

Special Report: Fecal DNA Analysis for Colon Cancer Screening*



Assessment
Program
Volume 21, No. 6
August 2006

Executive Summary

Background

Various methods for screening for colorectal cancer in persons at average risk are available. Highly effective methods (e.g., colonoscopy) are invasive and costly, and would require greater capacity within the healthcare system than currently exists to screen all adults over 50 years of age. Low-cost, noninvasive methods (e.g., fecal occult blood testing or FOBT) have been shown to be effective at reducing mortality from colorectal cancer, but have much poorer sensitivity for neoplasia than colonoscopy, and required sample collection procedures may reduce compliance. New methods of screening that are noninvasive, highly sensitive, and patient friendly would be ideal. Fecal DNA testing has been proposed as an alternative screening method.

Objective

This Special Report will provide information relevant to the evaluation of fecal DNA screening for colon cancer in asymptomatic patients at average risk. This report will summarize:

- the current context of existing and emerging screening tests for colorectal cancer, including current published recommendations;
- the molecular basis for fecal DNA screening and the commercially available fecal DNA screening test, PreGen-Plus™;
- direct and indirect evidence comparing the performance of PreGen-Plus™ testing to other methods of colon cancer screening;
- evidence regarding the likelihood of compliance with fecal DNA screening; and
- available cost-effectiveness analyses of fecal DNA screening.

Methods

MEDLINE® (via PubMed) was searched through June 2006, using the following search terms: “(neoplas* OR cancer) AND (fecal DNA OR stool DNA OR (stool AND DNA)).” Selected studies were fully published in a peer-reviewed journal; publicly available, FDA-reviewed information was also acceptable. Studies were selected as evidence if they reported:

- analytical performance characteristics of fecal DNA assays;
- clinical performance, specifically the results of prospective studies that targeted an average risk screening population, compared fecal DNA to a currently accepted screening method (per USPSTF guidelines), and administered colonoscopy to all patients;
- patient preferences or compliance for fecal DNA tests compared to conventional screening tests; or
- cost-effectiveness of fecal DNA testing versus conventional methods.

* This Special Report evaluates the available evidence on fecal DNA testing. It does not address whether the TEC criteria are met so that the evidence can be examined in the broader context of conventional and other emerging screening tests, published recommendations, patient compliance, and cost-effectiveness.

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Results

In a prospective, multicenter screening study of 5,486 asymptomatic, average-risk individuals, all of whom received colonoscopy, 80% of participants completed all tests adequately. For these evaluable participants, fecal DNA sensitivity for cancer was 52% versus 13% for conventional FOBT. Sensitivities for large adenomas for both tests were similar at 11–15%; specificities of the two tests were also similar.

Although this study was well designed, it also has several limitations including an older test methodology no longer used in the PreGen-Plus™ commercial version, a loss of 20% of enrolled participants from evaluation, unusually low FOBT sensitivity (evidence from other large screening trials suggest sensitivity in the range of 30–40%), and no improvement in detection of large adenomas. Sources of false-positive results are also not yet well understood and the optimal screening interval is unknown. A newer version of this assay, using different markers, was recently presented at Digestive Disease Week 2006 and suggests improved sensitivity for cancer in highly selected patients; sensitivity for large adenomas was not reported in the abstract. Information on the performance of this new assay in a screening population will be needed.

In the multicenter screening study, the percentage of noncompliant participants was 12% for fecal DNA analysis versus 8% for FOBT ($p < 0.001$). These results suggest that participants preferred FOBT testing, but it is unclear whether these numbers reflect only compliance or also include problems with obtaining sufficient DNA from supplied samples. In contrast, a questionnaire returned by 84% of all evaluable participants indicated that the preferred screening test was fecal DNA for 45% of respondents, FOBT for 32%, and colonoscopy for 15%. Additional evidence is needed to determine patient compliance with various tests in clinical practice.

Two studies using the same cost-effectiveness model and testing various assumptions found that fecal DNA testing is nearly always dominated by conventional testing strategies. Assuming sensitivities for cancer and large adenoma of 65% and 40%, respectively, and specificity of 95%, the screening interval would have to be 2 years (current recommendation 5 years) and the cost of fecal DNA testing \$195 (currently \$400–500) to make fecal DNA comparable with colonoscopy.

Conclusions

Fecal DNA testing is a noninvasive colorectal cancer screening technology that may eventually offer sensitivity for cancer closer to that of colonoscopy than that of conventional, guaiac-based FOBTs. Although the impact of fecal DNA screening on cancer morbidity and mortality has not yet been studied, it seems reasonable to assume that attaining sensitivities equal to or better than that of FOBT would result in similar or improved outcomes. However, several questions remain before fecal DNA screening can be widely recommended:

- Can sensitivity for large adenoma be significantly increased compared to FOBT?
- Can false-positive rates be maintained appropriately low for a screening program?
- What is the final configuration of the PreGen-Plus™ test and what are its published performance characteristics in an average-risk screening population?
- What is the optimal screening interval?
- Which patients should not be screened with fecal DNA testing?
- Does the test improve compliance with colorectal cancer screening?
- Is the test cost-effective?

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Published in cooperation with Kaiser Foundation Health Plan and Southern California Permanente Medical Group.

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Various methods for screening for colorectal cancer in persons at average risk are available. Highly effective methods (e.g., colonoscopy) are invasive and costly, and would require greater capacity within the healthcare system than currently exists to screen all adults over 50 years of age. Low-cost, noninvasive methods (e.g., fecal occult blood testing or FOBT) have been shown to be effective at reducing mortality from colorectal cancer, but have much poorer sensitivity for neoplasia than colonoscopy, and required sample collection procedures may reduce compliance. New methods of screening that are noninvasive, highly sensitive, and patient friendly would be ideal. Fecal DNA testing has been proposed as an alternative screening method.

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- available cost-effectiveness analyses of fecal DNA screening.

Background

Colorectal Cancer

Colorectal cancer is the third most common type of cancer in terms of incidence and mortality in both men and women in the U.S. The American Cancer Society (ACS) estimates that there will be about 147,000 new cases in 2006 in the United States; the lifetime probability of developing colorectal cancer is approximately 1 in 18. Colorectal cancer is expected to cause about 56,500 deaths in 2006 (American Cancer Society 2006).

The death rate from colorectal cancer has been decreasing for the past 20 years. This may be because there are fewer cases, because more of the cases are found early and also because

treatments have improved. Increased adenomatous polypectomy rates as a result of screening likely have a favorable effect on incidence (Gupta et al. 2005). About 90% of all cases are thought to be preventable (American Cancer Society 2002).

Conventional Screening Methods

Screening tests for detecting occult blood in the stool or screening sigmoidoscopy or colonoscopy can detect cancer at early, highly curable stages. Screening can also identify some adenomatous polyps and prevent the occurrence of cancer by allowing the removal of polyps before malignant transformation. The U.S. Preventive Services Task Force (USPSTF) identifies several viable methods of screening average-risk adults age 50 or older; no single method is preferred. These methods are: periodic guaiac-based fecal occult blood test (FOBT, e.g., Hemoccult II®, Hemoccult SENSE®), sigmoidoscopy alone or in combination with FOBT, colonoscopy, and double-contrast barium enema (U.S. Preventive Services Task Force 2002).

