Analysis of Human DNA in Stool Samples as a Technique for Colorectal Cancer Screening

Summary

Detection of genetic abnormalities associated with colorectal cancer in stool samples has been proposed as a screening test for colorectal cancer. This technology is another potential alternative to currently available screening approaches such as fecal occult blood testing, fecal immunochemical testing (FIT) or colonoscopy.

The evidence on the accuracy of stool DNA as a screening test for colorectal cancer consists of a number of studies that have compared stool DNA analysis to colonoscopy and one large population screening study that compared stool DNA testing and FIT, using colonoscopy as the reference standard. The population screening study reported stool DNA analysis has higher sensitivity and lower specificity than FIT. The largest study was done with a test that is no longer commercially available, and the evidence on the commercially available test is limited to smaller studies. These earlier studies report a low to moderate sensitivity and a high specificity for stool DNA analysis the test. The sensitivity varies widely in the available studies and as a result there remains some uncertainty as to the true sensitivity of stool DNA testing the test. A new test that uses next generation sequencing technology has reported a higher sensitivity, but prospective studies are lacking and this test is not yet commercially available.

In addition to uncertainty about the diagnostic accuracy of the test stool DNA analysis, clinical utility of this test has not yet been demonstrated; since there is no evidence that stool DNA testing this test improves outcomes. The studies of diagnostic accuracy for detecting cancer and cancer precursors does not establish efficacy for prevention of colorectal cancer. Effective screening for colorectal cancer requires a screening program with established screening intervals and appropriate follow-up for positive tests. For fecal DNA testing, compliance with testing, i.e., successful completion and return of the test, is not known and may substantially impact outcomes. As a result, analysis of DNA in stool samples is considered not medically necessary as a screening technique for colorectal cancer.

Related Policies

None
Policy

*This policy statement applies to clinical review performed for pre-service (Prior Approval, Precertification, Advanced Benefit Determination, etc.) and/or post-service claims.

DNA analysis of stool samples is considered not medically necessary as a screening technique for colorectal cancer in both patients with average to moderate risk and in patients considered at high risk for colorectal cancer.

Background

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. Mutations in APC (adenomatous polyposis coli) 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 Lynch syndrome (formerly known as hereditary nonpolyposis colorectal cancer [HNPCC]) and in a subgroup of patients with sporadic colon carcinoma. Tumor-associated gene mutations and epigenetic markers can be detected in exfoliated intestinal cells in stool specimens. Since cancer cells are shed into stool, tests have been developed that detect these genetic alterations in the DNA from shed colorectal cancer cells isolated from stool samples. This has been proposed for use in screening two populations of patients for colon cancer:

1. Known or suspected carriers of Lynch syndrome mutations, considered at high risk of developing colorectal cancer

In this setting, testing of fecal samples could be used to monitor patients over time for development of colorectal cancer. The test could be used either in lieu of routinely scheduled surveillance colonoscopies or during intervals between scheduled colonoscopies. Those patients testing positive for cancer-related genetic alterations could be further evaluated with colonoscopy.

2. In patients at average risk of colorectal cancer

In this setting, testing of fecal samples could be offered in lieu of, or as an adjunct to, other recommended colorectal cancer screening tests, including fecal occult blood testing, flexible sigmoidoscopy, colonoscopy, or double contrast barium enema.

Regulatory Status

Several types of tests have been evaluated in studies and some have been marketed. One of these, PreGen-Plus™, tests for 21 different mutations in the p53, APC, and K-ras genes; the BAT-26 MSI marker; and incorporates the DNA Integrity Assay (DIA®). PreGen-Plus™ has not been cleared by the U.S. Food and Drug Administration (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 Amendments of 1988 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 (i.e., laboratory-developed tests). Historically, FDA has not regulated laboratory-developed tests. However, on January 13, 2006, FDA sent correspondence to LabCorp indicating that PreGen-Plus™ may be subject to FDA regulation as a medical device. As a consequence, and as a result of studies showing better performance of other tests, this test is no longer offered.

On Aug 12, 2014, Exact Sciences received FDA premarket approval for its latest automated fecal DNA testing product, Cologuard™ (P130017). Cologuard™ is intended for the qualitative detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool. A positive result may indicate the presence of colorectal cancer or advanced adenoma and should be followed by diagnostic colonoscopy. Cologuard™ is indicated to screen adults of either sex, 50 years or older, who are at average risk for colorectal cancer. Cologuard™ is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals.

Another currently available test is called ColoSure™, developed by OncoMethylome, which detects aberrant methylation of the vimentin (hV) gene. This test is offered as a laboratory-developed test, not subject to FDA regulation.

