

New Stool Screening Tests for Colorectal Cancer

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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.

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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

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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 ($1 - \text{specificity}$) [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 ‘Non-hemoglobin 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
Immuno-chemical (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

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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].

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

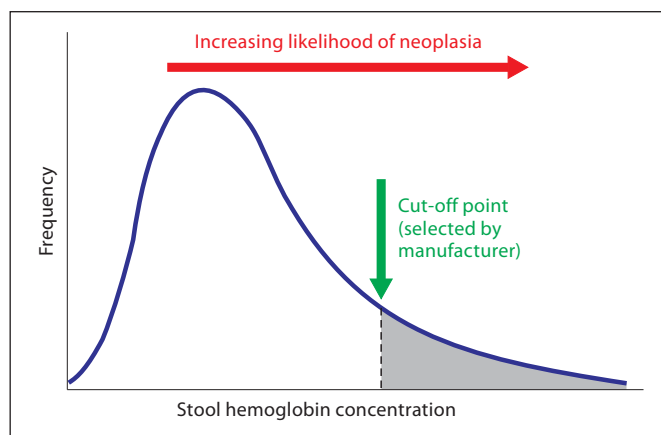


Fig. 1. Theoretical distribution of fecal hemoglobin concentrations in a target screening population showing a tail to the right as those with pathology will have higher concentrations than those with a normal colon. As the hemoglobin concentration increases, there is a continuous increase in the likelihood 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.

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

RCTs using the GFOBT Hemoccult II that a significant impact on mortality was achieved with a positivity rate of just 2% [7, 8]. So, we can be confident that working with a positivity rate higher than that will achieve better cancer detection and be more likely to deliver a similar or better reduction in mortality.

To summarize, the advantage of fecal hemoglobin measurement is that it returns full control of sensitivity and specificity to the end-user [3] who can establish the level of fecal hemoglobin that would trigger colonoscopy. At the population level, such an approach allows optimization of the test specificity:sensitivity ratio, so adapting the colonoscopy rate to the facilities and resources available for screening.

Nonhemoglobin Molecular Markers in Stool

Detection of molecular or genetic events that either cause cancer or else reflect development of neoplasia could theoretically be useful in selecting who undergoes colonoscopy. Because of the molecular heterogeneity of DNA in neoplasms, selecting the best panel of markers represents a challenge.

The first large-scale evaluation of the value of fecal DNA testing (using a 21-mutation multitarget panel) as a first-step test in two-step screening for colorectal cancer has recently been reported [20]. This version of the stool DNA test (PreGen Plus) is costly (in excess of USD 400) relative to GFOBT and FIT (up to USD 30) and requires a somewhat cumbersome stool collection that needs to be rapidly delivered to the processing laboratory [21]. The comparator tests were colonoscopy as the diagnostic reference standard and Hemoccult II (unhydrated) as the proven first-step screening test [20]. Results were analyzed in a subgroup of 2,507 which included all subjects found to have neoplasia as well as a range of subjects with normal colon or benign disease. The cancer detection rate using the fecal DNA panel compared to Hemoccult II was 16 of 31 (52%) versus 4 of 31 (13%, $p = 0.003$), respectively. While sensitivity was greater than that with Hemoccult II, it was lower than would be hoped for and the reported sensitivity of Hemoccult II was substantially lower than that reported in other studies for one-time testing [10]. The reasons for this seem likely to relate to the fact that Hemoccult II tests were developed at many different sites rather than at a centralized site paying full attention to quality assurance. The performance of both tests for detecting advanced adenomas was similarly disappointing: 61 of 403 (15.1%) for fecal DNA versus 43 of

403 (10.7%) for Hemoccult II. In those with negative (no finding of adenoma or cancer) colonoscopy, 5.6% had tested positive on fecal DNA compared to 4.8% on Hemoccult II (specificities of 94.4 and 95.2%, respectively). Fecal DNA testing did not identify the majority of neoplastic lesions found at colonoscopy.

Another recent study failed to find an association between *K-ras* mutations in the stool and development of colorectal cancer [22].

In this format (i.e. as PreGen Plus [20]) the fecal DNA test has not met expectations of sensitivity or specificity, and while it might be more sensitive for cancer than Hemoccult II, it is quite unclear as to whether it represents an advance over the newer FOBT types and especially FIT.