The FOBT is a low complexity, low cost, noninvasive population screening test; use of annual or biennial FOBT in screening studies is associated with improved outcomes (Hardcastle et al. 1996; Kronborg et al. 1996; Mandel et al. 1999). FOBT depends on release of blood into the gut lumen from disrupted vessels at the tumor surface. However, this test will miss most polyps and some cancers, and will produce some false-positive results due to non-neoplastic sources of bleeding. Patients must follow drug and dietary restrictions prior to FOBT sampling. Sigmoidoscopy requires minimal bowel preparation, can be done every 5 years, and has few complications, but views only the lower third of the colon. Colonoscopy can usually view the entire colon, allows biopsy and polyp removal, and need be done only every 10 years. Limitations of the use of colonoscopy for population screening are the requirement of full bowel preparation, possibility of bowel tears and infection, time required, expense, and healthcare system capacity.

Compliance with screening recommendations using any of these tests is low; approximately 25–40% of adults over age 50 report receiving screening tests, depending on age and gender. As a result, only 37% of cases are diagnosed when the disease is still localized; diagnosis at later disease stages results in substantially lower survival. Thus, given the limitations of

existing tests and current uptake of testing, other methods of population screening are desirable.

Emerging Screening Methods

Newer screening tests may offer advantages to conventional methods; these include immunochemical FOBT (iFOBT), computed tomographic (CT) colonography (“virtual colonoscopy”), and fecal DNA testing. These emerging methods are in various stages of evaluation and/or limited use.

Immunochemical FOBT. Immunochemical FOBTs employ antibodies to detect the globin portion of human hemoglobin (see Table 2). Because globin is degraded during passage through the upper GI tract, the iFOBT is specific for bleeding that is limited to the colon and rectum, in contrast to conventional FOBT. No drug or dietary restrictions are necessary prior to sampling, and sample is collected by swirling a brush in toilet water containing stool (samples from 2 separate stools required). Several commercially produced iFOBTs have received U.S. Food and Drug Administration (FDA) approval. However, the published evidence base depends largely on tests that are not available in the U.S.

A TEC Assessment of iFOBT versus FOBT (2005, Volume 19, No. 5) concluded that iFOBTs could not be evaluated as an assay class because of different test methodologies and variation in detection limits among different tests. This limited the evidence for iFOBTs available in the U.S. to 2 studies comparing two different iFOBTs, InSure™ and FlexSure® (no longer available), and 1 study comparing the iFOBT MonoHaem® to the FOBT Hemoccult II®; the evidence was judged insufficient for drawing conclusions.

An updated search discovered no additional large screening studies of persons at average risk that used FDA-approved iFOBT tests as the primary screen with comparison to endoscopy. Population-based studies of the InSure™ iFOBT are ongoing in Australia.

In August 2005, Beckman Coulter, Inc. launched Hemoccult® ICT, a new version of FlexSure®. Studies of FlexSure® reviewed in the 2004 TEC Assessment enrolled predominantly symptomatic or high-risk patients. Two relatively small studies (403 and 1,410 participants) of persons at average risk resulted in highly

disparate estimates of FlexSure® sensitivity for significant neoplasia (62% and 35%; Rozen et al. 1997 and Rozen et al. 2000, respectively). Whether the FlexSure® test was modified prior to launch as Hemoccult® ICT is unknown. The Clearview ULTRA (Inverness Medical) iFOBT was approved by the FDA in August 2004; no studies related to this test were found.

Instant-View®, an FDA-approved iFOBT, was recently studied as a follow-up test prior to colonoscopy for average-risk screenees who are positive by FOBT (Fraser et al. 2006). The study reported that Instant-View® discriminated well between cancer and other pathology, and that it would reduce colonoscopies by 30%. However, a significant proportion of advanced adenomas were missed, which would reduce the effectiveness of the screening program on cancer incidence.

In April 2002, the American Cancer Society updated its colorectal cancer guideline to include iFOBT as a screening option and a few others have followed suit (see Professional Society Recommendations, following). In January 2004, the Centers for Medicare and Medicaid Services (CMS) expanded their National Coverage Decision for colorectal cancer screening to include one iFOBT every 12 months (Centers for Medicare and Medicaid Services 2004). The cost of iFOBT is reported to be in the range of \$25–50 (Ouyang et al. 2005). The federal reimbursement cap for iFOBT tests increased to \$22.22 in 2005.

CT Colonography. Computed tomographic colonography involves helical computed tomographic scanning of the colon and computerized image manipulation to generate high-resolution 2-dimensional and 3-dimensional images of the inner surface of the colon. A TEC Assessment of CT colonography for cancer screening (2004, Volume 19, No. 6) found that “The current evidence does not allow conclusions as to the comparative efficacy of CT colonography and colonoscopy. Comparison of sensitivity and specificity with colonoscopy is incomplete evidence, because the 2 techniques are intended to be used differently. The bowel preparation currently required for CT colonography is the same as conventional colonoscopy, and depending on the criteria for referral, a variable proportion of patients require colonoscopy, which necessitates another bowel preparation. CT colonography only identifies for removal polyps of a

certain minimum size threshold, and is meant to be used more frequently. It is uncertain what the appropriate size threshold for referral and frequency of screening is appropriate. Actual longitudinal studies or modeling studies are needed to assess comparative efficacy in preventing colon cancer mortality. Improvements in the technical aspects of CT colonography include developments in stool tagging and digital subtraction, which may obviate the need for bowel preparation.”

In June 2005, the National Institute for Health and Clinical Excellence (U.K.) issued an Interventional Procedure Guidance (National Institute for Health and Clinical Excellence 2005), concluding that “current evidence on the safety and efficacy of computed tomographic colonography (virtual colonoscopy) appears adequate to support the use of this procedure.” A meta-analysis of 14 studies (1,324 patients) resulted in a per-patient sensitivity for advanced adenoma of 88% and a specificity of 95%. The Guidance noted the rapidly evolving nature of the technology and the significant effect of training and experience on results.

A recent, evidence-based review summarized the evidence available by March 2005 (Banerjee and Van Dam 2006). Of the 3 included studies with the highest quality rating (multicenter studies of over 500 patients using multidetector scanners and segmental unblinding), none studied an exclusively average-risk population. Sensitivities for large adenoma were disappointingly low in 2 studies, and physician training and experience were identified as important factors. Patient preference is likely to be in favor of CT colonography only if conventional bowel preparation can be avoided. The review highlights the evolving nature of the CT colonography technique. It will be necessary to standardize the procedure, interpretation, and reporting (Zalis et al. 2005), as well as physician training and test standardized CT colonography in large, average-risk populations. In addition, cost-effectiveness studies suggest that CT colonography may not be cost-effective compared to colonoscopy unless the cost decreases considerably.

