Comparative Effectiveness Review
Number 52

Effective Health Care Program

Fecal DNA Testing in Screening for Colorectal Cancer in Average-Risk Adults

Executive Summary

Background

Colorectal cancer (CRC) is the third most common cancer in both men and women and is the third leading cause of cancer deaths in the United States.[1] Incidence and mortality rates for CRC have declined over the past two decades, corresponding with an increase in self-reported screening rates.[1] However, screening rates remain suboptimal. While different U.S. guideline-issuing organizations agree on the majority of recommended CRC screening options, there are differences between some recommended options, such as fecal DNA testing. In 2008, the United States Preventive Services Task Force (USPSTF) found that evidence was insufficient to recommend fecal DNA testing for CRC screening.[2,3] However, the American Cancer Society (ACS), the U.S. Multi-Society Task Force (MSTF) on Colorectal Cancer, and the American College of Radiology (ACR) collectively recommended fecal DNA testing as an alternative screening method. The ACS-MSTF-ACR's recommendation was based on a lower threshold of evidence than that of the USPSTF.[4,5]

Fecal DNA tests are designed to detect molecular abnormalities in cells from cancer or precancerous lesions that are shed into the stool. Fecal DNA testing to screen for CRC has evolved significantly over time, both in improvements in understanding relevant molecular abnormalities associated with CRC and technological advances to allow for

Effective Health Care Program

The Effective Health Care Program was initiated in 2005 to provide valid evidence about the comparative effectiveness of different medical interventions. The object is to help consumers, health care providers, and others in making informed choices among treatment alternatives. Through its Comparative Effectiveness Reviews, the program supports systematic appraisals of existing scientific evidence regarding treatments for high-priority health conditions. It also promotes and generates new scientific evidence by identifying gaps in existing scientific evidence and supporting new research. The program puts special emphasis on translating findings into a variety of useful formats for different stakeholders, including consumers.

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improved detection of molecular abnormalities in DNA in the stool.[6] Molecular abnormalities that have served as the basis for CRC screening tests have focused on three major genetic mechanisms: chromosomal instability due to abnormalities in mutational hotspots like *APC*, *KRAS*, and *TP53*; microsatellite instability due to loss of function of mismatch repair genes that can result in accumulation of errors within the DNA sequence; and DNA methylation, an epigenetic alteration, in which promoter sites of genes are hypermethylated leading to suppression of gene transcription.[7]

Thus far a single company, Exact Sciences, has been the major commercial developer of fecal DNA testing in the United States (Table A). Currently, only one fecal DNA test, ColoSure™, is commercially available. This test is a single marker fecal DNA assay for methylated vimentin distributed by LabCorp. Marketing for commercially available fecal DNA testing specifies that the test is intended for individuals who are not eligible (either unable or unwilling) for more invasive CRC screening (i.e., colonoscopy, flexible sigmoidoscopy, or CT colonography).[8]

Objectives

This report includes six Key Questions to systematically review the evidence on fecal DNA testing to screen for CRC in average-risk adults (Figure A).

Key Question 1. Clinical Utility. What is the effectiveness of fecal DNA testing (alone or in combination with other screening tests) to screen for CRC in reducing morbidity (CRC incidence) or mortality (all-cause or CRC-specific)?

Key Question 2. Clinical Validity.

2.1. What are the absolute test-performance characteristics (e.g., sensitivity, specificity) of fecal DNA testing for CRC screening, as compared to colonoscopy?

a. To detect CRC?

b. To detect precancerous lesion(s)?

2.2. What is the relative test performance of fecal DNA testing as compared to other established screening modalities in current practice?

a. To detect CRC?

b. To detect precancerous lesion(s)?

Key Question 3. Interval of Screening. What is the test performance of fecal DNA testing across different screening interval(s)?

Key Question 4. Analytic Validity.