**Rationale**

As with any diagnostic test, the key outcomes are the diagnostic performance (i.e., sensitivity, specificity, positive and negative predictive value) compared to a gold standard, and consideration of how the results of the test will be used to benefit patient management. Of the various screening options (i.e., fecal occult blood testing, fecal immunochemical testing, flexible sigmoidoscopy, double contrast barium enema, colonoscopy), colonoscopy is considered the gold standard. For example, in patients considered at high risk for colorectal cancer, due either to a family history or Lynch syndrome (formerly known as hereditary nonpolyposis colorectal cancer [HNPCC]) mutation, colonoscopy at varying intervals is recommended by the American Society of Colorectal Surgeons, the American Gastroenterological Society, and the American Cancer Society. Therefore, for patients at high risk of colorectal cancer with suspected or known Lynch syndrome mutations, the diagnostic performance of DNA analysis of stool samples will be compared with colonoscopy. In addition, the role of DNA analysis in the context of the recommended colonoscopic screening must be explored. Will this test be offered in lieu of colonoscopy, such that patients with a negative test can defer a scheduled colonoscopy, or will this test be offered as an adjunct to colonoscopy screening, for example during the intervals between colonoscopies?

For patients at average to moderate risk for colorectal cancer, these organizations also recommend colonoscopy starting at age 50 years, with an interval of 10 years, as one screening option. In addition, other screening techniques are also considered options, and the choice of screening option may be dictated in part by patient preference. Many authors have noted the low patient acceptance of current colorectal cancer screening options, particularly flexible sigmoidoscopy and colonoscopy; at the present time, only approximately 40% of eligible patients undergo screening for colon cancer. Advocates of genetic testing of stool samples have hypothesized that the relative simplicity of collecting a stool sample might increase the overall compliance with screening recommendations. Therefore, for patients at average to moderate risk of colon cancer, genetic testing of stool samples will be compared to
colonoscopy and also to fecal immunochemical testing and fecal occult blood testing, the other entirely noninvasive techniques. Patient acceptance of the different options is also a relevant outcome as a technique to increase screening compliance.

**Literature Review**

No clinical trials have been published that evaluate use of DNA stool tests in those at high risk for colon cancer.

The largest population screening study was published in 2014 by Imperiale et al and compared the fecal DNA test (previously developed and evaluated in the studies by Ahlquist et al and Lidgard et al discussed below) with fecal immunochemical testing (FIT) in 12,000 asymptomatic persons at average risk for colorectal cancer. The results of this study supported the Food and Drug Administration approval of this fecal DNA test (Cologuard™) in August 2014. This multitarget stool DNA test consists of quantitative measurements of molecular assays for aberrantly methylated BMP3 and NDRG4 promoter regions, mutant KRAS, and β-actin in a logistic-regression algorithm. All enrolled subjects were scheduled to undergo screening colonoscopy. Stool specimens were collected and tested no more than 90 days prior to the screening colonoscopy. Screening colonoscopy findings were considered the reference standard for determining the diagnostic characteristics of the fecal DNA test and FIT for detecting colorectal cancer and cancer precursors. In 9989 evaluable subjects, fecal DNA test sensitivity for cancer was 92.3% and 73.8% for FIT. For advanced precancerous lesion, fecal DNA test sensitivity was 42.4% and 23.8% for FIT. In analyses of specific types of lesions, sensitivity of the fecal DNA test did not vary by cancer stage or cancer location. Among patients with advanced precancerous lesions, the sensitivity of fecal DNA testing was higher for distal lesions than for proximal lesions. Fecal DNA test sensitivity increased as lesion size increased. The specificity of the fecal DNA test was lower than that of FIT. For identification of patients with insignificant lesions and negative colonoscopy, specificity of the fecal DNA test was 86.6% versus 94.9% for FIT. For identification of patients with negative colonoscopy, specificity of the fecal DNA test was 89.8% versus 96.4% for FIT.

