



TITLE: Fecal DNA Screening for Colorectal Cancer

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FECAL DNA SCREENING FOR COLORECTAL CANCER

INTRODUCTION

The California Technology Assessment Forum is requested to review the scientific evidence for the use of fecal DNA testing to screen for colon cancer.

BACKGROUND

Colorectal cancer is the third most common cancer diagnosed in the U.S. and the second leading cause of cancer death. In 2005, an estimated 145,290 new cases will be diagnosed and 56,290 deaths are expected, accounting for approximately 10 % of cancer deaths this year in the U.S. (American Cancer Society 2005).

The risk of colon cancer increases with age. More than 90 % of cases are diagnosed in individuals over the age of 50. Other risk factors include family history, inflammatory bowel disease, smoking, alcohol consumption, obesity, physical activity, a high-fat diet and low intake of fruits and vegetables. In addition, there are certain genetic factors that increase the risk of colon cancer, including familial adenomatous polyposis, Gardner's syndrome, hereditary nonpolyposis colorectal cancer and Ashkenazi Jewish descent.

Death from colon cancer is largely preventable. When colorectal cancer is diagnosed at an early, localized stage, five-year survival is 90%. However, less than 40% of cases are diagnosed at this early stage (Smith, von Eschenbach *et al.* 2001). More than 95% of colorectal cancers are thought to arise from adenomas. Adenomas are common with a prevalence of 25-50% in people over 50 years of age (Winawer, Fletcher *et al.* 1997). However, most colorectal adenomas are small (less than 1 cm). It is estimated that the length of time it takes for an adenoma to progress to a carcinoma ("dwell time") is on average about 10 years (Winawer, Fletcher *et al.* 1997).

Safe and effective methods to screen for colorectal cancer and adenomas have been available for many years. The most common methods include fecal occult blood testing (FOBT), flexible sigmoidoscopy, colonoscopy and double contrast barium enema. Three randomized clinical trials have demonstrated that FOBT screening every one to two years reduces mortality from colorectal cancer (Mandel, Bond *et al.* 1993; Hardcastle, Chamberlain *et al.* 1996; Kronborg, Fenger *et al.* 1996). More than 10 years ago, the National Polyp Study demonstrated that endoscopic removal of adenomatous polyps decreased the incidence of colorectal cancer by 76-90% (Winawer, Zauber *et al.* 1993). There is significant controversy around the most efficacious and efficient strategy to screen for colorectal cancer. Recent systematic reviews of the

available screening options concluded that the available evidence is insufficient to recommend one test over another (Pignone, Rich *et al.* 2002; Walsh and Terdiman 2003).

It is clear that all average risk men and women over the age of 50 should undergo some form of screening for colorectal cancer, but most Americans do not adhere to this recommendation. One study reported that only 44% of Americans eligible for colorectal screening reported ever having had FOBT, sigmoidoscopy or colonoscopy (2001). Many explanations have been proposed for the low adherence with screening including low rates of physicians offering screening, poor patient adherence with physician recommendations and patient dissatisfaction with the available screening options (Vernon 1997; Nadel, Shapiro *et al.* 2005). New screening options such as fecal DNA testing may help to address this important public health problem.

FOBT

As indicated above, the best evidence for efficacy of colorectal cancer screening comes from three large randomized controlled studies of FOBT in average risk populations in the U.S., England and Denmark. FOBT is designed to detect bleeding from colorectal cancers and adenomas, which tend to bleed more than normal colonic mucosa. The test most commonly used in the U.S. is the Hemoccult II. This test detects the peroxidase-like activity of hemoglobin in the presence of guaiac. FOBT requires patients to collect two samples from each of three consecutive stools at home. Due to concerns about false positive test results, patients are instructed to avoid non-steroidal anti-inflammatory drugs (NSAIDS) for a week prior to testing and to avoid red meat for three days. Vitamin C intake from food or vitamin supplements may cause false negative test results and is also restricted to less than 250 mg per day for three days prior to testing. Recent studies have suggested that the dietary restrictions may not affect test accuracy significantly (Pignone, Campbell *et al.* 2001).

Systematic reviews report that FOBT has a sensitivity of approximately 40% with specificity ranging from 96-98%. Rehydration increased the sensitivity to 50-60%, but decreased specificity to 90% (Ransohoff and Lang 1997). Most current guidelines do not recommend the use of rehydration because the reduced specificity increased potential harms and cost by increasing the number of patients undergoing colonoscopy for evaluation of a false positive test. A single, in-office FOBT is much less sensitive than the traditional three-card FOBT (Collins, Lieberman *et al.* 2005) and is not recommended for use as a screening test for colorectal cancer.