Fecal DNA Analysis. Several genetic alterations have been associated with colorectal cancer; in the proposed multistep model of carcinogenesis, the tumor suppressor gene *p53* and the proto-oncogene *K-ras* are most frequently altered (Fearon 1990). Mutations

in APC genes and epigenetic markers (e.g. hypermethylation of specific genes) have also been detected. Colorectal cancer is also associated with DNA replication errors in microsatellite sequences (termed microsatellite instability or MSI) in patients with hereditary nonpolyposis colorectal cancer (HNPCC) and in a subgroup of patients with sporadic colon carcinoma (reviewed in Boland et al. 1998). A different marker found in DNA from cancer cells exfoliated into the gut lumen is so-called long-fragment DNA (L-DNA), thought to reflect disrupted apoptosis (normal mode of cell death) in cancer cells.

Tumor-associated gene mutations and epigenetic markers can be detected in exfoliated intestinal cells in stool specimens. Because tumor cells are continuously exfoliated, detection of DNA mutations is more likely in random stool samples than is detection of intermittently released occult blood. Comparison of *p53* and *K-ras* mutations found in stool samples with mutations found in tumor tissue in patients with known colon cancer indicates that mutations found in stool samples reflect those of the tumor (Sidransky 1992; Hasegawa 1995; Smith-Ravin 1995; Nollau 1996; Ratto 1996; Villa 1996). Use of single markers to screen stool samples from highly selected patients in exploratory studies typically results in sensitivities of 40-60% for cancer, with lower results for advanced adenoma (for examples, see Table A in the Appendix).

Colorectal neoplasms are heterogeneous in the mutations they express; no single mutation is expressed by all colorectal cancers (reviewed in Ahlquist 2002). Thus, recent published studies have evaluated the potential of combining various tumor and microsatellite markers in panels to screen stool samples for evidence of DNA mutations characteristic of colon cancer. These studies of stool samples from highly selected patients resulted in sensitivities of about 60-70% for cancer (for examples, refer to Table 1B in the Appendix).

PreGen-Plus™ Fecal DNA Test

Description. PreGen-Plus™ is a commercially available multitarget fecal DNA screening assay for colon cancer. The test is based, “in part, on certain patents and technologies of EXACT Sciences” (EXACT Sciences Web site, <http://www.exactsciences.com/applied/faqs.html>). Patients are supplied with a specimen collection kit and are required to collect an

entire bowel movement; a minimum of 30 g of stool is required for assay. No drug or dietary restrictions are necessary prior to sampling. After DNA purification from stool and amplification using the polymerase chain reaction, the test analyzes 25 markers. These markers consist of 21 separate point mutations in the *k-ras* oncogene, and the APC and *p53* tumor suppressor genes; BAT-26, the most commonly altered marker of microsatellite instability; and L-DNA (DNA Integrity Assay®).

Studies leading to the development of the current test are briefly summarized in Table B of the Appendix. The studies by Syngal et al. (2006) and Tagore et al. (2003) tested the same marker panel as currently used in PreGen-Plus™ on stool samples from highly selected patients, resulting in sensitivities of 63% and 64%, respectively, for cancer; 26% and 57% for advanced adenoma (defined as ≥ 1 cm for Syngal et al. 2006 and containing high-grade dysplasia for Tagore et al. 2003).

Berger et al. (2005a) tested 20 PreGen-Plus™ panel markers on tissue samples from 101 patients with proximal (right-sided) colon cancer and no distal (left-sided) disease. Panel mutations were found in over 80% of proximal tumors that would not be detected by flexible sigmoidoscopy. Similarly, tissues from 52 advanced adenomas were also tested (Berger et al. 2005b), with a detection rate of 88%. If sufficient tumor DNA can be routinely recovered from stool samples of patients with cancer or advanced adenomas, the PreGen-Plus™ panel of markers appears to have the potential for a highly sensitive, noninvasive screening test.

Modifications. Primary difficulties in designing fecal DNA assays are recovering enough DNA and in sufficiently undegraded form for reliable analysis. Whitney et al. (2004) reported an improved method of DNA purification (Effipure™, manufactured by EXACT Sciences) from stool that increased DNA yield 5.4-fold, increased assay sensitivity from 53% to 70% in highly selected samples, and maintained specificity. Olson et al. (2005) determined that storing stool specimens at room temperature for more than 36 hours significantly reduced DNA yield; of all PreGen-Plus™ panel markers, L-DNA is the most sensitive to sample handling conditions. The authors developed and tested a stabilization specimen buffer that significantly retards DNA degradation during specimen

storage. While these modifications were not available for large screening studies (Imperiale et al. 2004, NIH clinical trial; see Review of Evidence and Discussion), they were incorporated into the commercial version of the assay, which was first available in August 2003.

The assay continues to evolve. A second generation assay utilizing many fewer markers was recently presented (see Discussion) and is expected to replace the current assay in the near future (Q1 2006 EXACT Sciences Corporation Earnings Conference Call April 25, 2006; Web cast).

Availability. PreGen-Plus™ testing is available by physician order via an exclusive arrangement with Laboratory Corporation of America® Holdings (LabCorp®), a commercial reference laboratory. PreGen-Plus™ testing is also available without a physician order from DNA Direct (<http://www.dnadirect.com>) and from Direct Laboratory Services, Inc. (<http://www.directlabs.com>). According to both Web sites, LabCorp test kits are shipped to the patient.

Intended Use. According to LabCorp, PreGen-Plus™ is intended “for the detection of clinically significant colorectal neoplasia in asymptomatic average risk patients 50 years old and older... PreGen-Plus™ is not intended to replace a colonoscopy in those patients who are willing to undergo the procedure. Additionally, while it may be used adjunctively or in non-compliant patients, it is not intended as a primary screening tool for individuals at increased risk for developing disease.” (http://www.labcorp.com/pdf/PreGen_Plus_reduce_mortality.pdf)

Cost. The list price of PreGen-Plus™ is \$495, but actual cost depends on the provider contract with LabCorp. DNA Direct and Direct Laboratory Services list prices for PreGen-Plus™ are \$499 and \$595, respectively.

Regulatory Status. PreGen-Plus™ has not been cleared by the FDA. Although the scientific studies that are the basis of the PreGen-Plus™ test were conducted or funded by EXACT Sciences, LabCorp is identified as the test developer. LabCorp is regulated under the Clinical Laboratory Improvement Amendment of 1988 (CLIA) and is certified as qualified to perform high-complexity testing. As a result, LabCorp may develop tests in-house and offer them as laboratory services (so-called “home-

brew” tests). Historically, the FDA has not regulated home-brew tests and has not clearly claimed authority to do so.

However, on January 13, 2006, the FDA sent correspondence to LabCorp indicating that PreGen-Plus may be subject to FDA regulation as a medical device. The FDA expressed particular concern that Effipure™, supplied by EXACT Sciences to LabCorp and used to enhance purification of DNA from stool samples, may be viewed as a device in itself, potentially requiring FDA approval.

LabCorp is developing an alternative, in-house DNA purification procedure that would not require the use of Effipure™ and has responded to the FDA with their plan (Q1 2006 EXACT Sciences Corporation Earnings Conference Call April 25, 2006; Web cast). As this report was released, the final regulatory status of PreGen-Plus™ was unknown.

Other Technology Assessments

The California Technology Assessment Forum (CTAF; Tice 2005) determined that fecal DNA testing met their criteria based on the fact that FOBT is an accepted screening test with evidence of clinical benefit and one high quality study (Imperiale et al. 2004; see Review of Evidence) showing fecal DNA testing to have a higher sensitivity than FOBT. At the time of the CTAF assessment, the FDA regulatory status was not in question.