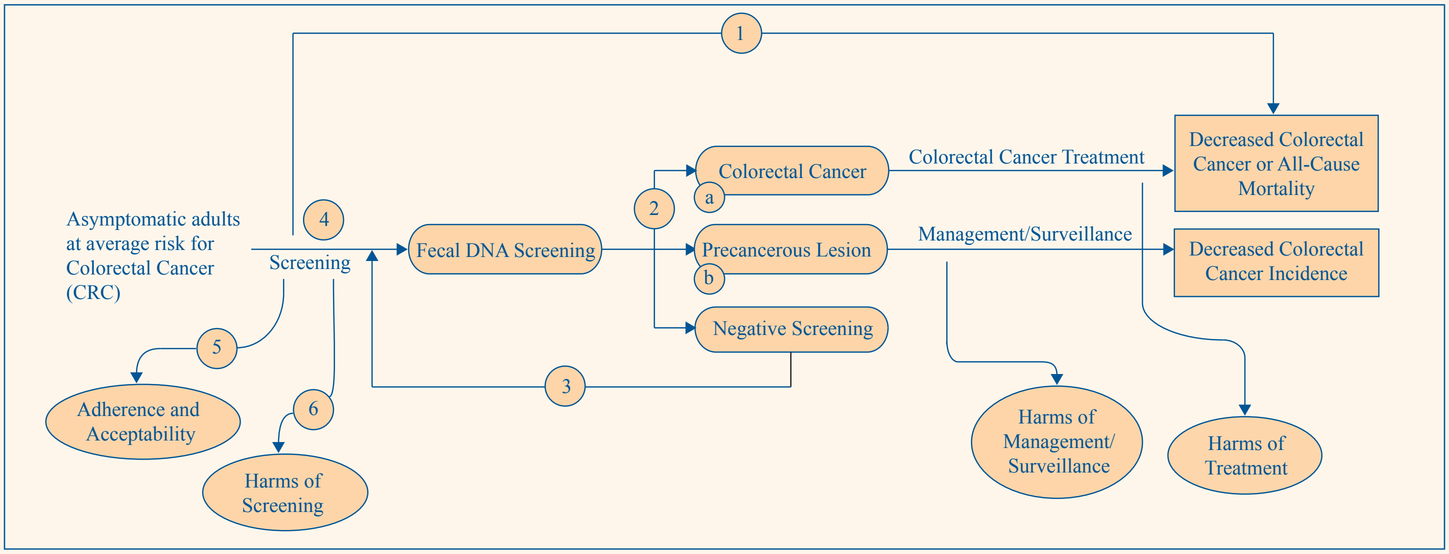
4.1. What is the analytic validity (analytic sensitivity, specificity, and reproducibility) of currently available fecal DNA assays?

4.2. What are the important analytic and pre-analytic factors that can affect fecal DNA assay validity?

Key Question 5. Acceptability of Testing. What is the acceptability and adherence of fecal DNA screening in comparison to other stool-based screening tests, or in comparison to more invasive modalities of screening?

Key Question 6. Harms. What are the potential harms of fecal DNA testing?

Figure A. Analytic framework of the benefits and harms of fecal DNA testing in screening for colorectal cancer



Note: Numbers and letters correspond to the Key Questions.

Methods

Input From Stakeholders

This topic was initiated based on a public nomination submitted to the Agency for Healthcare Research Quality Effective Health Care program. Several individuals expressed concern about the optimal timing of this review during public review due to the current development of new fecal DNA screening test. Despite these comments, it was determined that a review would still be helpful to stakeholders in the interim. A Technical Expert Panel (TEP) helped in the refinement of our review protocol and provided details about fecal DNA test development.

Data Sources and Selection

We performed comprehensive literature searches in the following databases from 2000 through August 11, 2011: MEDLINE, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Database of Abstracts of Reviews of Effects, and the Health Technology Assessments Database. Searches of these databases were supplemented with manual searching of reference lists of relevant review articles and suggestions made by TEP members. We also performed a focused search of the grey literature, including: unpublished data from recent conference abstracts (2009–2011), regulatory documents, and information regarding ongoing and future research via clinical trial registry entries. Additional unpublished literature was sought via a Scientific Information Packet (SIP) request to LabCorp.

Two reviewers independently screened abstracts against a set of a priori inclusion criteria. Included studies were limited to asymptomatic screening populations, published since 2000 in English language. Full-text articles of abstracts meeting inclusion criteria were retrieved and dual-reviewed against the inclusion criteria. Disagreements were resolved with consultation of a third reviewer.

Data Extraction and Quality Assessment

Data from all included studies were abstracted into standardized evidence tables by one reviewer and checked by a second reviewer. Separate abstraction forms were created for key questions. We abstracted important details relating to study design, population characteristics, test and comparators, and all relevant outcomes.

We applied the study design-specific quality criteria of the USPSTF to assess the methodological quality of included studies.[9] We supplemented these quality criteria with methods from the Evaluation of Genomic Applications in Practice and Prevention Working Group (specific to genetic testing),[10] the Newcastle Ottawa Scale (specific to cohort studies),[11] and the QUADAS criteria (specific to diagnostic accuracy studies).[12] Two independent reviewers assigned a quality rating of the internal validity for each study. Disagreements were resolved by discussion and consensus or by consulting a third, independent reviewer.

