each of these, although hemoglobin-based tests are by far the most prominent (see the section 'Current Types of FOBT: Guaiac and Immunochemical Tests' below).

Mucins are an example of secreted products. No mucin-based test has, however, achieved significant usage.

The products of cell exfoliation create considerable options for detection. Certainly, cytological studies show neoplastic cells to be present in feces [4]. Tests for these might be based on DNA (see the section 'Nonhemoglobin Molecular Markers in Stool' below), RNA or proteins. A recent American Gastroenterological Association Future Trends Committee report on emerging screening and diagnostic technologies for colorectal cancer [5] identified a range of tests and procedures that might be appropriate. These include proteomics or the analysis of broad protein patterns, making it possible to assess small amounts of protein for the presence of identified cancer markers using new protein assessment tools and computerized artificial intelligence analysis.

The nature of the major fecal screening tests, either established or under study, is summarized in table 1. The efficacy of screening for colorectal cancer is supported by the highest level of evidence, namely randomized, controlled trials, at the population level for guaiac-based FOBT (GFOBT) [6–8]. Evidence supporting the other test technologies is not as strong, as summarized in table 1 and further outlined below in the sections 'Current Types of FOBT: Guaiac and Immunochemical Tests' and 'Nonhemoglobin Molecular Markers in Stool'.

Justification of New Test Development

Evaluation of Test Performance

Before considering the new developments in stool tests, it is worthwhile to consider whether we need new tests at all. To do this, we need to briefly consider what outcomes are important to the success of a screening program, i.e. what measures relate to a reduction in mortality and/or incidence in a cost-effective and acceptable fashion?

The measures of effectiveness of a screening program have been detailed elsewhere [1] and informative measures can be classified as:

- Behavioral, i.e. participation rates in screening
- Test performance, such as sensitivity (including neoplasia detection rates), specificity (including false positives) and predictive values
- Programmatic, namely reductions in incidence and mortality.

The most immediate measurable events when screening will be participation rate, test positivity rate, adenoma detection, downstaging of the detected cancers and, at a later stage, prolonged survival after treatment [1]. Presymptomatic detection of localized cancer will result in a reduction in morbidity and/or mortality [6–8]. If screening detects preinvasive lesions, namely dysplasia, it will reduce cancer incidence [9].

The published RCTs using GFOBT provide information on each of these measures; new tests can be tested relative to these.

Performance of GFOBT

An early measurable outcome in a screening program is *participation*, i.e. willingness of an individual offered screening to undertake the testing process. The RCTs of GFOBT have achieved rates of 53–67% when approaching the entire population, but other studies show lower rates [1, 6–8]. Clearly, the impact of a screening program on population outcomes would be greater if more people did a screening test [10]. It is also important to emphasize that FOBTs must be undertaken repeatedly for benefit to be shown, so ease of use is crucial.

Rates of detection of adenomas and cancers, together with stage of cancer, are the next obvious outcomes. In themselves they are difficult to meaningfully interpret when expressed relative to the size of the target population, but if two tests are compared directly, the results provide a relative indication of sensitivity for the target lesions. Improved sensitivity for cancer will translate into a greater reduction in mortality.

The published RCTs using the standard GFOBT, Hemoccult, have observed modest population mortality reductions (from colorectal cancer) of 14–21% when analyzed on an intention-to-screen basis [6–8]. This modest impact is a direct consequence of the low *sensitivity* of Hemoccult for cancer, estimated in a range of studies to be around 33% and no greater than 50% [1, 11]. Clearly, a more sensitive test seems likely to have a greater impact on mortality as a larger number of cancers will be detected by screening.

Cumulative *incidence* rates for colorectal cancer did not differ between the controls and screened groups in the RCTs using GFOBT after 13 years of follow-up. However, after 18 years of follow-up, the Minnesota study observed a significant impact on incidence whether screening was annual or biennial [9]. It seems likely that the higher sensitivity of rehydrated Hemoccult and the resultant higher colonoscopy rate [6] has resulted in a better detection (and thus removal) of adenomas. Obviously, improved sensitivity for adenomas would result in a greater impact on incidence.

Unfortunately, increasing the sensitivity of GFOBT leads to a marked deterioration in specificity [11] and this would also increase cost of the program as the colonoscopy rate is a major determinant of cost.

To summarize, we need new tests because there is much room to improve participation rates of those being invited to screen, to improve sensitivity for cancer, to improve sensitivity for adenomas, and to achieve the improved sensitivity without unacceptable deterioration in specificity.