In 2014, the Blue Cross Blue Shield Association Technology Evaluation Center evaluated fecal DNA analysis for colorectal cancer screening in a special report. The report found the Imperiale study to be of good quality but noted while fecal DNA testing had higher sensitivity than FIT for various types of colorectal lesions, these results represent the diagnostic characteristics of the fecal DNA test in a one-time cross-sectional study. How these study results may translate to reduced colorectal mortality in a screening program are uncertain. The study of the diagnostic characteristics of a test for detecting cancer and cancer precursors does not establish efficacy for prevention of colorectal cancer. Effective screening for colorectal cancer requires a screening program with established screening intervals and appropriate follow-up for positive tests. Given what is known about relative efficacy of different screening strategies from the results of modelling studies, the fecal DNA test would produce equivalent or better outcomes than FIT if both were used annually. However, the fecal DNA test has a considerably higher false-positive rate and would therefore consume greater health care resources than FIT at this screening frequency. Formal modelling studies of the fecal DNA test are needed to estimate the efficacy of the test in preventing colorectal cancer and help determine the optimal strategy for its use.
In 2014, Shah et al published a systematic review of biomarkers for early detection of polyps and colorectal cancer.6 This review included 44 studies published from 2007 through June 2013 and thus did not include the 2014 Imperiale study. Sixty-seven different tumor markers were included in the studies. Shah et al found overall sensitivities of fecal DNA markers ranged from 53% to 87% for detecting colorectal cancer detection. The sensitivity of detecting colorectal cancer and adenoma increased when fecal DNA markers were combined. The authors noted a need for well-structured population-based studies to validate biomarkers for colorectal cancer and adenoma detection further.

The largest An earlier 2004 study of those at average risk for colon cancer is that of Imperiale et al who reported on the results of a prospective trial of 5486 enrolled subjects.7 However, this study evaluates a test that is no longer available and that uses completely different DNA markers than the Colosure™ test. Thus, the results do not represent the performance of the Colosure™ test. It is worth reviewing here because it is the central piece of evidence used by some organizations to endorse such screening.

Subjects underwent fecal occult blood testing (FOBT), fecal DNA analysis using a precommercial version of the test, and colonoscopy, considered the gold standard for this trial. Of the 5486 enrolled, 4404 completed all aspects of the study and, from this group, 2507 underwent comparative analysis. The subgroup was chosen by including all subjects who were found to have adenocarcinoma (n=31) and a random selection of subjects with adenomas, polyps, or normal findings. The sensitivity of fecal DNA analysis and FOBT for all cancers and adenomas with high-grade dysplasia was 40.8% versus 14.1%, respectively. Specificity in subjects with a negative finding on colonoscopy was 94.4% for fecal DNA and 95.3% for FOBT. This study is the first large study of fecal DNA testing in an asymptomatic average-risk population. The following limitations are noted:

- The Imperiale et al study is not an intention-to-treat analysis. Approximately 20% of subjects were not evaluated (12% did not provide an adequate stool sample for DNA testing; 8% did not complete FOBT cards; 14% did not complete colonoscopy). Missing data were not imputed.
- The observed sensitivity for cancer of the Hemoccult II FOBT in this study was lower at 13% than reported in other studies. Imperiale et al also note in their discussion section that “the difference between our results (on Hemoccult sensitivity) and those of other reports is potentially important and deserves further study.”
- The Hemoccult II FOBT tests were performed at each of the 81 study sites (including private-practice and university-based settings); quality control procedures were not described. In contrast, the DNA test was conducted in a single laboratory. Screening would require dissemination of the DNA test to more laboratories, which, as the authors note, could introduce greater variability in results.

However, the results of this study suggest that fecal DNA analysis offers an improved sensitivity, and thus the question arises as to whether fecal DNA should be considered an alternative to FOBT for patients who are unwilling to undergo, or do not have access to colonoscopy. The authors comment on the large percentage of patients who forgo recommended screening for colorectal cancer, particularly the gold standard of colonoscopy, and propose that a simple noninvasive screening test with an improved sensitivity compared to FOBT would be a viable alternative.
These issues are addressed in an accompanying editorial by Woolf, who urges caution in interpreting the results of the Imperiale et al study.8 For example, Woolf notes the wide confidence intervals around the sensitivity of fecal DNA, ranging from 35% to 68%, which preclude any firm estimates of the magnitude of benefit associated with fecal DNA testing. Fecal DNA testing does provide some advantages in that, unlike FOBT, the patient does not have to undergo a specialized diet prior to the test. However, the patient must collect, refrigerate, and mail an entire bowel movement, which may be unacceptable to some patients. Woolf suggests that increasing screening rates is an important outcome but one that may be achieved by improving the accessibility and delivery of current screening methods.

Subsequently, Schroy and Heeren conducted a study of patient perceptions of stool-based DNA testing of those participating in the Imperiale et al study.9 A total of 4042 subjects completed the survey, an 84% response rate. The survey consisted of 25 questions using a 5-point ordinal scale or a yes-no format. Stool-based testing received the same or higher mean ratings as fecal occult blood, and higher ratings than colonoscopy, except for perceived accuracy.