An application to the CMS for a National Coverage Decision (NCD) on PreGen-Plus testing is on hold, pending resolution of the test regulatory status. Whether a technology assessment will accompany this NCD could not be determined.

Professional Society Recommendations

Several professional groups have developed recommendations, guidelines, or practice parameters regarding colorectal cancer screening, summarized in Table 1. Four of 7 organizations reviewing all screening tests identify colonoscopy as the preferred screening method. No organizations specifically recommend fecal DNA or CT colonography screening, although two organizations recognize fecal DNA testing as a possible option only when patients refuse conventional screening methods. A few organizations list iFOBT as an option.

Methods

Search Methods

MEDLINE® (via PubMed) was searched through June 2006, using the following search terms: “(neoplas* OR cancer) AND (fecal DNA OR stool DNA OR (stool AND DNA))” limited to Human and the English language. The search yielded 240 references, which were searched for studies of DNA markers in fecal samples to discriminate between patients with cancer/advanced adenoma and patients with no evidence of neoplasia.

Study Selection

Selected studies were fully published in a peer-reviewed journal; publicly available, FDA-reviewed information was also acceptable. Studies were selected as evidence if they reported:

- analytical performance characteristics of fecal DNA assays;
- clinical performance, specifically the results of prospective studies that targeted an average risk screening population, compared fecal DNA to a currently accepted screening method (per USPSTF guidelines), and administered colonoscopy to all patients; or
- patient preferences or compliance for fecal DNA tests compared to conventional screening tests
- cost-effectiveness of fecal DNA testing vs. conventional methods.

Medical Advisory Panel Review

This Special Report was reviewed by the Blue Cross and Blue Shield Association Medical Advisory Panel (MAP) on June 22, 2006. To maintain the timeliness of the scientific information in this Report, literature search updates were performed subsequent to the Panel’s review (see “Search Methods”). If the search updates identified any additional studies that met the criteria for detailed review, the results of these studies were included in the text where appropriate. There were no studies that would change the conclusions of this report.

Review of Evidence

Performance

Analytic Performance. Reproducibility of the PreGen-Plus™ assay was tested by collecting and analyzing 3 different stool specimens on 3 different days from each of 16 presurgery

Table 1. Options for Colorectal Cancer Screening: Recommendations from Professional Organizations¹

Organization	Publication	Colonoscopy	Flexible Sigmoidoscopy	Barium Enema	FOBT	iFOBT	fDNA	CT Colonography
U.S. Preventive Services Task Force	Pignone et al. 2002 Guideline	Option	Option	Option	Option			
American Cancer Society	Smith et al. 2006 Guideline	Option	Option		Option	Option	“not recommended at this time”	“not recommended at this time”
National Comprehensive Cancer Network	(Online) Colorectal Cancer Screening Guidelines v1.2006	Preferred	Plus FOBT Option	Option			“under development”	“technique continues to evolve”
American Gastroenterological Association	Winawer 2003 and Regueiro 2005 Future Trends Committee Report	Option	Option	Option	Option		“option only for those reluctant to participate in more conventional approaches”	“less cost-effective than other existing options”
American College of Gastroenterology	(Online) Colon Cancer Resource Kit for Physicians & Patients (files from various dates)	Preferred	Plus FOBT Option				?Option ²	Not endorsed
American Society for Gastrointestinal Endoscopy	Davila et al. 2006 Guideline	Preferred	Option		Option	Option	“cannot be recommended at this time”	“not currently recommended”
American Society of Colon and Rectal Surgeons	Ko et al. 2006 Practice Parameter	Option	Option	Option	Option	Option	“may be a promising approach”	“not yet ready for widespread screening”
National Academy of Clinical Biochemistry	Brunner et al. 2006 (Online) Guideline for laboratory tests	(N/A to laboratory guidelines)	(N/A to laboratory guidelines)	(N/A to laboratory guidelines)	“specifically recommends the Haemoccult SENSEA”	“should be at least as accurate [as] FOBT”	“cannot be recommended at present”	

¹ “Option” indicates that the test is supported as a valid option, without preference or ranking. When specified, the “preferred” test is noted

² “The fecal DNA test is an appropriate option to consider in patients who are unwilling to undergo colonoscopy or in whom colonoscopy is not feasible.” ACG press release December 23, 2004. Available at: <http://www.acg.gi.org/media/releases/dec232004.asp>

patients with newly diagnosed colorectal cancer (Brand et al. 2004). Twenty-eight of 30 (93%) sample results were the same as for the baseline sample. No panel markers were positive in follow-up samples that were not positive at baseline. In 3 cases, a marker positive in the baseline sample was negative on follow-up samples. Based on the results of this study, a single, complete bowel movement is recommended for testing.

Clinical Performance. Imperiale et al. (2004) conducted a prospective, multicenter[†] screening study in which all 5,486 eligible participants were supplied with fecal DNA and FOBT (Hemoccult II) collection kits and were asked to send samples for both tests; all participants were to receive colonoscopy. Participants were compensated except for costs of colonoscopy. Participants were asymptomatic persons ≥ 50 years old at average risk for colorectal cancer. Excluded were persons who had undergone endoscopy or barium enema within preceding 10 years or who had had a positive FOBT within the preceding 6 months. All fecal DNA testing was performed at the EXACT Sciences laboratory; FOBT cards were returned to physicians' offices for analysis by the physician or designee according to the manufacturer's instructions and current guidelines (not rehydrated).

Missing or inadequate DNA specimens or FOBT cards prompted follow-up prior to analysis. Of 5,486 enrolled, 4,404 (80%) participants had adequate DNA specimens and colonoscopy, and completed all FOBT panels. Evaluable participant results were analyzed according to a scheme (see Figure, Appendix) in which only a random sample of participants with no or minor polyps were included. All participants with cancer or advanced adenomas were included in the analysis.

Results are shown in Table 2. The prevalence of cancer among the 4,404 evaluable participants was 31 cases or 0.7%. Test specificities for the 2,507 participants evaluated were similar. However, fecal DNA sensitivity for cancer was four times that of FOBT; for advanced adenoma with high-grade dysplasia fecal DNA sensitivity was more than two times that of FOBT. Sensitivities were similar for all advanced adenoma, defined as 1 cm in size or more.

Patient Preference/Compliance

In the Imperiale et al. (2004) study, 12% of participants did not return adequate stool samples for fecal DNA analysis compared to 8% who did not complete Hemoccult II cards ($p < 0.001$), suggesting a preference for FOBT testing. However, the publication did not distinguish between failure to return any stool sample or FOBT cards versus returning insufficient sample for analysis or incomplete FOBT cards. Thus, it is difficult to determine whether the results for fecal DNA testing truly represent problems with compliance or difficulty with purifying sufficient DNA; the test used lacked later modifications that improved DNA yield (see Background, PreGen-Plus[™] fecal DNA test, Modifications).