Fecal DNA Testing

Fecal DNA testing is promoted as a more accurate, non-invasive alternative to FOBT for colorectal cancer screening. The studies and rationale supporting the potential of fecal DNA testing as a screening test have recently been reviewed in detail (Mak, Laloo, *et al.*, 2004). Briefly, many DNA mutations have been

identified that are associated with the progression from normal colonic mucosa to adenomatous polyps and finally colorectal carcinoma (Fearon and Vogelstein 1990; Boland, Sato *et al.* 1998). First, inactivation of the adenomatous polyposis coli (APC) gene facilitates the hyperproliferation of colonic epithelium. Activation of K-ras occurs in early adenoma followed by deletion of the deleted in colon cancer (DCC) gene and *P53* inactivation. Colonic epithelial and tumor cells are constantly shed and become incorporated into stool. Recent advances in technology allow human DNA to be separated from bacterial DNA in the stool, amplified more than a billion-fold by polymerase chain reaction (PCR), and then tested for mutations associated with colorectal neoplasia. Evidence has accumulated that such tests may be better screening tests than FOBT because DNA is shed continuously from the mucosa and it is stable in stool (Ahlquist and Shuber 2002).

No single mutation has been identified that is expressed in all colorectal cancers. Early work demonstrating that human DNA with mutations could be found in stool targeted single mutations in either K-ras or p53 (Sidransky, Tokino *et al.* 1992; Hasegawa, Takeda *et al.* 1995; Smith-Ravin, England *et al.* 1995; Eguchi, Kohara *et al.* 1996; Nollau, Moser *et al.* 1996; Villa, Dugani *et al.* 1996; Puig, Urgell *et al.* 1999). These mutations were generally present in less than half of all colorectal cancers and the stool assay was never 100% sensitive, even in stool from patients with cancers known to have the mutation of interest. Thus, any DNA-based screening test will need to use a panel of mutations to achieve high sensitivity. These studies also did not assess the specificity of these mutations which can be a problem as K-ras mutations, for instance, are known to occur in other disease states (Caldas, Hahn *et al.* 1994; Yamashita, Minamoto *et al.* 1995)., PreGen-Plus, a CLIA (Clinical Laboratory Improvement Amendments) cleared test, targets 21 point mutations in three genes known to be associated with colorectal cancer (3 in the K-ras gene, 10 in the APC gene and 8 in the p53 gene), a marker of microsatellite instability (shortened forms of Bat-26), and a test for long DNA (DNA Integrity Assay or DIA). Cells shed from normal mucosa undergo apoptosis. During apoptosis, their DNA is chopped into small fragments less than 200 base pairs in length. Tumors, on the other hand, often shed cells with incompletely cleaved DNA, resulting in long, high molecular weight pieces of DNA that can be detected by the DIA.

The patient receives a collection kit and container to mail the specimen to the clinical laboratory. The patient is required to collect one complete bowel movement of at least 30 grams. The specimen must be refrigerated or frozen until mailed. At the central laboratory, the samples are thawed, homogenized in a buffer solution and centrifuged to remove particulate matter. Crude DNA is then precipitated and resuspended. The specific DNA sequences of interest are purified using oligonucleotide-based hybrid capture. Then the DNA is amplified using real-time PCR. Finally, specific assays for each of the DNA alterations described above are performed.

The potential advantages of fecal DNA testing over FOBT testing included improved specificity because it eliminates false positives due to gastrointestinal bleeding from sources other than polyps and cancer. Sensitivity may also be improved because DNA is continuously shed from tumors at a greater rate than normal mucosa (Loktionov, O'Neill *et al.* 1998; Ahlquist 2000), while bleeding from polyps and tumors is only intermittent (Ahlquist, McGill *et al.* 1989). No dietary modifications are needed prior to collecting the stool. This may also lead to improved patient acceptance of the test.

Potential disadvantages include patient acceptance of the need to collect, refrigerate and send an entire bowel movement to the clinical laboratory. The test is also much more expensive (\$400-\$800) than FOBT (\$3-\$40) (Pignone, Rich *et al.* 2002; Song, Fendrick *et al.* 2004; Woolf 2004).

Technology Assessment (TA)

TA Criterion 1: The technology must have final approval from the appropriate regulatory bodies.

PreGen-Plus (Exact Sciences) falls under the jurisdiction of the CLIA program (Clinical Laboratory Improvement Amendments of 1988). As such, the FDA has determined that premarket clearance is not required.

TA Criterion 1 is met.

TA Criterion 2: The scientific evidence must permit conclusions concerning the effectiveness of the technology regarding health outcomes.

The Medline database including Current Contents, Cochrane clinical trials database, Cochrane reviews database and the Database of Abstracts of Reviews of Effects (DARE) were searched using the key words "DNA" and "feces" AND "colorectal neoplasm". The search was performed for the period from 1966 through December 2004. The bibliographies of systematic reviews and key articles were manually searched for additional references. The abstracts of citations were reviewed for relevance and all potentially relevant articles were reviewed in full.