Further enhancements of fecal DNA testing are now emerging. These take advantage of certain epigenetic changes that can characterize colorectal neoplasia together with improved methods for stabilization of DNA. These two approaches have been combined in a new-generation fecal DNA test, PreGenPlus v2, where the main components of the test comprise an optimized method for detecting undegraded DNA ('DIA', a characteristic of neoplasia) together with a marker for methylation of the vimentin gene [23]. In a clinical cohort of 40 patients with cancer, the DIA component returned a 65% sensitivity, the vimentin test a 73% sensitivity and together an 88% sensitivity. At this stage, the performance in screen-detected cancers is not clear, nor has performance relative to FIT or GFOBT been studied. It is intriguing that a test based on methylation might achieve such a sensitivity, since tumors bearing the so-called methylation genotype, the CIMP pathway, seem likely to constitute no more than 20% of all colorectal neoplasia [24]. Clearly, there is overlap between molecular pathways of colorectal oncogenesis.

Specificity of the PreGenPlus v2 test has been determined in 122 people with a normal colon [23]. Combined specificity was 82%. This is not an improvement over FIT and is clearly inferior. This finding further demonstrates that DNA-based tests are not necessarily specific for neoplasia. The explanation for this is not clear, but the most likely possibility is that some molecular lesions emerge before focal lesions become obvious at colonoscopy. Whether such 'occult' lesions are likely to progress at a later stage is unknown. If they were, this might represent an argument for more intensive colonoscopic surveillance of such individuals, but this is pure speculation at this stage.

To summarize, fecal DNA tests require much more work before it is clear what the best markers are, how

these compare to GFOBT and FIT, and what the relative costs will be.

Nonetheless, it seems most likely that if stool tests can be improved, the new generation stool screening tests will emerge from discovery of new markers that improve sensitivity or specificity relative to FIT. DNA-based tests will continue to improve, but RNA-based and proteomic tests might also prove valuable.

Conclusions

The benefits of stool screening tests are several: they, particularly GFOBT, are proven by RCTs to have an impact on mortality and incidence, and they are acceptable to a majority of the population in that people will undertake such testing. Stool tests provide a simple introduction into the screening algorithm – they serve to profile risk for neoplasia and direct those more likely to have neoplasia to receive colonoscopy.

There are several disadvantages: fairly frequent testing seems to be necessary and sensitivity for incident lesions still leaves room for improvement whether they are based on blood or tumor DNA. No stool test is specific for neoplasia.

Recent developments in FITs, namely quantification, provide for a flexible approach to screening in that they

do not commit the end-user to a particular sensitivity: specificity ratio and they have improved capacity to detect adenomas compared with GFOBT. As they are also more acceptable to people offered screening, FIT should replace GFOBT for screening. Perhaps, quantification represents the ultimate refinement of FIT and there might not be much room for further improvement.

Fecal DNA tests, especially the latest versions, need further evaluation in screening cohorts before we can be confident of their ability to detect cancers relative to FIT. They do appear to be more sensitive for cancer than the GFOBT Hemoccult II. As yet, fecal DNA tests have not been shown to have any advantage for adenoma detection and they are no more specific than GFOBT.

The challenge for fecal screening tests is to provide more reliable identification of those who might have an advanced adenoma in the colon. This would more effectively target colonoscopy to this group and reduce indiscriminate colonoscopic screening of everyone considered to fall within the at-risk age range. Fecal molecular tests have the potential to achieve this, but the ideal marker, or panel of markers, is yet to be identified.