Schroy et al. (2005) studied the test preferences of the participants in the Imperiale et al. (2004) study. Participants rated fecal DNA testing, FOBT, and colonoscopy on a variety of features such as understanding of instructions, ease of sample collection for stool tests, invasiveness, associated embarrassment and anxiety, perceived test accuracy, and likelihood of compliance with repeat testing. The questionnaire was distributed within 2 days after colonoscopy and returned by mail by 84% of all participants who completed all screening tests. Overall, the preferred screening test was fecal DNA for 45% of respondents, FOBT for 32%, and colonoscopy for 15%; 8% had no preference. In contrast, colonoscopy had the greatest perceived accuracy. Fecal DNA testing and FOBT received similar ratings for simplicity of instructions, perceived invasiveness, embarrassment, and test-related anxiety. Fecal DNA testing received better ratings than FOBT for simplicity of sample collection, but worse ratings for preparation-related anxiety.

The questionnaire used by Schroy et al. (2005) has been included in each PreGen-Plus[™] collection kit since the test was commercially introduced. Berger et al. (2006) analyzed returned questionnaires for the first 2 years of commercial availability; colonoscopy results for patients with an abnormal fecal DNA result were obtained by surveying physician offices. Questionnaires were returned by 18% of 6,730 patients tested in 44 states; 73% were less than 65 years in age, 8% were less than 50 years old. Fifty-two percent had never been screened

[†] Eighty-one sites including private-practice and university-based settings. Study sites recruited from local practices and advertised availability of screening

Table 2. Results of Fecal DNA Screening Compared to FOBT Screening; Colonoscopy was the Reference Standard (Imperiale et al. 2004)

Colonoscopy Finding	n	Sensitivity (%)		Specificity (%)	
		Fecal DNA	FOBT	Fecal DNA	FOBT
Adenocarcinoma	31	51.6 (34.8–68.0)	12.9 (5.1–28.9)		
Advanced adenoma w/ high-grade dysplasia	71	40.8 (30.2–52.5)	14.1 (7.8–24.6)		
All advanced adenoma (≥1 cm)	403	15.1 (12.0–19.0)	10.7 (8.0–14.1)		
Minor polyps	648	7.6 (5.8–9.9)	4.8 (3.4–6.7)		
No polyps on colonoscopy	1,423			94.4 (93.1–95.5)	95.2 (94.2–96.1)

for colorectal cancer. The majority (87%) of patients found stool sample collection to be very (64%) or somewhat (23%) easy. The vast majority also found the sample collection and return process to be very or somewhat easy. Most patients indicated they were very likely (80%) or likely (11%) to use the test again if ordered by their physician. Possible sources of bias due to, for example, low response rate and self selection, cannot be evaluated in this study.

Ongoing Studies

A trial funded by the National Cancer Institute (NCI; ClinicalTrials.gov identifier NCT00025025) is currently undergoing analysis; publication may be expected later in 2006. The objectives of this trial are to compare the performance characteristics of FOBT (Hemoccult) and PreGenPlus™ testing in screening for colorectal cancer. The trial will compare the detection rates of colorectal neoplasia using PreGenPlus™ alone, flexible sigmoidoscopy alone, and combination sigmoidoscopy and FOBT testing, with colonoscopy as reference standard for all. Additional objectives are to determine the causes of fecal DNA “false-positive” results and to compare the pathological and molecular features of colorectal cancer detected versus not detected by the fecal DNA testing. The trial is observational in nature; 4,434 patients had been accrued when recruiting ended in May 2005.

An unplanned, interim analysis (Ahlquist et al. 2005) was published as an abstract and

compares PreGenPlus™ results to those from FOBT testing. The reason for the interim analysis was the introduction of PreGenPlus™ test modifications; the analysis included only patients tested using the original version of the assay (n=2502). PreGenPlus™ detected 20% of 146 patients with “screen-relevant neoplasia” compared to 12% for Hemoccult FOBT (p=0.03). For cancer (n=23), the corresponding results were 35% for PreGenPlus™ vs. 39% for Hemoccult (p=0.76). These results await confirmation and full publication.

Cost-Effectiveness Analyses

Song et al. (2004) conducted a cost-effectiveness analysis of PreGen-Plus™ fecal DNA testing compared to conventional FOBT, sigmoidoscopy, and colonoscopy. Base case assumptions and modeling results are shown in Table 3. Compliance for all screening strategies was assumed to be 100% as unbiased information regarding patient preferences and participation rates was not available. Fecal DNA testing was cost-effective compared to no screening, but was dominated by conventional strategies; this result was unchanged in most sensitivity analyses. Colonoscopy and sigmoidoscopy plus FOBT were the most effective, gaining an incremental 6,190 and 6,270 life-years per 100,000 persons, respectively, vs. fecal DNA testing at 4,560, all compared with no screening. Assuming base case sensitivity and specificity, the screening interval would have to decrease to 2 years and

Table 3. Fecal DNA vs. Conventional Screening Methods: Cost-Effectiveness Analyses

Study	Test	Assumptions	Cancer Incidence Reduction	Incremental Cost/ Life-year Gained ¹	Life-years per Patient ¹	
Song et al. 2004	fecal DNA (base case)	Cancer sensitivity	65%	35%	\$47,700	18.731
		Adenoma sensitivity	40%			
		Specificity	95%			
		Cost	\$695			
		Screening interval	5 years			
	FOBT	Cancer sensitivity	40%	45%	\$7,200	18.742
		Adenoma sensitivity	10%			
		Specificity	92%			
		Cost	\$20			
	Sigmoidoscopy	Cancer sensitivity ²	90%	56%	\$15,500	18.734
		Adenoma sensitivity ²	80%			
		Specificity	95%			
		Screening cost	\$290			
	Colonoscopy	Cancer sensitivity	95%	71%	\$17,010	18.748
		Adenoma sensitivity	90%			
		Screening cost	\$820			
Parekh et al. 2006 [Abstract; update of Song et al. 2004]	fecal DNA	Cancer sensitivity	52%	24%	\$18,000	18.720
		Adenoma sensitivity	18%			
		Specificity	94%			
		Cost	\$300			
		Screening interval	5 years			
		Cancer sensitivity	82%	not reported	\$13,000	18.733
		Adenoma sensitivity	18%			
		Specificity	82%			
		Cost	\$300			
		Screening interval	5 years			
	FOBT			36%	not reported	18.742

¹ Compared to no screening for purposes of this table so that comparable information could be presented for all tests/assumptions. fDNA screening was dominated by conventional screening methods, see text

² For neoplasia within reach of sigmoidoscope

the cost of fecal DNA testing to \$195 to make fecal DNA comparable with colonoscopy.

Parekh et al. (2006) updated the Song et al. (2004) FOBT versus fecal DNA comparison with changes in base case test performance for fecal DNA testing (see Table 1) to those reported by Imperiale et al. (2004; see Review of Evidence) and a reduction in test cost to \$300. Colorectal treatment costs were also revised upward (see Table 3). Although the incremental cost per life-year gained relative to not screening decreased considerably in this analysis, with 100% adherence FOBT was still more effective and less costly than either no screening or fecal DNA screening. If fecal DNA sensitivity were greatly improved at the expense of specificity, the incremental cost per life-year relative to not screening would decrease further. FOBT and fecal DNA screening had comparable effectiveness at 35% and 100% compliance, respectively, but FOBT remained cost-saving and dominated fecal DNA screening.