Data Synthesis and Analysis

We conducted qualitative syntheses of study results for each key question. We did not conduct meta-analysis of results due to the limited number of studies for each key question and clinical differences between studies. For qualitative syntheses, we evaluated and summarized clinical and methodological characteristics of included studies, as well as important internal (quality) and external (applicability) study characteristics. The strength of evidence for primary outcomes was graded using the standard process of the Evidence-based Practice Centers, based on four major domains: risk of bias, consistency, directness, and precision of the evidence.[13]

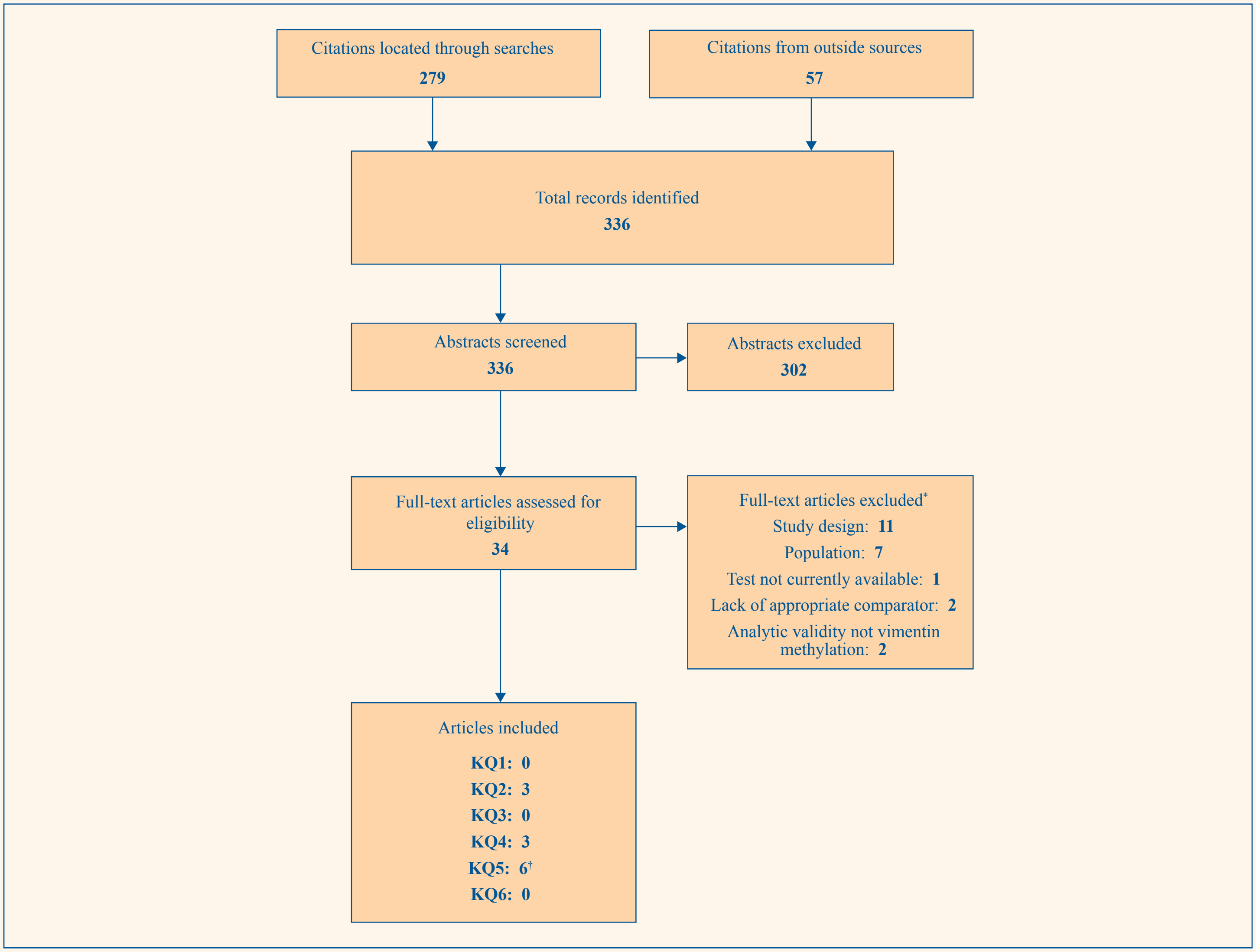
Results

Our literature search yielded 336 citations from electronic database searches and outside sources (Figure B). Based on the review of title and abstracts, we subsequently reviewed 34 full-text articles for their eligibility. We included 12 articles, three diagnostic accuracy studies (clinical validity) that met inclusion criteria for Key Question 2, three analytic validity studies for Key Question 4, and six studies of acceptability or preference of testing for Key Question 5. For Key Question 2, all three studies reported absolute test performance based on colonoscopy findings (KQ2.1), two of which also reported test performance compared to guaiac-based FOBT (KQ2.2). Two studies for Key Question 2 also reported adherence to testing and are discussed with Key Question 5 results. We found no studies that addressed clinical utility (Key Question 1), intervals of screening (Key Question 3), or specific harms of screening (Key Question 6).