Current Types of FOBT: Guaiac and Immunochemical Tests

The fact that microscopic bleeding may arise from curable cancers, and adenomas, provides the basis for screening using an FOBT [3]. However, the biology of bleeding is complex and the different FOBT technologies now available are influenced by the biological fate of blood in the gut [9].

Available FOBTs are based on two principal quite different technologies: chemical or immunochemical detection of one or other component of blood. The major features of these tests are outlined in table 2 [1, 12].

Chemical FOBT

The chemical tests (e.g. Hemoccult II) react to the peroxidase capacity inherent in the heme molecule [13].

Table 2. Characteristics of different types of FOBT [1, 12]

Type of FOBT	Chemical basis	Diet restrictions	Drug interference	Site of occult bleeding detected	Specificity for neoplasia[1]	Sensitivity for cancer[1]
Chemical (GFOBT)	guaiac detects peroxidase activity of heme	required: red meats; possibly certain raw plant foods[2]	vitamin C, possibly NSAIDs[3]	rectum > colon > stomach (in decreasing order of sensitivity)	90–98% depending on test brand and usage	35–67% with one-time testing; over 80% with repeated testing
Immunochemical (FIT)	anti-human hemoglobin antibody detects globin	none required	none	colon and rectum	around 95% depending on sensitivity level chosen[4]	65–90% with one-time testing; unclear for repeated testing

Presented in modified form with permission [1].
[1] Indicative estimates only.
[2] Delaying development for 72 h minimizes interference from plant foods and avoids need for their restriction with standard Hemoccult II. Red meats must be restricted when using a more sensitive GFOBT [12].
[3] Low-dose aspirin is not a problem, but therapeutic doses such as for rheumatic disorders may.
[4] Tests generally provide a qualitative result, but some newer FITs can be quantifiable.

Guaiac is the reagent in most chemical tests. These GFOBTs react to any peroxidase in feces (e.g. plant peroxidases or heme in red meat) and are affected by certain chemicals (e.g. vitamin C). GFOBTs may detect bleeding from any site in the gastrointestinal tract, including stomach [13], as heme remains relatively stable during transit.

GFOBTs that are more sensitive than Hemoccult, e.g. Hemoccult II Sensa, have been developed to improve sensitivity; in practice they appear to almost double sensitivity. While valuable, this is unfortunately at a cost of decreased specificity [11].

Fecal Immunochemical Tests

Fecal immunochemical tests (FITs) use antibodies specific for human globin. This technology provides several advantages. It is not affected by diet or vitamin C [5, 13, 14]. FITs as a class are subject to less variability in positivity rate than the sensitive GFOBT [15]. FITs are also highly selective for occult bleeding of colorectal origin because globin is rapidly degraded by digestive enzymes [13]. These provide specificity advantages over GFOBT, especially the more sensitive GFOBT.

These improvements in specificity have, depending on the brand of FIT, been combined with improvements in fecal sampling; these are discussed elsewhere in detail [12, 14, 16]. FITs have also been developed to provide for large scale development in the laboratory where quality assurance of test development is much easier to monitor and control. Laboratory development is preferred in many countries, especially for mass screening when many tests must be done and quality assurance is vital.

Comparisons of GFOBT with FIT

It is beyond the scope of this review to fully analyze all studies comparing these technologies. Several studies have been selected to demonstrate key issues about these two quite different technologies.

Population participation is essential for cancer detection [3]. FIT technology simplifies the testing process, removes the need for diet and drug restrictions, provides for preferred and more acceptable stool-sampling methods such as brushes or probes rather than a wooden spatula, and is achieved while collecting fewer fecal samples. Most branded versions of FIT require fewer than three fecal samples, the recommended number for GFOBT. Removal of dietary restrictions has been shown to enhance participation in screening with FIT relative to GFOBT, in one study by 28% [10]. Changing to a brush-sampling method also simplifies the process and enhances participation by 30%. Together, these two advances increase population participation by 66% [10].

A study of over 7,000 people undergoing screening in California was the first to provide a large-scale comparison of two types of GFOBT with an FIT [11]. It showed that a sensitive GFOBT, Hemoccult Sensa, doubled detection rate of Hemoccult II for cancer but required almost 5 times as many colonoscopies to achieve this. An FIT, no longer available commercially, also achieved double the sensitivity but with only a doubling of the colonoscopy rate. FITs provide for an improved sensitivity/specificity ratio; in other words, they can achieve better sensitivity without an unacceptable deterioration in specificity.