Discussion

The Imperiale et al. (2004) study constitutes the only fully published evidence meeting inclusion criteria for clinical utility of fecal DNA screening. The study has several strengths: a large, average-risk screening population was enrolled at 81 centers including both private practice and university-based settings; all participants received colonoscopy; and all investigators were blinded except for the gastroenterologists who had access to the FOBT results. However, the study also has several limitations (see also Woolf 2004), as follows:

- The fecal DNA test did not include the modifications that are used in the commercially available test;
- Overall, nearly 20% of participants could not be evaluated because not all tests were completed;
- Cost of colonoscopy was not covered by study; uninsured participants may be less likely to complete all parts of the study and more likely to have advanced disease;
- White persons were overrepresented (87% versus 75.1% of the U.S. population in the 2000 census);
- Fecal DNA testing was conducted centrally at a single, non-commercial laboratory site and FOBT was conducted locally at all sites;

- FOBT sensitivity was unusually low;
- Detection of advanced adenoma was not significantly better than FOBT.

The poor FOBT sensitivity is especially troubling. According to Pignone et al. (2002), “Studies that have measured the sensitivity and specificity of a single iteration of FOBT among truly asymptomatic subjects have found a sensitivity for an unrehydrated test to be approximately 40%.” Indeed, the interim analysis of the NCI trial of PreGenPlus™ (see Review of Evidence, Ongoing Studies) reported Hemoccult FOBT sensitivity for cancer at 39%. However, at least 2 often-quoted studies of unrehydrated FOBT screening and mortality outcomes (Hardcastle et al. 1996; Kronborg et al. 1996), as well as the NCI trial, processed all FOBT tests at a centralized laboratory, which may not be representative of testing in clinical practice. Nevertheless, the large difference between expected and actual FOBT performance in the Imperiale et al. (2004) study needs further investigation.

In contrast to FOBT, fecal DNA tests were not performed by the reference laboratory currently offering the test, nor did the test used in this study incorporate modifications that are reported to improve DNA yield and test sensitivity. Thus testing was not conducted as in current clinical practice. While the test modifications are expected to improve results beyond those achieved in this study, widespread screening with fecal DNA testing would probably require availability at many different test sites. As this is a home-brew assay and as such is not regulated by the FDA, the effect of increased test volume on reagent quality and test performance is unknown.

It is notable that fecal DNA testing did not improve upon FOBT testing in detecting advanced adenomas. Unless already applied or future test modifications improve these results, the test is not likely to have much impact on cancer incidence.

While sources of false-positive FOBTs are well-understood (e.g., upper gastrointestinal bleeding, certain foods), sources of false-positive DNA tests are less clear. Syngal et al. (2006) noted that L-DNA, included in the PreGen-Plus™ panel, was positive in 15% of patients at 1–3 months post-surgery, becoming negative in most cases by 6–9 months. The

authors postulated an association with inflammation and leukocyte DNA. Similarly, Berger et al. (2006) reported positive results for L-DNA but negative results for other panel markers for 5 patients with inflammatory bowel disease (IBD) or colitis on colonoscopy. Thus, inflammatory conditions related to the gastrointestinal tract may complicate interpretation of test results and currently the test is not recommended for patients with IBD or colitis. No long-term follow-up studies of fecal DNA-positive, colonoscopy-negative patients to determine patient outcome have been reported although long-term registries are reported to be in progress (Berger 2006).

Recently, results using the original and a “Version 2” PreGen-Plus™ assay (PV2) were presented at Digestive Disease Week 2006 by Itzkowitz et al. (2006). Both included the test modifications for improved specimen stability and DNA yield. For PV2, the authors initially tested several new biomarkers including hypermethylation on Vimentin 29 (V29). Samples from the NCI trial (see Review of Evidence, Ongoing Studies) were used to first determine the performance characteristics of the original assay used in Imperiale et al. (2004) with additional modifications: sensitivity improved from 52% to 72% for cancer. For PV2, the “optimal combination” was determined to be V29 plus L-DNA, which resulted in 88% sensitivity for cancer, much nearer the sensitivity of colonoscopy. Compared to the original assay, however, specificity was reduced at 82%. Sensitivity for large adenomas was not reported. PV2 is expected to replace the original PreGen-Plus™ assay as soon as is feasible (Q1 2006 EXACT Sciences Corporation Earnings Conference Call April 25, 2006; Web cast). Full evaluation of PV2 awaits publication in a peer-reviewed journal.

It is important to consider the standard against which fecal DNA testing is measured. In one view, the standard of comparison is the most commonly used, professionally recommended noninvasive test, i.e., FOBT (Tice 2005). The FOBT is an attractive standard because it is an inexpensive test for which there is extensive data confirming a positive effect on mortality in a regular screening program, but it is the least sensitive of all screening tests and sets the lowest bar for comparison. In another view, if the goal is to attain sensitivity near that of colonoscopy (and the cost is not far behind), should the test be compared to colonoscopy? Colonoscopy is, however, not only a screening

test but an intervention to prevent the development of cancer when large adenomas are visualized and removed, and colonoscopy is the recommended follow-up to a positive result for any other screening test. Thus, colonoscopy may be appropriately used as the reference standard, and thus sets the benchmark for cancer and adenoma detection.

Table 4 summarizes test performance, clinical utility, and cost for conventional and emerging screening methods including PreGen-Plus™ testing.

Finally, while claims for iFOBT and fecal DNA tests have been made for improved compliance for noninvasive screening, the supporting evidence is insufficient. For PreGen-Plus™ testing, compliance in the Imperiale et al. (2004) trial may contradict results from a patient preference questionnaire (Schroy et al. 2005). For iFOBT testing, a study of sampling preferences for InSure™ vs. FOBT tests was not conducted in the context of a healthcare setting (TEC Assessment 2005, Volume 19, No. 5). Thus, the impact of test preferences is uncertain; real-world compliance may best be studied in clinical practice settings, rather than in clinical trials for which participants are asked to complete all tests. However, test preferences may not be the most important factor for a successful screening program; compliance with any form of colorectal cancer screening has been shown to depend heavily on patient awareness and on recommendations and tracking by primary care providers (Klabunde et al. 2005).

Conclusions

Fecal DNA testing is a noninvasive colorectal cancer screening technology that may eventually offer sensitivity for cancer closer to that of colonoscopy than that of conventional, guaiac-based FOBTs. Although the impact of fecal DNA screening on cancer morbidity and mortality has not yet been studied, it seems reasonable to assume that attaining sensitivities equal to or better than that of FOBT would result in similar or improved outcomes. However, several issues remain before fecal DNA screening can be widely recommended:

- Can sensitivity for large adenoma be significantly increased compared to FOBT?
- Can false-positive rates be maintained appropriately low for a screening program?