Key Questions 2 and 6. Diagnostic Accuracy and Harms of Fecal DNA Testing

Despite the availability of numerous initial validation studies of fecal DNA testing, we only found three studies that examined the accuracy of fecal DNA testing in screening populations (Table B).[14-16] Two fair-quality diagnostic accuracy studies (n=5,004) in screening cohorts of average-risk patients undergoing colonoscopy evaluated a fecal DNA test (SDT-1) that was a prototype to a later

Figure B. Literature flow diagram



KQ = Key Question

[*]1 article was excluded for different reasons for different Key Questions.

[†]2 articles from KQ2 reported adherence to testing (and therefore are also discussed with KQ5).

version that was clinically available as PreGen Plus™ (Table A).[14,15] These two studies found different sensitivities for detection of CRC (25 percent [95% CI, 5 to 57] versus 51.6 percent [95% CI, 34.8 to 68.0]) (Table B). Both found similarly low sensitivities for detection of advanced adenomas (Table B).

The specificity for detection for CRC or advanced adenomas was approximately 93 to 96 percent (Table B). In one of the diagnostic accuracy studies, the specificity for the prototype to PreGen Plus (SDT-1) and Hemoccult II™ were not statistically significantly different, although the study had limited power to detect a difference (Table C).[15] One smaller study (n=441) evaluating the test accuracy of *KRAS* mutations,[16] and a subset analysis (n=217) of the diagnostic accuracy study by Ahlquist and colleagues,[14] evaluating a multi-marker test that included methylated vimentin (SDT-2), were both poor quality. None of these studies evaluated fecal DNA tests applicable to the currently available test, ColoSure.

We did not find any studies that specifically evaluated the harms of fecal DNA testing. The major hypothesized harms of fecal DNA testing are the sequelae from diagnostic inaccuracy (false positives and false negatives).

Key Question 4. Analytic Validity of Fecal DNA Testing

We found three poor-quality studies that specifically evaluated the analytic validity of currently available fecal DNA assays, a single-marker test for methylated

vimentin.[17-19] These studies showed that technological advances (i.e., methyl-BEAMing and methyl-binding domain enrichment) can improve the analytic sensitivity of assays to detect methylated vimentin in stool samples (Table D). None of the studies evaluated the repeatability, reproducibility, or analytic specificity of testing. These three studies were generally of poor quality, and the technological advances evaluated in these studies are not applicable to the previously studied (SDT-2) or currently available test (ColoSure) for methylated vimentin.

Key Question 5. Acceptability and Adherence of Testing

We found six fair- to poor-quality studies that evaluated the acceptability and two diagnostic accuracy studies that reported the adherence to fecal DNA testing.[14,15,20-25] From very limited evidence, it appears that fecal DNA testing is generally acceptable, although an important test attribute for acceptability appears to be the test's accuracy (Table E). In one fair-quality diagnostic accuracy study, fecal DNA adherence was lower than adherence to fecal occult blood test (FOBT).[15] No studies have evaluated the relative acceptability or adherence of fecal DNA tests to fecal immunochemical test (FIT) tests. It is likely that future fecal DNA testing will be in test accuracy, and possibly stool collection, such that the currently available evidence on acceptability and adherence to fecal DNA testing will no longer be relevant.

Discussion

Strength of Evidence

Despite considerable media attention and expert-based clinical recommendations that include fecal DNA testing for CRC screening, at present, fecal DNA tests have insufficient evidence about their clinical validity (diagnostic accuracy) in patients at average risk for CRC. Due to the differences in tests evaluated and differences in sensitivity between the two studies that evaluated the same test, the evidence for the test accuracy for fecal DNA testing is both inconsistent and imprecise. Fecal DNA test development has evolved significantly over the past decade. There have been advances in the understanding of molecular markers that reflect neoplastic change and advances in technologies to stabilize, extract, and amplify/detect low levels of human target DNA in stool samples. Therefore, the three studies on diagnostic accuracy of fecal DNA tests in screening populations do not reflect the current commercially available fecal DNA test (or soon to be available fecal DNA testing). Likewise, harms and acceptability of and adherence to fecal DNA testing in comparison to other screening modalities also have insufficient evidence and are largely not applicable to currently available fecal DNA tests. Because patients' (and clinicians') preference of test choice is influenced by test performance, acceptability and adherence to testing will need to be reexamined once test accuracy is known. Subtleties in stool collection may also affect acceptability and adherence, and therefore may change if future fecal DNA testing no longer requires a single whole-stool specimen.