The literature search identified 18 studies (Sidransky, Tokino *et al.* 1992; Hasegawa, Takeda *et al.* 1995; Smith-Ravin, England *et al.* 1995; Eguchi, Kohara *et al.* 1996; Nollau, Moser *et al.* 1996; Ratto, Flamini *et al.* 1996; Villa, Dugani *et al.* 1996; Deuter and Muller 1998; Puig, Urgell *et al.* 1999; Notarnicola, Cavallini *et al.* 2000; Doolittle, Emanuel *et al.* 2001; Ito, Kobayashi *et al.* 2002; Koshiji, Yonekura *et al.* 2002; Nishikawa, Maemura *et al.* 2002; Traverso, Shuber *et al.* 2002; Traverso, Shuber *et al.* 2002; Boynton, Summerhayes *et al.* 2003; de Kok 2003) evaluating the test characteristics of single gene DNA tests and seven studies

evaluating panels of several genes (Table). This review will focus on the multi-gene studies because there is consensus in the literature that no known DNA abnormality occurs in enough colorectal tumors to give adequate sensitivity and because the CLIA-approved test uses a panel of DNA tests. Three of the identified studies did not include all of the markers used in the CLIA approved test (Ahlquist, Skoletsky *et al.* 2000; Dong, Traverso *et al.* 2001; Rengucci, Maiolo *et al.* 2001) and one of the studies retests samples used in prior studies with a novel method for extraction of DNA (Whitney, Skoletsky *et al.* 2004). Only one (Imperiale, Ransohoff *et al.* 2004) of the three remaining studies evaluated the test characteristics in a screening population. The other two may be subject to spectrum bias (Tagore, Lawson *et al.* 2003; Brand, Ross *et al.* 2004). The results from a second, large (n=4,000) NIH sponsored study in a similar screening population should be available within a year.

No randomized controlled trials were identified, nor were there any studies that evaluated long term clinical outcomes. However, FOBT is an accepted screening test for colorectal cancer with randomized controlled trial evidence for clinical benefit. The one high quality study directly compared the test characteristics of fecal DNA testing to those of FOBT (Imperiale, Ransohoff *et al.* 2004).

TA Criterion 2 is met.

Level of evidence: 3, 5

TA Criterion 3: The technology must improve the net health outcomes.

The first study (Ahlquist, Skoletsky *et al.* 2000) reported results from two pilot studies of a precursor to the multi-factorial test now marketed as PreGen-Plus. They tested stool from 22 patients with colorectal cancer, 11 patients with large premalignant adenomatous polyps and 28 patients with normal colonoscopies. Stool was collected prior to colonoscopy and stored frozen at -80°C . DNA testing was performed blinded to outcome status. The assay panel included 15-point mutations on K-ras, APC and p53; Bat-26; and long DNA testing. Human DNA sufficient for analysis was recovered from all 61 stool specimens. The sensitivity was 91% (95% CI 71-99%) for cancer and 82% (95% CI 48-98%) for large adenomas. Specificity was 93% (95% CI 88-100%). Excluding K-ras from the assay panel did not affect the sensitivity for cancer, but increased the specificity to 100% (95% CI 88-100%).

The second study using this technology (Dong, Traverso *et al.* 2001) evaluated stool DNA from paired stools and primary tumor samples from 51 colorectal cancer patients. Stool was collected after the patients were diagnosed with cancer on colonoscopy. No patients with adenomas or normal colonoscopies were evaluated. The panel used in this study assayed 11-point mutations in K-ras and p53, as well as Bat-26. It

did not include mutations in APC nor the long DNA assay. This fecal DNA panel was positive in 36/51 patients (sensitivity 71%, 95% CI 56-83%) and in 36/39 (92%) of the tumors that tested positive for the mutations.

An Italian group (Rengucci, Maiolo *et al.* 2001) used a different technology platform to test for 11-point mutations in p53 and K-ras, and microsatellite instability. They used stool collected after diagnostic colonoscopy from 46 patients with colorectal cancer and 18 patients with normal colonoscopies. It was not possible to directly calculate the sensitivity of the combined set of markers from the article, but it was < 26% (12/46). The specificity was 100%.

None of these early studies used the full set of markers included in the CLIA approved panel. Certain methodological issues, such as collecting stool for DNA testing after the biopsy of the tumor, may have affected the sensitivity and specificity of the tests. There was a wide range of sensitivities reported (26-91%), though specificity was quite high (93-100%). However, these early studies were encouraging and larger prospective studies were started.

The first study (Tagore, Lawson *et al.* 2003) to report data using the CLIA approved PreGen-Plus assay enrolled eighty patients with advanced colorectal neoplasia and 212 controls. Stool samples were collected prior to colonoscopy. Patients with hereditary colorectal cancer syndromes were excluded. All samples were analyzed in the clinical laboratory at EXACT Sciences. The test was positive in 33/52 patients with invasive colorectal cancer (sensitivity 64%, 95% CI 49-76%). The sensitivity for advanced adenomas (lesions containing high-grade dysplasia, villous adenomas or tubular adenomas > 1 cm in size) was 57% (16/28, 95% CI, 37-76%). Specificity was 96% (95% CI, 93-98%) in patients with either no colorectal lesions or minor polyps. The authors concluded that with these positive results, a prospective study in an average-risk population was needed to validate these findings.