Disclosure Statement

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References

- Young GP, Allison J: Screening for colorectal cancer; in Yamada T, Alpers D, Kaplowitz N, Laine L, Owyang C, Powell D (eds): *Textbook of Gastroenterology*, ed 5. Philadelphia, Lippincott, Williams and Wilkins, 2007, in press.
- Watson JMG, Junger G: Principles and practice of screening for disease. *Public Health Pap* 1968;34.
- Young GP, Macrae FA, St John DJB: Clinical methods of early detection: basis, use and evaluation; in Young GP, Rozen P, Levin B (eds): *Prevention and Early Detection of Colorectal Cancer*. London, Saunders, 1996, pp 241–270.
- Osborn NK, Ahlquist DA: Stool screening for colorectal cancer: molecular approaches. *Gastroenterology* 2005;128:1–22.
- Regueiro CR: AGA Future Trends Committee report: colorectal cancer: a qualitative review of emerging screening and diagnostic technologies. *Gastroenterology* 2005;129:1083–1103.
- Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, Ederer F: Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *NEJM* 1993;328:1365–1371.
- Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O: Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996;348:1467–1471.
- Hardcastle JD, Chamberlain JO, Robinson MH, Moss SM, Amar SS, Balfour TW, James PD, Mangham CM: Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 1996;348:1472–1477.
- Mandel JS, Church TR, Bond JH, Ederer F, Geisser MS, Mongin SJ, Snover D, Schuman LM: The effect of fecal occult-blood screening on the incidence of colorectal cancer. *N Engl J Med* 2000;343:1603–1607.
- Cole SR, Young GP, Esterman A, Cadd B, Morcom J: A randomized trial of the impact of new fecal hemoglobin test technologies on population participation in screening for colorectal cancer. *J Med Screen* 2003;10:117–122.
- Allison JE, Tekawa IS, Ransom LJ, Adrain AL: A comparison of fecal occult blood tests for colorectal cancer screening. *N Engl J Med* 1996;334:155–159.
- Young GP, St John DJB, Winawer SJ, Rozen P: Choice of fecal occult blood tests for colorectal cancer screening: recommendations based on performance characteristics in population studies. *Am J Gastroenterol* 2002;97:2499–2507.
- Young GP, St John DJB: Faecal occult blood tests: choice, usage and clinical applications. *Clin Biochem Rev* 1992;13:161–167.
- Smith A, Young GP, Cole SR, Bampton P: Comparison of a brush-sampling fecal immunochemical test for hemoglobin with a sensitive guaiac-based fecal occult blood test in detection of colorectal neoplasia. *Cancer* 2006;107:2152–2159.

- ▶ 15 Wong BC, Wong WM, Cheung KL, Tong TS, Rozen P, Young GP, Chu KW, Ho J, Law WL, Tung HM, Lai KC, Hu WH, Chan CK, Lam SK: A sensitive guaiac faecal occult blood test is less useful than an immunochemical test for colorectal cancer screening in a Chinese population. *Aliment Pharmacol Ther* 2003;18:941–946.
- ▶ 16 Young GP: Molecular approaches to stool screening for colorectal cancer. *Curr Colorectal Cancer Rep* 2006;2:30–35.
- ▶ 17 Fraser CG, Matthews CM, Mowat NA, Wilson JA, Carey FA, Steele RJ: Immunochemical testing of individuals positive for guaiac faecal occult blood tests in a screening programme for colorectal cancer. *Lancet Oncol* 2006;7:101–103.
- ▶ 18 Levi Z, Rozen P, Hazazi R, Vilkin A, Waked A, Maoz E, Birkenfeld S, Leshno M, Niv Y: A quantitative immunochemical fecal occult blood test for colorectal neoplasia. *Ann Intern Med* 2007;146:244–255.
- ▶ 19 Smith A, Young G, Cole S, Morcom J, Chandler H, La Pointe L: A quantifiable fecal immunochemical test (FIT) for hemoglobin facilitates balancing sensitivity with specificity when screening for colorectal cancer. *Gastroenterology* 2004;126:S1346.
- ▶ 20 Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME: Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004;351:2704–2714.
- ▶ 21 Woolf SH: A smarter strategy? – reflections on fecal DNA screening for colorectal cancer. *N Engl J Med* 2004;351:2755–2758.
- ▶ 22 Haug U, Hillebrand T, Bendzko P, Low M, Rothenbacher D, Stegmaier C, Brenner H: Mutant-enriched PCR and allele-specific hybridization reaction to detect K-ras mutations in stool DNA. *Clin Chem* 2007;53:787–790.
- ▶ 23 Itzkowitz S, Jandorf L, Brand R, Rabeneck L, Schroy PC 3rd, Sontag S, Johnson D, Skoletsky J, Durkee K, Markowitz S, Shuber A: Improved fecal DNA test for colorectal cancer screening. *Clin Gastroenterol Hepatol* 2007;5:111–117.
- ▶ 24 O'Brien MJ, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, Amorosino M, Farraye FA: Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol* 2006;30:1491–1501.