Evidence Gaps and Future Research

The most critical evidence gap for fecal DNA testing to screen for CRC is the lack of appropriately designed diagnostic accuracy studies applicable to currently available fecal DNA testing. At a minimum, clinical decision making should be based upon evidence from test validation studies conducted in the intended population (i.e., asymptomatic screening population) for which the test is proposed. Empiric evidence shows that distorted selection of participants (including nonrepresentative patients) and use of case-control study designs overestimate overall test accuracy due to both variation and spectrum bias.[26,27] Based on this review, we found discordant results from the three included diagnostic accuracy studies in comparison to the initial validation studies identified but excluded from this review. For example, initial validation studies for the prototype of PreGen Plus had sensitivity for CRC estimates around 90 percent, and subsequent test validation studies in screening populations showed much lower sensitivities (about 25 to 50 percent).[28] When better-quality, more-applicable diagnostic accuracy studies in screening populations become available, clinicians and decision makers can use robust models that have been developed by the National Cancer Institute Cancer Intervention and Surveillance Modeling Network for evaluating CRC screening (e.g., MISCAN, SimCRC) to estimate net benefit of testing (of a program of testing, and harms of testing due to diagnostic inaccuracies) and optimal intervals of testing, compared to other currently used or promising screening modalities. Other important evidence gaps include the relative acceptability of and adherence to fecal DNA testing, compared with FIT (which is a stool based test that does not require dietary or medication restrictions), and issues around fecal DNA testing analytic validity, specifically accuracy, and repeatability and reproducibility. In addition, reporting of potentially important details that may affect analytic validity of assays should be routinely reported in clinical evaluation (clinical

validity) studies. Especially given the constant changes in test development, test developers and researchers need to be transparent and explicit about differences in the assays evaluated in studies and the actual assays that are clinically available.

Limitations

The limitations in this review are primarily from the limitations in the primary research (small body of variable, often poor quality studies) and the evolving nature of fecal DNA testing (resulting in a mismatch between primary research and available testing). However, there are few important limitations in the scope and timing of this review. Our review focused on fecal DNA testing to screen for CRC, and therefore did not address other potential roles of fecal DNA testing. Also, our review did not include stool-based testing using RNA or other genetic/genomic based testing in plasma. However, these newer types of genetic/genomic testing to screen for CRC are more developmental than fecal DNA testing. Finally, this review will likely be out of date as new tests and evidence supporting these tests becomes available within the next 2 years.

Abbreviations

95% CI	95 percent confidence interval
ACR	American College of Radiology
ACS	American Cancer Society
CLIA	Clinical Laboratory Improvement Amendments
CRC	Colorectal cancer
CT colonography	Computed tomographic colonography
DIA	DNA integrity assay
DNA	Deoxyribonucleic acid
EHC Program	Effective Health Care Program
FDA	U.S. Food and Drug Administration
FIT	Fecal immunochemical test
FOBT	Fecal occult blood test (usually used to refer to guaiac based tests like Hemoccult II™ or Hemoccult SENSA™ versus immunochemical based tests for hemoglobin)
KQ	Key Question
LDT	Laboratory-developed test
MBD	Methyl-binding domain
NR	Not reported
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
sDNA	Stool DNA test
SIP	Scientific Information Packet
TEP	Technical Expert Panel

Glossary

Absolute test performance—Performance of a test (sensitivity, specificity) when compared to the gold standard.

Accuracy—Ability of assay to measure what it purports to measure determined independently by a reference method.

Adenoma—Benign tumor from epithelial tissue.

Advanced adenomas—Adenomas 1 cm or greater, or with villous components (tubulovillous or villous), or with high-grade or severe dysplasia.

Aliquots—A measured portion of a sample taken for analysis.

Analytic factors—Test methods and performance of procedures, and monitoring and verification of accuracy and reliability of test results.

Analytic sensitivity (lower limit of detection)—Ability of assay to detect all true positive specimens, for quantitative tests this is defined as the smallest quantity of a substance that can be reliably detected or quantified.

Analytic specificity—Ability present in the sample of assay to measure the target substance when potentially interfering or cross-reacting substances are present in the sample.

Analytic validity—An assay's ability to accurately and reliably measure the genotype (or analyte) of interest.

Assay—An analysis conducted to verify the presence (and amount) of a substance.

Chromosomal instability—The gain or loss of whole chromosomes or fractions of chromosomes.

Clinical utility—A test's ability to improve clinical outcomes and the test's usefulness and value it adds to patient management decision-making, compared with current management without genetic testing.

Clinical validity—A test's ability to accurately and reliably predict the clinically defined disorder or phenotype of interest.

DNA integrity—Potential biomarker for colorectal cancer because DNA shed from cancer cells have been characterized as having longer DNA fragments as compared to DNA shed from noncancer cells.

Epigenetics—Changes in gene expression caused by mechanisms other than changes in the DNA sequence.

Guaiac based fecal occult blood test (FOBT)—An assay to detect the presence of hemoglobin in the feces that is not visibly apparent in which feces is applied to a thick piece of paper attached to a thin film coated with guaiac (a phenolic compound).