A small study (Brand, Ross *et al.* 2004) set out to assess whether analyzing three stool specimens obtained on three different days with PreGen-Plus was superior to a single specimen for the detection of CRC. Sixteen patients with newly diagnosed CRC underwent stool collection on three different days prior to surgical resection. Each specimen was analyzed using the fecal DNA test. Eleven of the sixteen patients (69%) had at least one mutation detected in their first stool specimen. No new mutations were detected in subsequent. The authors concluded that there was no additional benefit from performing fecal DNA testing on more than one specimen per patient.

The most important study to consider when evaluating the effectiveness of fecal DNA testing was recently published in the New England Journal of Medicine (Imperiale, Ransohoff *et al.* 2004). It is the only study that compared the test characteristics of fecal DNA testing with those of the Hemoccult II FOBT in average-risk,

asymptomatic persons 50 years of age or older. This is the only study evaluating fecal DNA testing in a screening population. Eligible subjects submitted one stool specimen for DNA analysis, underwent standard Hemoccult II testing and then underwent colonoscopy. Of 5,486 subjects enrolled, 4,404 completed all aspects of the study. Their average age was 68.6 years old and 87% were greater than 60 years old. 87% of the participants were white, 8% black and 45% were men. A subgroup of 2,507 subjects was analyzed, including all those with a diagnosis of invasive adenocarcinoma or advanced adenoma plus randomly chosen subjects with no polyps or minor polyps. The fecal DNA panel detected 16 of 31 invasive cancers, whereas Hemoccult II identified 4 of 31 (51.6 % vs. 12.9 %, $P=0.003$). Half of the cancers (15/31) were TNM Stage I. None of the cancers were TNM Stage IV. Among 403 subjects with advanced adenoma (defined as a tubular adenoma at least 1 cm in diameter, a polyp with a villous histological appearance or a polyp with high-grade dysplasia), the DNA panel was positive in 61 (15.1 %), whereas Hemoccult II was positive in 43 (10.7 %). Specificity in subjects with negative findings on colonoscopy was 94.4 % for the fecal DNA panel and 95.2 % for Hemoccult II. Although the majority of neoplastic lesions identified by colonoscopy were not detected by either noninvasive test, the multitarget analysis of fecal DNA detected a greater proportion of important colorectal neoplasia than did Hemoccult II without compromising specificity.

The study has many strong points. To avoid spectrum bias, the fecal DNA test was evaluated in the population in which it will be used (asymptomatic individuals > 50 without known familial risk for colon cancer). It was directly compared to one of the standard alternatives (unrehydrated Hemoccult II) in the same patients. It appears that both stool tests were performed blinded to the results of all other tests, though this is only clearly stated for the fecal DNA test. The gold standard (colonoscopy) may not have been performed blinded to the stool test results. This may have resulted in some work-up bias, as 770 of the participants in the study (14%) did not complete their colonoscopy. It is also concerning that 641 participants (12%) did not provide an adequate sample for DNA analysis, even though repeat samples were sought. This would decrease the effective sensitivity of the test in clinical practice, as potentially 12% of individuals with colorectal cancer would not provide adequate samples for the test. A lower proportion ($n=426$, 8%) of the study participants were unable to complete the FOBT. This also calls into question whether the fecal DNA test is actually more acceptable to patients than FOBT, even though they may express greater preference for the fecal DNA test (Schroy and Heeren 2005 in press).

Several other concerns have been raised about the results of this study (Woolf 2004). The sensitivity of both fecal DNA and FOBT were lower than previously reported. Pooled estimates from prior studies suggested that the sensitivity of fecal DNA testing for cancer would be about 67% with 95% confidence interval extending from 60% to 74% (Whitney, Skoletsky *et al.* 2004); this study (Imperiale, Ransohoff *et al.* 2004) reported a sensitivity of only 52% (95% CI 35-68%), well outside the 95% CI of prior estimates. The

confidence interval around the estimate of sensitivity is wide, reflecting the small number of cancers (n=31) detected in this screening population. Similarly the sensitivity of FOBT (13%, 95% CI 5-29%) is much lower than the 30% to 40% reported for prior studies of unrehydrated FOBT (Ransohoff and Lang 1997). This may reflect spectrum bias in the prior studies. For example, in the study of Dong *et al.*, 24% of the colorectal cancers were Stage IV and only 2% were Stage I, compared with 0% Stage IV cancers and 48% Stage I in this study. Furthermore, in many of the early studies, the stool for fecal DNA testing was collected after the diagnosis of cancer. Diagnosis usually involves biopsy of the tumor, which may lead to increased shedding of tumor cells into the stool for some time after the biopsy. The specificity of fecal DNA testing (94%) was also lower than prior reports (97%, 95% CI 93-99%) (Whitney, Skoletsky *et al.* 2004) and was lower than that of FOBT in this study (95%).