Published evidence on the currently available Colosure™ test is relatively slim. Two studies allow calculation of the performance characteristics of the hypermethylated vimentin (hV) gene alone. In a study by Itzkowitz et al, separately assembled groups of patients with colorectal cancer (n=40) and patients with normal colonoscopy (n=122) were tested with hV.10 Sensitivity was 72% and specificity was 87%. In a second study by Itzkowitz et al, separately assembled groups of patients with colorectal cancer (n=82) and patients with normal colonoscopy (n=363) were tested with hV and a 2-site DNA integrity assay.11 The purpose of the study was to calculate diagnostic performance characteristics of this combined test, but the results are also presented for hV alone. Using data-derived cutoff values, the sensitivity for cancer was 77% and the specificity was 83%. Other studies of hypermethylated vimentin using different assays have shown sensitivities of 38% and 41% for detecting colorectal cancer.12,13

None of these studies is adequate to evaluate a test that is to be used in the screening setting. The study samples are enriched with cancer cases that may not represent the prevalence or spectrum of disease present in a screening situation. The sensitivity and specificity values calculated from these studies should not be generalized to actual clinical populations. Patients with any other clinically relevant abnormalities such as polyps have been excluded from many of the studies. The cutoff values have been determined post hoc by examining the data.

Another study by Ahlquist et al, evaluated a screening test in which one component of the test was hV.14 However, hV was only 1 of 3 different types of markers used in this multicomponent test. Data were not analyzed separately for hV, thus the results of this study do not represent the performance of hV alone. In addition, normal patients were not tested, meaning that specificity could not be calculated. Without knowing what the corresponding specificity is, the sensitivity of a test is uninformative because it can be manipulated by simply changing the cutoff value for a positive test.

A next-generation stool test has been developed by Exact Sciences and has been evaluated in a study by Ahlquist et al.3 This test detects 4 methylated genes, a mutant form of K-ras, and the alpha-actin gene. In a study of 252 patients with colorectal cancer, 133 patients with adenomas of 1 cm or larger, and 293 subjects with normal colonoscopy, the test detected 85% of colon cancer cases and 54% of
subjects with adenomas, with 90% specificity. Another smaller study of this same test showed a sensitivity of 87% for detecting colorectal cancer and 82% sensitivity for detecting adenomas.\textsuperscript{15} 

Lidgard et al reported on another study by Exact Sciences in 2013.\textsuperscript{4} In this multicenter, blinded, case-control study of 1003 patients, there were 207 cases with colorectal cancer or advanced adenomas (>1 cm), and 796 control patients with no polyps or nonadvanced adenomas (<1 cm). In the case group, 93 subjects had colorectal cancer, 84 had advanced adenoma 1 cm or larger and 30 had sessile serrated adenoma 1 cm or larger. In the control group, 155 subjects had nonadvanced adenomas and 641 did not have any colonic lesions. Stool samples were drawn from 544 patients prior to bowel prep for colonoscopy, and from 459 patients 1 week after colonoscopy but before any treatment had been given. An automated fecal DNA assay measured \( \beta \)-actin, mutant K-ras, aberrantly methylated BMP3 and NDRG4, along with fecal hemoglobin. Using a logistic regression algorithm that incorporates 11 markers into one regression score and a fixed specificity of 90%, the fecal DNA test identified 84 of 86 (98% sensitivity) colorectal cancers and 41 of 73 (56% sensitivity) advanced adenoma cases.

These automated fecal DNA tests are not yet commercially available. The test characteristics need to be evaluated in a prospective manner in general population samples, rather than in case-controlled, predefined cancer cases and normal controls.

**Ongoing and Unpublished Clinical Trials**

An online search of ClinicalTrials.gov on October 25, 2014, identified 52 open studies on fecal DNA for detection of colorectal cancer. The largest study will evaluate fecal DNA testing prior to colonoscopy in 1600 patients to evaluate the test’s efficacy in detecting advanced adenoma (NCT01647776). This study has an estimated completion date of November 2015.

The automated fecal DNA assay is currently being evaluated in the Multi-Target Colorectal Cancer Screening Test for the Detection of Colorectal Advanced Adenomatous Polyps and Cancer (DeeP-C) study (NCT01397747). The DeeP-C study is evaluating the sensitivity and specificity of the Exact Sciences colorectal screening test in 12,776 subjects compared to colonoscopy. This study was completed in 2013, but results have not yet been published.