Table 4. Average-Risk Screening: Comparison of Methods (from Pignone et al. 2002, unless otherwise indicated)

Test	Repeat Interval	Issues	One-time Sensitivity		% Patients Requiring Colonoscopy	Mortality Reduction	Potential for Cancer Prevention	Cost ¹
			Cancer	Adenoma ≥ 1 cm				
FOBT, not rehydrated (biennial testing)	1–2 yr	Needs frequent repeat testing Complex program Poor one-time sensitivity	30–40%	10%	5–10%	15–18%	+	\$10–25
FOBT, rehydrated ² (biennial testing) (annual testing)	1–2 yr	(see above)		50% 24%	28% 38%	21% 33%	+	\$10–25
Immunochemical FOBT ³	?? (assume 1–2 yr)	Limited evidence		35–62% (for cancer or advanced adenoma combined)	5–6%	??	??	\$25–50
Sigmoidoscopy	5 yr	Misses proximal lesions 0.1% risk of perforation ⁴	68–78% (for cancer or advanced adenoma combined)		??	50–60%	++	\$190–330
Colonoscopy	10 yr	0.2% risk of perforation ⁵ Requires qualified endoscopist High cost	>90%	>90%	N/A	??	++++ 50–90% incidence reduction ⁵	\$630–1,200
Fecal DNA ⁶	??	Limited evidence Assay still evolving Screening interval unknown High cost	52%	11%	8%	??	??	\$400–500

1 From Song et al. 2004 and Ouyang et al. 2005

2 Rehydrated FOBT is not currently recommended because of high false-positive rates

3 Technology Evaluation Center (TEC) (2005). *Immunochemical versus Guaiac Fecal Occult Blood Tests*. Vol. 19, No. 5. Chicago, IL: Blue Cross and Blue Shield Association4 Gatto NM, Frucht H, Sundararajan V et al. Risk of perforation after colonoscopy and sigmoidoscopy: a population-based study. *J Natl Cancer Inst*, 2003;95(3):230-65 Muller AD, Sonnenberg A. Prevention of colorectal cancer by flexible endoscopy and polypectomy. A case-control study of 32,702 veterans. *Ann Intern Med*, 1995;123(12):904-10Winawer SJ, Zauber AG, Ho MN et al. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. *N Engl J Med*, 1993;329(27):1977-81

6 Imperiale et al. 2004

- What is the final configuration of the PreGen-Plus™ test and what are its published performance characteristics in an average-risk screening population?
- What is the optimal screening interval?
- Which patients should not be screened with fecal DNA testing?
- Does the test improve compliance with colorectal cancer screening?
- Is the test cost-effective?

It has been suggested that any test a patient will take is the best test, as about 57% of adults have never been screened (Centers for Disease Control and Prevention 2006). However, improved screening uptake also depends on efforts to increase awareness among patients, improve recommendation rates for primary care physicians, and include patient compliance tracking in office practice. Physicians will need complete information regarding test performance, and can adjust recommendations based on patient preferences and cost-effectiveness.

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Appendix

Table A. Studies of Molecular Markers in Fecal DNA for Cancer Screening, Using Preselected Patients and Controls (All Studies Excluded Patients with FAP or HNPCC); Not Associated with Development of EXACT Sciences Test

Study	Patients	n	Markers Tested	Sensitivity for Cancer	Specificity
A. Single Molecular Markers					
Chen 2005	Patients with colorectal cancer (n=94) and average-risk controls (n=198)	292	Methylation of vimentin exon 1	46%	90%
Lenhard 2005	Patients with colorectal cancer (n=26), advanced adenoma (>1 cm; n=13); control patients with hyperplastic polyps (n=9) inflammatory bowel disease (n=9), or normal colonoscopy (n=32)	89	Methylation status of 'hypermethylated in cancer 1' gene	42% (Adenoma, 31%)	98%
Wan 2004	Patients with large intestinal cancer (n=23), adenomatous polyps (n=20), and patients with normal colonoscopy (n=20)	63	K- <i>ras</i> codon 12 mutations	56% (Adenoma, 30%)	95%
Muller 2004	Patients with colorectal cancer or normal colonoscopy: Training set, 23+26; Test set 13+13	49 + 26	SFRP2 methylation	Training: 90% Test: 77%	Both: 77%
Calistri 2004	Patients with colorectal cancer (n=86) or normal colonoscopy (n=38)	94	L-DNA ¹	76%	93%
Nishikawa 2002	Patients with colorectal cancer (n=31) or normal colonoscopy (n=5)	36	K- <i>ras</i>	42%	100%
Ito 2002	Patients with colorectal cancer (n=18) or advanced adenoma (n=2)	20	K- <i>ras</i>	30%	
Puig 2000	Patients with colorectal cancer (n=12), adenoma (n=25), other GI disease or normal colonoscopy (n=30)	67	K- <i>ras</i>	55% (Adenoma, 27%)	100%

1 Similar to DNA integrity assay in EXACT Sciences' PreGen-Plus panel

Table A. Studies of Molecular Markers in Fecal DNA for Cancer Screening, Using Preselected Patients and Controls
(All Studies Excluded Patients with FAP or HNPCC); Not Associated with Development of EXACT Sciences Test (cont'd)

Study	Patients	n	Markers Tested	Sensitivity for Cancer	Specificity
B. Combinations of Molecular Markers					
Kutzner 2005	Patients with colorectal cancer (n=57) and "control donors" (n=44)	101	APC, BAT26, L-DNA ¹	65%	91%
Matsushita 2005	Patients with colorectal cancer (n=116) and controls with normal colonoscopy (n=83)	199	APC, K-ras, p53, BAT26	71%	88%
Leung 2004	Patients with colorectal cancer (n=20), advanced adenoma (n=6), or normal colonoscopy (n=20)	46	Methylation of APC, ATM, HMTF, MGMT, hMLH-1, and GSTP1 genes	70% (Adenoma, 67%)	100%
Calistri 2003	Patients with colorectal cancer (n=53) or controls with negative follow-up (n=38)	91	p53, K-ras, APC, 5 microsatellite markers, DNA amplification of p53 and APC	62%	97%
Rengucci 2001	Patients with colorectal cancer (n=46) or controls with normal colonoscopy (n=18)	64	p53, K-ras, 5 microsatellite markers	26%	100%

¹ Similar to DNA integrity assay in EXACT Sciences' PreGen-Plus panel

Table B. Studies of Molecular Markers in Fecal DNA for Cancer Screening, Using Preselected Patients and Controls (All Studies Excluded Patients with FAP or HNPCC); Associated with Development of EXACT Sciences Test