One additional study (Whitney, Skoletsky *et al.* 2004) tested the impact of a new purification method that would increase the yield of human DNA from stool. DNA from 86 cancer and 100 controls was purified from the stool with a new method for DNA recovery based on sequence-specific capture with acrylamide gel immobilized capture probes, as well as with a previously developed magnetic bead-capture procedure. The same 21 point mutation plus satellite instability plus long DNA assay panel was used after DNA purification by both methods. The new purification method gave an average 5.4-fold increase in the quantity of human DNA retrieved from fecal samples. The increased recovery of DNA corresponded to an increase in assay sensitivity from 53% (CI: 42-64%) to 70% (CI: 59-79%; P = 0.0005), with no change in specificity. This new sample preparation method may improve the sensitivity of fecal DNA testing.

TA Criterion 3 is met.

TA Criterion 4: The technology must be as beneficial as any established alternatives.

There are several established alternatives to fecal DNA screening for colorectal cancer including FOBT, sigmoidoscopy, colonoscopy and barium enema, though the latter is rarely used. Fecal DNA testing, like FOBT, was developed as a non-invasive alternative to endoscopy. The Imperiale (2004) study directly compared fecal DNA testing to FOBT in a screening population. The fecal DNA test had a higher sensitivity for both invasive colorectal cancer (52% vs. 13%, p=0.003) and advanced adenomas (15% vs. 11%, p not reported). The specificity was slightly lower for both normal colonoscopies (94% vs. 95%, p NS) and minor polyps (92% vs. 95%, p NS). From these numbers, it is estimated that among the 4,404 patients in the group that could be evaluated, 334 patients were positive on fecal DNA testing and would have been recommended for colonoscopy. This would identify 16 cancers and 64 advanced adenomas. In the same group, FOBT would be positive in 239 and would identify 4 cancers and 45 advanced adenomas. Thus, 95

additional colonoscopies would be performed to identify 12 additional cancers and 19 additional advanced adenomas.

Thus, fecal DNA testing appears to be more effective at identifying high-risk colorectal neoplasms with only modest increased risk (an additional 95 colonoscopies for every 4,404 patients screened). These numbers would change somewhat based on the prevalence of cancer and advanced adenomas in the population screened, but they represent reasonable estimates. The major uncertainty in the application of fecal DNA screening is the frequency with which it should be performed. The randomized controlled trials of FOBT suggest that the mortality reduction is much larger when a one-year rather than two-year screening interval is used. However, comparable data are not available for fecal DNA. Repeated testing over several days clearly increases the sensitivity of FOBT for colorectal cancer (Collins, Lieberman *et al.* 2005), but the same was not found to be true for fecal DNA testing (Brand, Ross *et al.* 2004). In the absence of clinical trial data, modeling with decision analysis will be needed to determine the optimal interval for repeat testing with fecal DNA. One published decision analysis (Song, Fendrick *et al.* 2004), assumed fecal DNA sensitivity of 65% for invasive cancer, 40% for advanced adenoma and 95% specificity found screening with fecal DNA to be effective compared to no screening when a screening interval of five years was used. However, these estimates were more optimistic than those found in the Imperiale (2004) study and the authors reported that fecal DNA screening was inferior to conventional alternatives like FOBT and colonoscopy. However, at a screening interval of two years, it was comparable to colonoscopy.

TA criterion 4 is met.

TA Criterion 5: The improvement must be attainable outside the investigational setting

In the Imperiale study (2004) over 4,000 patients at 81 centers successfully provided an adequate stool sample. It is of some concern that 641 patients (12% of initial sample) were unable to provide an adequate sample in an investigational setting, but 426 patients were also unable to give an adequate sample for FOBT. Given that the testing was done at a single central lab and that this will remain the case for the foreseeable future, it is likely that the sensitivity and specificity observed in Imperiale study (2004) can be obtained outside of the investigational setting.

TA Criterion 5 is met.