More recently, a new brush-sampling FIT (InSure) has been directly compared with Hemoccult Sensa in several

clinical and screening cohorts undertaking paired sampling of stools [14]. The FIT returned a true-positive result significantly more often in those with cancer (n = 24, 87.5 vs. 54.2%) and in those with significant adenomas (n = 61, 42.6 vs. 23.0%). The false-positive rate for any neoplasia was marginally higher with the FIT than the GFOBT (3.4 vs. 2.5%, 95% CI of difference 0–1.8%), while positive predictive values were 41.9 and 40.4%, respectively.

A recent study involving 1,486 subjects in Scotland further supports the observations that specificity remains acceptable with FITs even though they have improved sensitivity [17].

Table 2 shows performance estimates of the different types of the FOBT, i.e. GFOBT and FIT. As a general rule, FITs are at the more sensitive end of the range while GFOBTs vary widely across the range.

Obviously, FITs overcome most of the disadvantages presented by GFOBT, are superior to GFOBT in terms of participation as well as performance and should replace GFOBT in two-step screening [14, 16].

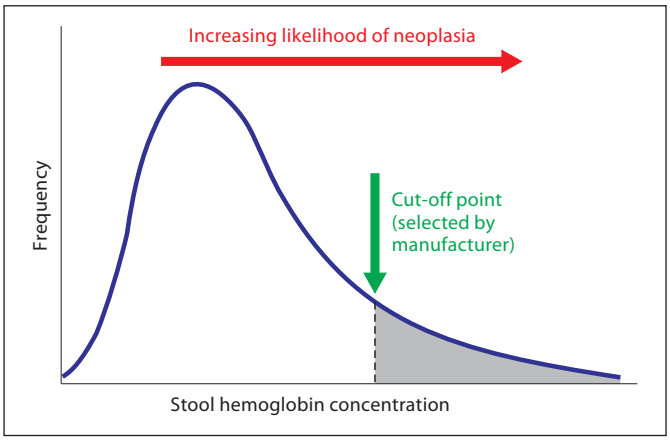

Fig. 1. Theoretical distribution of fecal hemoglobin concentrations in a target screening population showing a tail to the right as those with pathology will have higher concentrations than those with a normal colon. As the hemoglobin concentration increases, there is a continuous increase in the likelihood of finding neoplasia. Qualitative tests are set to react at a given hemoglobin concentration and so the likelihood of neoplasia varies with the cut-off selected. The proportion of the population falling in the grey-shaded area will be those who are colonoscoped.

Quantitative Immunochemical Tests

Several of the latest FITs, namely OC-Micro and InSure, provide for quantification of fecal hemoglobin [18, 19]. The relationship between fecal hemoglobin concentration and pathology has been explored in these studies and gives more insight into strategies for managing FIT-based screening programs. Several interesting guiding principles emerge from these studies:

- As pathology progresses, hemoglobin concentration increases (cancers bleed more than advanced adenomas which bleed more than small adenomas).
- Patients with advanced adenomas do show higher fecal hemoglobin concentrations than those without neoplastic pathology.
- Quantification enables one to select a cut-off corresponding to a particular chosen sensitivity/specificity ratio.

These studies clearly show that the greater the amount of marker present in the stools, the more likely is neoplasia to be present. If we represent a theoretical distribution of fecal hemoglobin concentrations in a target population (fig. 1), we would find that as the hemoglobin concentration increases there is a continuous increase in the likelihood of finding neoplasia. Qualitative FOBT are designed to return a positive at a set hemoglobin concentration, the 'cut-off' that defines positivity. Cut-offs vary between manufactured tests and so the likelihood of neoplasia being present varies according to where it is on the curve in figure 1. Qualitative tests fail to provide for flexibility in varying the cut-off. The same principle should apply for any other molecular marker in feces unless it is totally specific for neoplasia.

Several groups [18, 19] have shown how quantification provides flexibility by constructing an ROC curve, expressing the relationship between sensitivity and specificity at different hemoglobin concentrations. In practice, the hemoglobin cut-off used to trigger colonoscopy can be adjusted to correspond to a particular chosen sensitivity:specificity ratio. No longer is the test performance as set by a manufacturer important, since the flexibility provided by quantification allows those running screening programs to select whatever sensitivity:specificity ratio they want, while knowing that the lower the cut-off hemoglobin concentration selected, the greater is the chance of detecting significant neoplasia.

An even simpler way to apply this flexibility is to choose a hemoglobin cut-off that delivers a positivity rate that is manageable in terms of the resultant colonoscopy rate. For instance, if it is considered that 5% of the target population can be realistically colonoscoped, then the cut-off can be selected to achieve that. The real concern is what constitutes an acceptable rate. We know from

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.