**Summary of Evidence**

The evidence on the accuracy of stool DNA as a screening test for colorectal cancer consists of a number of studies that have compared stool DNA analysis to colonoscopy and one large population screening study that compared stool DNA testing and FIT, using colonoscopy as the reference standard. The population screening study reported stool DNA analysis has higher sensitivity and lower specificity than FIT. The largest study was done with a test that is no longer commercially available, and the evidence on the commercially available test is limited to smaller studies. These Earlier studies report a low to moderate sensitivity and a high specificity for stool DNA analysis the test. The sensitivity varies widely in the available studies and as a result there remains some uncertainty as to the true sensitivity of stool DNA testing the test. A new test that uses next generation sequencing technology has reported a higher sensitivity, but prospective studies are lacking and this test is not yet commercially available.
In addition to uncertainty about the diagnostic accuracy of the test stool DNA analysis, clinical utility of this test has not yet been demonstrated, since there is no evidence that stool DNA testing this test improves outcomes. The studies of diagnostic accuracy for detecting cancer and cancer precursors do not establish efficacy for prevention of colorectal cancer. Effective screening for colorectal cancer requires a screening program with established screening intervals and appropriate follow-up for positive tests. For fecal DNA testing, compliance with testing, i.e. successful completion and return of the test, is not known and may substantially impact outcomes. As a result, analysis of DNA in stool samples is considered **not medically necessary** as a screening technique for colorectal cancer.

### Supplemental Information

#### Practice Guidelines and Position Statements

Recommendations of specialty organizations regarding fecal DNA testing largely base their statements on the 2004 study by Imperiale et al summarized previously, which used a different test than the currently offered Colosure™ test. Current recommendations were also made prior to publication of the 2014 Imperiale et al study and the FDA approval of the Cologuard™ test.

Updated guidelines for colon cancer screening were also issued in 2008 by a group consisting of the American Cancer Society, the U.S. Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. This guideline endorses the use of fecal DNA testing as an acceptable means of colon cancer screening. However, unlike all the other recommendations in this guideline that recommended specific time intervals between tests, the recommended interval for fecal DNA testing is “uncertain.” The document notes that the manufacturer of the one commercially available test recommends a 5-year interval after an examination with normal results. Such an interval was judged by the committee to be only suitable for a test that has high sensitivity for both cancer and adenomatous polyps—a standard that has not been documented for fecal DNA to date. The evidence supporting the joint guideline consisted of the previously summarized study by Imperiale et al., and additional older studies of diagnostic performance that did not use screening populations but used previously diagnosed or advanced cancer patients.

The National Comprehensive Cancer Network (NCCN) guidelines for colorectal cancer screening note fecal DNA testing is not considered a first-line screening test but may be an option for those unwilling or unable to undergo screening colonoscopy. The NCCN guidelines also indicate more research is needed to determine the optimal interval for fecal DNA testing.

In 2012, the American College of Physicians issued a guidance statement on colorectal cancer screening. Fecal DNA testing is listed as an option for screening in the guidance statement. However, the screening interval is noted to be uncertain.

The 2008 American College of Gastroenterology guidelines on colorectal cancer screening also indicate fecal DNA testing is an alternative option for screening every 3 years. This is based on a grade 2B weak recommendation from moderate quality evidence.
U.S. Preventive Services Task Force Recommendations

The U.S. Preventive Services Task Force (USPSTF) published recommendations for colorectal cancer screening with fecal DNA testing in October 2008. The USPSTF concluded evidence is insufficient to assess the benefits and harms of fecal DNA testing as screening modalities for colorectal cancer (Grade I statement). They limited their evidence review to only 1 study, the previously summarized study by Imperiale et al. The USPSTF recommendations are in the process of being updated.

Medicare National Coverage

On October 9, 2014, a Decision Memo was issued indicating Medicare Part B will cover the Cologuard™ test once every three years for beneficiaries who meet all of the following criteria:

- Age 50 to 85 years,
- Asymptomatic (no signs or symptoms of colorectal disease including but not limited to lower gastrointestinal pain, blood in stool, positive guaiac fecal occult blood test or fecal immunochemical test), and
- At average risk of developing colorectal cancer (no personal history of adenomatous polyps, colorectal cancer, or inflammatory bowel disease, including Crohn’s Disease and ulcerative colitis; no family history of colorectal cancers or adenomatous polyps, familial adenomatous polyposis, or hereditary nonpolyposis colorectal cancer).

All other screening stool DNA tests not otherwise specified above remain nationally non-covered.

References

This policy was approved by the FEP® Pharmacy and Medical Policy Committee on March 20, 2015 and is effective April 15, 2015.

Signature on file

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