Table: Test Characteristics of Panels of Genetic Markers for the Diagnosis of Colorectal Neoplasms

Study	Test	Population	Cancer			Advanced Adenoma			Minor Polyp			Normal		
			N	N +	Sens	N	N +	Sens	N	N +	Sens	N	N +	Spec
Alquist 2000	15 mutations + Bat-26+ long DNA		22	20	91	11	9	82				28	2	93
Dong 2001	Only 11 mutations (in K-ras and p53) + Bat-26	Stool collected after diagnosis, prior to surgery.	51	36	71	-	-	-	-	-	-	-	-	-
Rengucci 2001	Only 11 mutations (in K-ras and p53) + Bat-26	Stool collected 72 hours after biopsy diagnosis, prior to surgery.	46	<12	<26	-	-	-	-	-	-	18	0	100
Tagore 2003	PGP: 21 mutations + Bat-26+ long DNA	80 cancers and polyps + 212 screening colonoscopies.	52	33	63	28	16	57	99	6	6.1	113	2	98
Brand 2004	PGP: 21 mutations + Bat-26+ long DNA	Stool collected after diagnosis, prior to surgery.	16	11	69	-	-	-	-	-	-	-	-	-
Imperiale 2004	PGP: 21 mutations + Bat-26+ long DNA	Screening, stool collected prior to diagnosis.	31	16	52	403	61	15	648	49	7.6	1,423	79	94
Whitney 2004	PGP: 21 mutations + Bat-26+ long DNA. Novel DNA recovery method.	Stored samples from prior studies.	60	86	70	-	-	-	-	-	-	100	96	96

N: Number of patients studied
 N+: Number of tests positive
 Sens: Sensitivity
 Spec: Specificity

Advanced Adenoma: High-grade dysplasia, villous adenoma or tubular adenoma ≥ 1 cm.
 Minor polyp: Tubular adenoma < 1 cm, hyperplastic polyp.
 Normal: No polyps seen on colonoscopy.

CONCLUSION

Early results suggested that fecal DNA testing would have much higher sensitivity and specificity than FOBT for colonic neoplasms, including advanced adenomas. Unfortunately, when evaluated in a large screening population, the specificity was slightly, but not significantly lower (94% vs. 95%). And while the sensitivity for invasive cancer was much higher (52% vs. 13%), the sensitivity for advanced adenomas was only slightly higher (15% vs. 11%)--a major disappointment. In the same study, patients expressed a preference for fecal DNA testing over FOBT, but a much larger proportion of the target population did not complete the fecal DNA testing compared to FOBT (12% vs. 8%). While there is no RCT evidence that fecal DNA testing decreases mortality rates from colorectal cancer, it appears to have comparable specificity and higher sensitivity than FOBT, a screening test that has been shown to decrease colorectal cancer mortality. It remains unclear how often fecal DNA testing should be performed when chosen instead of FOBT or endoscopic screening. It is recommended that FOBT be done annually, but FOBT is much less sensitive and expensive than fecal DNA testing. An additional complication is the fact that repeat FOBT screening has been shown to increase the sensitivity of the test for colon cancer and advanced adenomas, but similar data are lacking for fecal DNA testing. Further modeling work should be done to clarify the optimal use of fecal DNA testing. Publication of data from the ongoing NIH study of fecal DNA testing will also provide more confidence in the estimates for the sensitivity and specificity of fecal DNA testing in a screening population.

Recommendation

It is recommended that the use of the PreGen-Plus fecal DNA test meets Technology Assessment Criteria 1 through 5 to screen asymptomatic individuals for colorectal cancer.

February 16, 2005

The CTAF panel voted unanimously to accept the recommendation as written.

OPINIONS OF OTHERS

Blue Cross Blue Shield Association (BCBSA)

The BCBSA Technology Evaluation Center has not conducted a review specific to this technology to date.

Centers for Medicare and Medicaid Services (CMS)

CMS does not currently have a national coverage decision regarding fecal DNA testing.

American Gastroenterological Association (AGA)

An AGA representative did attend the meeting and indicated that the AGA could not support the recommendation at this time.

American Cancer Society (ACS)

An ACS representative was not able to attend the meeting. The ACS did however indicate that they could not support the recommendation at this time.

California Society of Pathologists

The California Society of Pathologists did not provide representation at the meeting, nor was a formal opinion/position available.

ABBREVIATIONS USED IN THIS REVIEW:

FOBT: Fecal Occult Blood Testing

NSAIDS: Non-steroidal Anti-inflammatory Drugs

ACP: Adenomatous Polyposis Coli

DCC: Deleted in Colon Cancer

DIA: DNA Integrity Assay

PCR: Polymerase Chain Reaction

DARE: Database of Abstracts and Reviews of Effects

REFERENCES

1. (2001). "Trends in screening for colorectal cancer--United States, 1997 and 1999." *MMWR Morb Mortal Wkly Rep* **50**(9): 162-6.
2. Ahlquist, D. A. (2000). "Molecular stool screening for colorectal cancer. Using DNA markers may be beneficial, but large scale evaluation is needed." *Bmj* **321**(7256): 254-5.
3. Ahlquist, D. A., D. B. McGill, *et al.* (1989). "Patterns of occult bleeding in asymptomatic colorectal cancer." *Cancer* **63**(9): 1826-30.
4. Ahlquist, D. A. and A. P. Shuber (2002). "Stool screening for colorectal cancer: evolution from occult blood to molecular markers." *Clin Chim Acta* **315**(1-2): 157-68.
5. Ahlquist, D. A., J. E. Skoletsy, *et al.* (2000). "Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel." *Gastroenterology* **119**(5): 1219-27.
6. American Cancer Society (2005). *Cancer Facts and Figures 2005*. Atlanta, American Cancer Society.
7. Boland, C. R., J. Sato, *et al.* (1998). "Genetic instability and chromosomal aberrations in colorectal cancer: a review of the current models." *Cancer Detect Prev* **22**(5): 377-82.
8. Boynton, K. A., I. C. Summerhayes, *et al.* (2003). "DNA integrity as a potential marker for stool-based detection of colorectal cancer." *Clin Chem* **49**(7): 1058-65.
9. Brand, R. E., M. E. Ross, *et al.* (2004). "Reproducibility of a multitarget stool-based DNA assay for colorectal cancer detection." *Am J Gastroenterol* **99**(7): 1338-41.
10. Caldas, C., S. A. Hahn, *et al.* (1994). "Detection of K-ras mutations in the stool of patients with pancreatic adenocarcinoma and pancreatic ductal hyperplasia." *Cancer Res* **54**(13): 3568-73.
11. Collins, J. F., D. A. Lieberman, *et al.* (2005). "Accuracy of screening for fecal occult blood on a single stool sample obtained by digital rectal examination: a comparison with recommended sampling practice." *Ann Intern Med* **142**(2): 81-5.
12. de Kok, J. B. (2003). "Quantification and integrity analysis of DNA in the stool of colorectal cancer patients may represent a complex alternative to fecal occult blood testing." *Clin Chem* **49**(12): 2112-3.
13. Deuter, R. and O. Muller (1998). "Detection of APC mutations in stool DNA of patients with colorectal cancer by HD-PCR." *Hum Mutat* **11**(1): 84-9.
14. Dong, S. M., G. Traverso, *et al.* (2001). "Detecting colorectal cancer in stool with the use of multiple genetic targets." *J Natl Cancer Inst* **93**(11): 858-65.
15. Doolittle, B. R., J. Emanuel, *et al.* (2001). "Detection of the mutated K-Ras biomarker in colorectal carcinoma." *Exp Mol Pathol* **70**(3): 289-301.
16. Eguchi, S., N. Kohara, *et al.* (1996). "Mutations of the p53 gene in the stool of patients with resectable colorectal cancer." *Cancer* **77**(8 Suppl): 1707-10.
17. Fearon, E. R. and B. Vogelstein (1990). "A genetic model for colorectal tumorigenesis." *Cell* **61**(5): 759-67.

18. Hardcastle, J. D., J. O. Chamberlain, *et al.* (1996). "Randomised controlled trial of faecal-occult-blood screening for colorectal cancer." *Lancet* **348**(9040): 1472-7.
19. Hasegawa, Y., S. Takeda, *et al.* (1995). "Detection of K-ras mutations in DNAs isolated from feces of patients with colorectal tumors by mutant-allele-specific amplification (MASA)." *Oncogene* **10**(7): 1441-5.
20. Imperiale, T. F., D. F. Ransohoff, *et al.* (2004). "Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population." *N Engl J Med* **351**(26): 2704-14.
21. Ito, Y., S. Kobayashi, *et al.* (2002). "Frequent detection of K-ras mutation in stool samples of colorectal carcinoma patients after improved DNA extraction: comparison with tissue samples." *Int J Oncol* **20**(6): 1263-8.
22. Koshiji, M., Y. Yonekura, *et al.* (2002). "Microsatellite analysis of fecal DNA for colorectal cancer detection." *J Surg Oncol* **80**(1): 34-40.
23. Kronborg, O., C. Fenger, *et al.* (1996). "Randomised study of screening for colorectal cancer with faecal-occult-blood test." *Lancet* **348**(9040): 1467-71.
24. Loktionov, A., I. K. O'Neill, *et al.* (1998). "Quantitation of DNA from exfoliated colonocytes isolated from human stool surface as a novel noninvasive screening test for colorectal cancer." *Clin Cancer Res* **4**(2): 337-42.
25. Mak, T., F. Laloo, *et al.* (2004). "Molecular stool screening for colorectal cancer." *Br J Surg* **91**(7): 790-800.
26. Mandel, J. S., J. H. Bond, *et al.* (1993). "Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study." *N Engl J Med* **328**(19): 1365-71.
27. Nadel, M. R., J. A. Shapiro, *et al.* (2005). "A national survey of primary care physicians' methods for screening for fecal occult blood." *Ann Intern Med* **142**(2): 86-94.
28. Nishikawa, T., K. Maemura, *et al.* (2002). "A simple method of detecting K-ras point mutations in stool samples for colorectal cancer screening using one-step polymerase chain reaction/restriction fragment length polymorphism analysis." *Clin Chim Acta* **318**(1-2): 107-12.
29. Nollau, P., C. Moser, *et al.* (1996). "Isolation of DNA from stool and bodily fluids for PCR amplification." *Biotechniques* **20**(5): 784-8.
30. Notarnicola, M., A. Cavallini, *et al.* (2000). "K-ras and p53 mutations in DNA extracted from colonic epithelial cells exfoliated in faeces of patients with colorectal cancer." *Dig Liver Dis* **32**(2): 131-6.
31. Pignone, M., M. K. Campbell, *et al.* (2001). "Meta-analysis of dietary restriction during fecal occult blood testing." *Eff Clin Pract* **4**(4): 150-6.
32. Pignone, M., M. Rich, *et al.* (2002). "Screening for colorectal cancer in adults at average risk: a summary of the evidence for the U.S. Preventive Services Task Force." *Ann Intern Med* **137**(2): 132-41.
33. Puig, P., E. Urgell, *et al.* (1999). "Improved detection of K-ras codon 12 mutations in fecal exfoliated cells." *Lab Invest* **79**(5): 617-8.
34. Ransohoff, D. F. and C. A. Lang (1997). "Screening for colorectal cancer with the fecal occult blood test: a background paper. American College of Physicians." *Ann Intern Med* **126**(10): 811-22.