Immunochemical based fecal occult blood test (FOBT) or fecal immunochemical test (FIT)—An assay to detect the presence of hemoglobin in feces that is not visibly apparent in which a fecal sample is collected (e.g., with a brush, probe, stick) and transferred to a test card or slide (dry sampling) or deposited into a liquid buffer (wet sampling). Occult blood is then detected using an antibody specific for human hemoglobin.

Initial test validation—study designed to determine ability and diagnostic accuracy of a test in persons with the target condition (as opposed to validation in the test's intended population); for this report in persons with known CRC or colorectal adenomas; these studies are most often case-control studies in which cases are persons with known CRC or colorectal cancer versus healthy controls.

Methylation—The addition of a methyl group.

Microsatellite instability—DNA damage due to defects in the normal DNA repair process.

Pre-analytic factors—factors that may affect test performance prior to analysis specimen collection, processing, handling, and delivery to testing site.

Relative test performance—Diagnostic accuracy (sensitivity, specificity) when compared to another test that is not the gold standard.

Repeatability—Replication of results when the assay is performed multiple times on a single specimen.

Transcription—the copying of DNA into mRNA in gene expression.

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Table A. Development of fecal DNA testing for colorectal cancer screening

Test Details	Prototype sDNA Version 1.0	PreGen Plus™ sDNA Version 1.1	sDNA Version 2.0	sDNA Version 2.1	ColoSure™ sDNA Version 2.2	Next-Generation Version 3.0†
Market availability	Not implemented for clinical use	2003-2008 as a CLIA regulated LDT	Not implemented for clinical use	Not implemented for clinical use	2008-present as a CLIA regulated LDT	Not available‡
Genetic markers*	21 point mutations in *APC*, *KRAS*, and *TP53* One microsatellite instability marker, *BAT-26* One long DNA marker, DNA Integrity Assay (DIA)	Same 23 molecular markers as prototype (sDNA 1.0)	Vimentin methylation Point mutations in *APC* and *KRAS*	Vimentin methylation DIA	Vimentin methylation	*NDRG4* and *BMP3* methylation 7 point mutations *KRAS* exon 2 Also includes Fecal Immunochemical Test (FIT)
Evidence: Test development and/or initial validation	Ahlquist, 2000[29] Tagore, 2003[30] Calistri, 2003[31] Brand, 2004[32] Syngal, 2006[33]	Whitney 2004[34] Olson 2005[35]	Itzkowitz, 2007[20]	Itzkowitz, 2008[36]	Chen, 2005[19] Itzkowitz, 2007[20§] Itzkowitz, 2008[36§] Baek, 2009[37§] Li, 2009[17] Zou, 2010[38]	Expected 2011-2012
Evidence: Test validation in target population	Imperiale, 2004[15] Ahlquist, 2008[14]		Ahlquist, 2008[14]			Expected 2013

sDNA = stool DNA; CLIA = Clinical Laboratory Improvement Amendments; LDT = laboratory-developed test; DIA = DNA integrity assay; FIT = fecal immunochemical test

*Full information on genes can be found at http://www.ncbi.nlm.nih.gov/gene.

†Exact Sciences. Second-Quarter 2011 Earnings Call; 2011 August 2. Madison, WI: Exact Sciences Corporation; 2011.

‡FDA submission for premarket approval or clearance planned for late 2012.

§Studies addressed multiple markers but included data on vimentin as an individual marker.

Table B. Diagnostic accuracy of fecal DNA testing in screening populations (KQ2)

Author, Year	CRC Prevalence	Test	Test Positivity	Completion Rate	Type of Lesion Detected	Sensitivity (95% CI)	Specificity (95% CI)
Ahlquist, 2008[14]	0.5% (19/3,764)	SDT-1 (prototype sDNA version 1.0)	5.2% (129/2,497)	98.2% (3,766/3,834)	CRC	25% (5-57%)	95% (94-96%)
					Advanced adenomas	19% (5-42%)	Not applicable
					CRC + advanced adenomas	20% (14-26%)	96% (95-97%)
		SDT-2 (sDNA version 2.0)	35% (77/217)	98.2% (3,766/3,834)	CRC	58% (36-80%)*	NR
					Advanced adenomas	39% (26-52%)*	NR
					CRC + advanced adenomas	40% (32-49%)	NR
Haug, 2007[16]	1.6% (NR)	*KRAS* testing	8% (70/875)	NR	CRC	0% (NR)	NR
					Advanced adenomas	0% (NR)	NR
Imperiale, 2004[15]	0.7% (31/4,404)	SDT-1 (prototype sDNA version 1.0)	8.2% (205/2,505)	88.3% (4,845/5,486)	CRC	51.6% (34.8 to 68.0%)	92.8% (92.0-93.5%)*
					Advanced adenomas	15.1% (12.0 to 19.0%)	Not calculated
					CRC + advanced adenomas	17.7% (NR)	93.6% (92.9-94.3%)*
		Hemoccult II™	5.8% (146/2,505)	92.2% (5,060/5,486)	CRC	12.9% (5.1 to 28.9%)	94.6% (94.0-95.3%)*
					Advanced adenomas	10.7% (8.0 to 14.1%)	Not calculated
					CRC + advanced adenomas	10.8% (NR)	95.2% (94.6-95.8%)*

CRC = colorectal cancer; NR = not reported (and unable to calculate); SDT-1 = sDNA version 1.0; SDT-2 = sDNA version 2.0

*Weighted sensitivities and CI calculated.