Study	Patients	n	Tumor Size ¹ (Range) (cm)	Markers Tested	Sensitivity	Specificity	Comments
Syngal 2006	Patients with newly diagnosed colorectal carcinoma (n=68) or advanced adenoma \geq 1 cm (n=23)	91	Median 3.0 (0.1–11)	21 mutations in K-ras, p53, APC; BAT26; DNA integrity (same as PreGen-Plus panel)	Cancer: 63% Advanced Adenoma: 26%		Sensitivity for stage I 39% vs. 72% for Stage II+III+IV; Sensitivity 65% for distal lesions vs. 38% for proximal lesions Retest at 1-3 mos. post-resection 18% pos. Retest at 6-9 mos. post-resection 7% pos.
Tagore 2003	Patients with invasive colorectal cancer or adenomas with high-grade dysplasia (n=80) and patients negative for colonoscopy (n=212)	292	?	21 mutations in K-ras, p53, APC; BAT26; DNA integrity (<i>same as PreGen-Plus panel</i>)	Cancer: 64% Advanced Adenoma: 57%	96%	Sensitivity for stage I, II, III, and IV disease, 75%, 67%, 42%, and 50%
Boynton 2003	Consecutive patients with colorectal cancer (n=25) or negative colonoscopy (n=77)	102	?	DNA integrity	Cancer: 57%	97%	
Traverso 2002a [Research Letter]	46 patients with proximal colon cancer; 14 with proximal advanced adenomas (\geq 1 cm); 69 with positive FOBT, rectal bleeding or personal/family history but normal colonoscopy	134	?	BAT26 (microsatellite instability marker)	Cancer: 37% (23-52) Advanced Adenoma: 0%	100%	DNA alterations identical between stool sample and tumor tissue
Traverso 2002b	28 patients with Dukes' stage B2; 28 control patients with no known colorectal tumor; 18 patients with adenomas at least 1 cm in diameter	74	Median 1.6 Mean 2.1 (0.2–6.7)	APC mutations in the APC gene region codon 1210–1581	Dukes' B2: 61% Advanced Adenoma: 50%	100%	

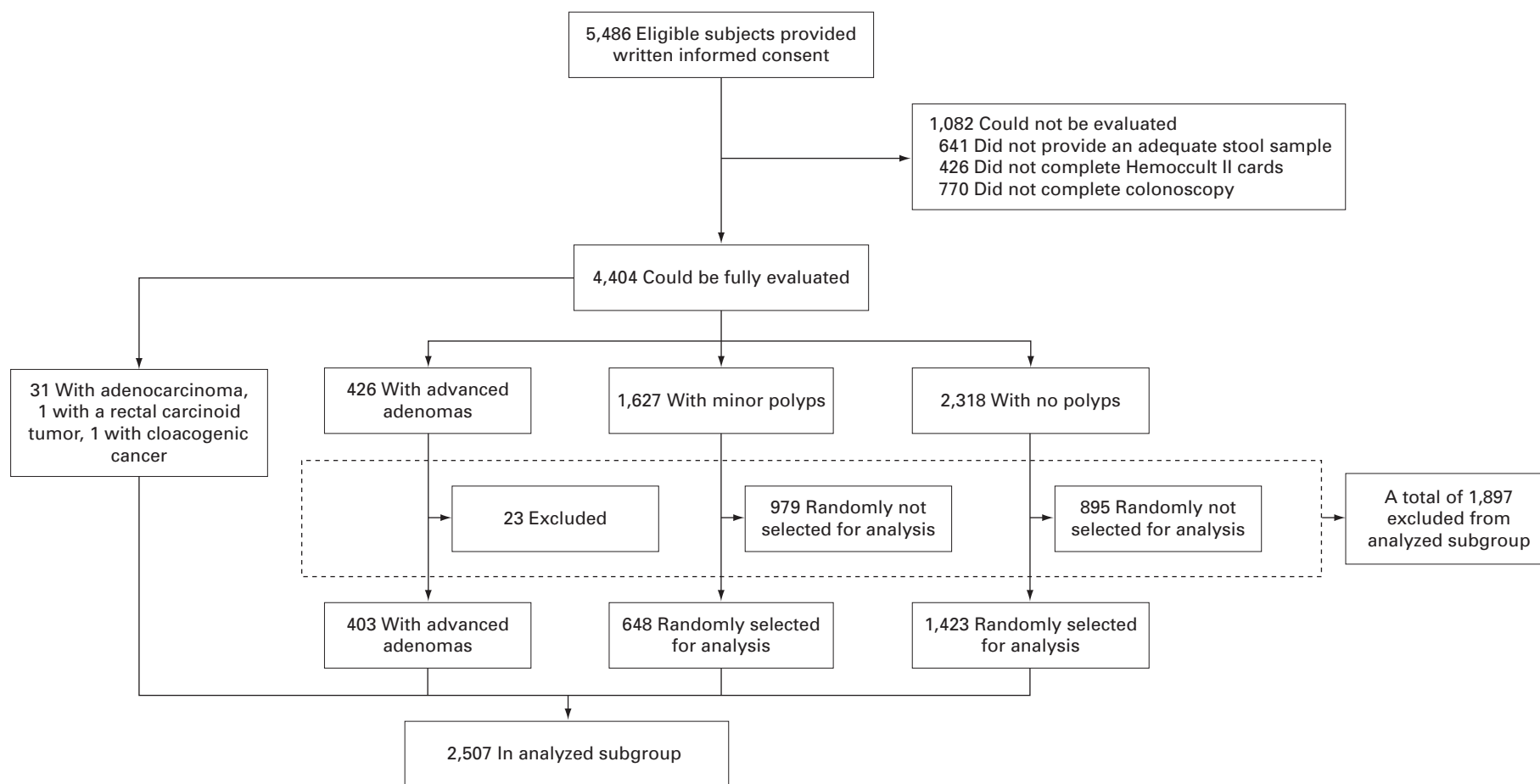
Table B. Studies of Molecular Markers in Fecal DNA for Cancer Screening, Using Preselected Patients and Controls (All Studies Excluded Patients with FAP or HNPCC); Associated with Development of EXACT Sciences Test (cont'd)

Study	Patients	n	Tumor Size ¹ (Range) (cm)	Markers Tested	Sensitivity	Specificity	Comments
Dong 2001	Patients with diagnosed intact colorectal adenocarcinoma	51	Median 3.75 (1.3–14)	TP53, BAT26, K-RAS by mismatch ligation assay	71%		Sensitivity for stage A, B, C, and D disease, 100%, 82%, 67%, and 58% DNA alterations identical between stool sample and tumor tissue
Ahlquist 2000	Pilot 1: 21 patients with colorectal adenocarcinoma, 9 advanced adenomas (≥ 1 cm), and 10 with normal colonoscopies	40	Median 4.9 (2.6–11)	15 mutations in K-ras, p53, APC; BAT26; DNA integrity	90%	100%	Use pilot 1 to establish cutoff for positivity
	Pilot 2: 8 patients with colorectal adenocarcinomas, 2 with advanced adenomas, 18 with normal colonoscopies	28 ²	Median 3.9 (2.5–11)		100%	89%	
	Combined	61	Mean 4.1 (2.5–11)		Cancer: 91% Adenoma: 82%	93%	
					without K-ras: Cancer: 91% Adenoma: 73%	without K-ras: 100%	

¹ For patients with carcinoma

² Includes 7 patients with cancer from pilot 1

Figure. Analysis Scheme for Imperiale et al. 2004 (Copyright © 2004 Massachusetts Medical Society. All rights reserved.)



Disposition of Subjects Enrolled in the Study

Some subjects had more than one reason for not being evaluated. A total of 641 subjects did not undergo colonoscopy; 129 subjects underwent a colonoscopy that did not reach the cecum or that did not adequately visualize the colonic mucosa. Twenty-three subjects with advanced adenomas were originally classified as having minor polyps and were excluded from the analyzed subgroup. On subsequent audit of the data by the clinical research organization, these subjects were reclassified as having advanced adenomas. Thus, only 403 subjects with advanced adenomas were included in the analyzed subgroup.



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