35. Ratto, C., G. Flamini, *et al.* (1996). "Detection of oncogene mutation from neoplastic colonic cells exfoliated in feces." *Dis Colon Rectum* **39**(11): 1238-44.
36. Rengucci, C., P. Maiolo, *et al.* (2001). "Multiple detection of genetic alterations in tumors and stool." *Clin Cancer Res* **7**(3): 590-3.
37. Schroy, P. C., 3rd and T. C. Heeren (2005 in press). "A comparative study of stool-based DNA testing, fecal occult blood testing and colonoscopy: Patient perceptions and screening preferences." *Am J Prev Med*.
38. Sidransky, D., T. Tokino, *et al.* (1992). "Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors." *Science* **256**(5053): 102-5.
39. Smith, R. A., A. C. von Eschenbach, *et al.* (2001). "American Cancer Society guidelines for the early detection of cancer: update of early detection guidelines for prostate, colorectal, and endometrial cancers. Also: update 2001--testing for early lung cancer detection." *CA Cancer J Clin* **51**(1): 38-75; quiz 77-80.
40. Smith-Ravin, J., J. England, *et al.* (1995). "Detection of c-Ki-ras mutations in faecal samples from sporadic colorectal cancer patients." *Gut* **36**(1): 81-6.
41. Song, K., A. M. Fendrick, *et al.* (2004). "Fecal DNA testing compared with conventional colorectal cancer screening methods: a decision analysis." *Gastroenterology* **126**(5): 1270-9.
42. Tagore, K. S., M. J. Lawson, *et al.* (2003). "Sensitivity and specificity of a stool DNA multitarget assay panel for the detection of advanced colorectal neoplasia." *Clin Colorectal Cancer* **3**(1): 47-53.
43. Traverso, G., A. Shuber, *et al.* (2002). "Detection of APC mutations in fecal DNA from patients with colorectal tumors." *N Engl J Med* **346**(5): 311-20.
44. Traverso, G., A. Shuber, *et al.* (2002). "Detection of proximal colorectal cancers through analysis of faecal DNA." *Lancet* **359**(9304): 403-4.
45. Vernon, S. W. (1997). "Participation in colorectal cancer screening: a review." *J Natl Cancer Inst* **89**(19): 1406-22.
46. Villa, E., A. Dugani, *et al.* (1996). "Identification of subjects at risk for colorectal carcinoma through a test based on K-ras determination in the stool." *Gastroenterology* **110**(5): 1346-53.
47. Walsh, J. M. and J. P. Terdiman (2003). "Colorectal cancer screening: scientific review." *JAMA* **289**(10): 1288-96.
48. Whitney, D., J. Skoletsky, *et al.* (2004). "Enhanced retrieval of DNA from human fecal samples results in improved performance of colorectal cancer screening test." *J Mol Diagn* **6**(4): 386-95.
49. Winawer, S. J., R. H. Fletcher, *et al.* (1997). "Colorectal cancer screening: clinical guidelines and rationale." *Gastroenterology* **112**(2): 594-642.
50. Winawer, S. J., A. G. Zauber, *et al.* (1993). "Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup." *N Engl J Med* **329**(27): 1977-81.
51. Woolf, S. H. (2004). "A smarter strategy? Reflections on fecal DNA screening for colorectal cancer." *N Engl J Med* **351**(26): 2755-8.

52. Yamashita, N., T. Minamoto, *et al.* (1995). "Frequent and characteristic K-ras activation in aberrant crypt foci of colon. Is there preference among K-ras mutants for malignant progression?" *Cancer* 75(6 Suppl): 1527-33.