Reference standard: colonoscopy.

Table C. Limitations and quality concerns for diagnostic accuracy studies of fecal DNA testing

Author, Year	Quality Rating	Quality Concerns	Applicability Concerns
Ahlquist, 2008[14]	SDT-1: Fair SDT-2: Poor FOBT: Poor	Small sample size for SDT-2 with limited sampling of controls, authors tried to weight sensitivity for proportion of screen relevant neoplasia in the entire population, but did not presented weighted adjustment for all outcomes. Poor precision around outcome measures. Subset of patients did not get instructions on dietary restrictions required for FOBT, very low sensitivities reported for FOBT which are not consistent with best known estimates.	Mostly White patient population (in comparison to general U.S. population). Neither SDT-1 or SDT-2 were ever available for clinical use and both are very different tests compared to currently available (and soon to be available) testing.
Haug, 2007[16]	Poor	Application of reference standard was opportunistic (patient who got colonoscopy were referred for colonoscopy). Average time between index and reference tests not presented, patients had to have colonoscopy within 2 years.	Unclear how patient selection was performed, n eligible not reported. Higher CRC prevalence in patients analyzed, higher percent of patients with first degree relative with CRC in n analyzed than full study population.
Imperiale, 2004[15]	Fair	Analysis focused on subset of patients, only basic demographic data presented detailing differences between full cohort and analyzed subset. Poor precision around outcome measures. Very low sensitivities reported for FOBT which are not consistent with best known estimates.	Exclusion of 20% of enrolled study population due to incomplete testing, characteristics for excluded persons not reported, n eligible not reported. Persons 65 years of age and over were disproportionately represented in the study population. Test evaluated was never available for clinical use and is a very different test compared to currently available (and soon to be available) testing.

CRC = colorectal cancer; FOBT = fecal occult blood test; SDT = sDNA version 1.0; SDT-2 = sDNA version 2.0

Table D. Analytic validity of fecal DNA testing

Author, Year	Experimental Aim	Outcomes	Quality Concerns	Applicability Concerns
Li, 2009[17]	To test methyl-BEAMing in the detection of methylated vimentin DNA in plasma and stool from CRC patients	Lower limit of detection: 0.1% (1/1,000 copies) methylated DNA detected using methyl-BEAMing versus no detection <6.2% without methyl-BEAMing. Accuracy (compared to next-generation sequencing): enumeration of methylation by methyl-BEAMing (0.018%) and reference standard (0.015%) in cancer cell lines; enumeration of methylation by methyl-BEAMing (10.8%) and reference standard (11.35%) in stool sample ("substantiated in 3 other samples").	Poor: Small sample size (n=1 series of dilution) and poor reporting, unclear if experiments were repeated and results replicated. Poor: Small sample sizes, unclear if experiment in cancer cell lines repeated and results replicated; experiment in stool samples (n=5), results only appear to be reported for 4 of 5 samples.	Mostly performed in plasma samples not stool samples. Methyl-BEAMing method does not appear to be used in assay studied (KQ2) or currently available testing.
Zou, 2007[18]	To test whether method using methyl-binding domain (MBD) could increase assay sensitivity for detecting methylated markers in stool	Lower limit of detection (in stool with cell line DNA added): methylated vimentin was detectable in stool aliquots to which 10 and 50 ng cancer cell line DNA, but not those with 0 and 2 ng using MBD enrichment; versus not detectable in any stool aliquot without MBD enrichment. Lower limit of detection (in stool from CRC patients): methylated vimentin was detected in 4 CRC stool samples (4-832 ng human DNA), but not detected in the other 4 samples (0.5-10 ng human DNA) using MBD enrichment; versus only 1 CRC stool sample (832 ng human DNA) without MBD enrichment.	Poor: Small sample size (n=1 series of dilution), unclear if experiments were repeated and results replicated. Poor: Small sample size (n = 8).	Unknown if MBD column is used in assay studied (KQ2) or currently available testing.
Chen, 2005[19]	To test the technical limits to the sensitivity of assay of methylated vimentin	Lower limit of detection (in normal mucosa with cell line DNA added): PCR could detect as little as 25-50 pg of methylated DNA in the presence of a 500-to 1,000-fold excess of normal mucosal DNA.	Poor: Small sample size (n=1 series of dilution), unclear if experiments were repeated and results replicated.	Not conducted in stool samples.

CRC = colorectal cancer; MBD = methyl-binding domain; PCR = polymerase chain reaction

Table E. Patient preferences and acceptability of fecal DNA testing

Author, Year	Study Aim	Study Design N Participants	Outcomes	Quality Concerns	Applicability Concerns
Marshall, 2009[25]	To compare patient and physician preferences about CRC screening tests	Cross-sectional survey N = 1,588 patients N = 200 physicians	Patients' test preferences: non-invasive, do not require repeated measurements over time, no pain, no preparation, no complications, and high accuracy. Physicians' test preferences: change in sensitivity from 40 to 90%, pain, process, specificity, complication risk, preparation, and testing frequency.	Fair: response rate not reported.	Financial compensation given for survey; FITs were not included as a screening option.
		Model	Patients' preferred tests: fecal DNA, colonoscopy and CT colonography. Physicians' prediction of patient's preferred tests: colonoscopy, CT colonography, and fecal DNA.	Poor: lack of reporting about model inputs, lack of inclusion of all relevant testing (i.e., FIT), no sensitivity analyses around important model inputs.	
Marshall, 2007[24]	To assess patient preferences about CRC screening tests	Cross-sectional survey N = 547	Patients' test preferences: non-invasive, no preparation, no pain, and high accuracy.	Fair: 52% response rate.	Canadian participants age 40-60 years old; CT colonography option is without bowel preparation (bowel prep is part of protocol in U.S. based practice); FITs were not included as a screening option.
		Model	Relative importance of test preferences (most to least important): sensitivity, specificity, preparation, process, pain. Preferred tests (most to least preferred): CT colonography, colonoscopy, double contrast barium enema, flexible sigmoidoscopy, fecal DNA, FOBT.	Poor: lack of reporting about model inputs, incorrect model inputs (CT colonography without bowel preparation), lack of inclusion of all relevant testing (i.e., FIT), no sensitivity analyses around important model inputs.	
Itzkowitz 2007[20]	To determine the sensitivity and specificity of SDT-2 (also collected patient satisfaction)	Cross-sectional survey of participants in diagnostic accuracy study N = 162	Most patients found it easy to perform the test and would repeat the test if recommended by their doctor.	Poor: not primary aim of study, no response rate reported, no details about questionnaire (items assessed), limited reporting of results.	Participants likely knew their diagnosis (if they had CRC or not) at the time of fecal DNA testing and responding to questionnaire.

Table E. Patient preferences and acceptability of fecal DNA testing (continued)

Author, Year	Study Aim	Study Design N Participants	Outcomes	Quality Concerns	Applicability Concerns
Schroy, 2007[23]	To assess patient preferences about CRC screening tests	Cross-sectional survey N = 263	Test preferences (most to least important): accuracy, frequency, discomfort, time, complications, preparation, need for follow-up testing. Preferred tests (most to least preferred): colonoscopy, fecal DNA, FOBT, FOBT plus flexible sigmoidoscopy, flexible sigmoidoscopy, double contrast barium enema.	Poor: response rate not reported; participants provided with incorrect (overestimated) information on fecal DNA test accuracy during educational counseling; willingness to pay outcome assessed, but cost of tests were not provided to participants during educational counseling.	Participants were given financial compensation, FIT (and CT colonography) were not included as screening options.
Berger, 2006[22]	To assess patients' screening experience with fecal DNA testing	Convenience survey N = 1,211	Most of the survey respondents found fecal DNA testing easy to perform sample collection, obtain collection materials, and return specimen.	Poor: 18% response rate, no relative outcomes in comparison to other screening tests.	Participants all ordered fecal DNA testing kit (within first 2 years it was commercially available), 73% of respondents were less than 65 years.
Schroy, 2005[21]	To compare patients' perceptions of fecal DNA, FOBT, colonoscopy	Cross-sectional survey of participants in diagnostic accuracy study N= 4,042	Test preferences: colonoscopy was perceived more accurate than stool based tests but less favorable in terms of invasiveness, anxiety (around preparation and test), likeliness to repeat test; very small but statistically significant differences between fecal DNA and FOBT. Preferred tests (most to least preferred): fecal DNA (45%), FOBT (32%), colonoscopy (15%), no preference (8%), $p<0.001$.	Fair: 84% response rate, conclusions drawn on statistical significance (unclear clinical significance).	Participants in diagnostic accuracy study had to be adherent to testing and were given financial compensation; only FOBT and colonoscopy were evaluated as screening options.

CRC = colorectal cancer; CT colonography = computed tomography colonography; FIT = fecal immunochemical test; FOBT = fecal occult blood test; SDT-2 = sDNA version 2.0

Full Report

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