Effective Health Care Program

Comparative Effectiveness Review
Number 52

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

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

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Preface

The Agency for Healthcare Research and Quality (AHRQ) conducts the Effective Health Care Program as part of its mission to organize knowledge and make it available to inform decisions about health care. As part of the Medicare Prescription Drug, Improvement, and Modernization Act of 2003, Congress directed AHRQ to conduct and support research on the comparative outcomes, clinical effectiveness, and appropriateness of pharmaceuticals, devices, and health care services to meet the needs of Medicare, Medicaid, and the Children's Health Insurance Program (CHIP).

AHRQ has an established network of Evidence-based Practice Centers (EPCs) that produce Evidence Reports/Technology Assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care. The EPCs now lend their expertise to the Effective Health Care Program by conducting comparative effectiveness reviews (CERs) of medications, devices, and other relevant interventions, including strategies for how these items and services can best be organized, managed, and delivered.

Systematic reviews are the building blocks underlying evidence-based practice; they focus attention on the strength and limits of evidence from research studies about the effectiveness and safety of a clinical intervention. In the context of developing recommendations for practice, systematic reviews are useful because they define the strengths and limits of the evidence, clarifying whether assertions about the value of the intervention are based on strong evidence from clinical studies. For more information about systematic reviews, see

http://www.effectivehealthcare.ahrq.gov/reference/purpose.cfm

AHRQ expects that CERs will be helpful to health plans, providers, purchasers, government programs, and the health care system as a whole. In addition, AHRQ is committed to presenting information in different formats so that consumers who make decisions about their own and their family's health can benefit from the evidence.

Transparency and stakeholder input from are essential to the Effective Health Care Program. Please visit the Web site (http://www.effectivehealthcare.ahrq.gov) to see draft research questions and reports or to join an e-mail list to learn about new program products and opportunities for input. Comparative Effectiveness Reviews will be updated regularly.

We welcome comments on this CER. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by email to epc@ahrq.hhs.gov.

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Structured Abstract

Objectives. To review the evidence on fecal DNA testing to screen for colorectal cancer in adults at average risk for colorectal cancer.

Data sources. We searched 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 from 2000 through August 11, 2011 and grey literature including recent conference abstracts, regulatory documents, unpublished information from the manufacturer, and expert suggestions.

Review methods. Two investigators independently reviewed all abstracts and full-text articles against a set of *a priori* inclusion criteria and assessed the quality of included articles using established criteria. Disagreements were resolved through consultation of a third investigator. We evaluated and summarized clinical and methodological characteristics and internal and external validity of studies. Finally, we assessed the overall strength of evidence for each outcome based on risk of bias, consistency, directness, and precision of the evidence.

Results. Despite the availability of numerous excluded initial validation studies of fecal DNA testing, we found only three studies that examined the test accuracy of fecal DNA testing in screening populations. Initial validation studies were excluded due to their use of highly selected patient populations. Two fair-quality diagnostic accuracy studies (n=5004) evaluating a multi-marker fecal DNA found differing sensitivities to detect CRC (25 percent [95% CI, 5 to 57 percent] versus 51.6 percent, [95% CI, 34.8 to 68.0]). Sensitivity for advanced adenomas was similarly low in both studies. Another small study and a subset analysis of one of the larger studies were both poor quality and evaluated different tests. We found no studies that specifically evaluated the harms of fecal DNA testing. While three poor-quality analytic validity studies showed that technological advances can improve the analytic sensitivity of assays, it is unclear if these advances are applicable to the currently available test. Six fair-to poor-quality studies that evaluated acceptability found that fecal DNA testing is generally acceptable, although an important test attribute for acceptability appears to be the test's accuracy (which is yet unknown). No studies have evaluated the relative acceptability of fecal DNA tests to FIT tests.

Conclusions. Fecal DNA tests have insufficient evidence about its diagnostic accuracy to screen for colorectal cancer in asymptomatic, average-risk patients. There is also insufficient evidence for the harms, analytic validity, and acceptability of testing in comparison to other screening modalities. Existing evidence has little or no applicability to currently available fecal DNA testing.

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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 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 . ey 4 uestions 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?
   D. To detect CRC?
   E. 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.

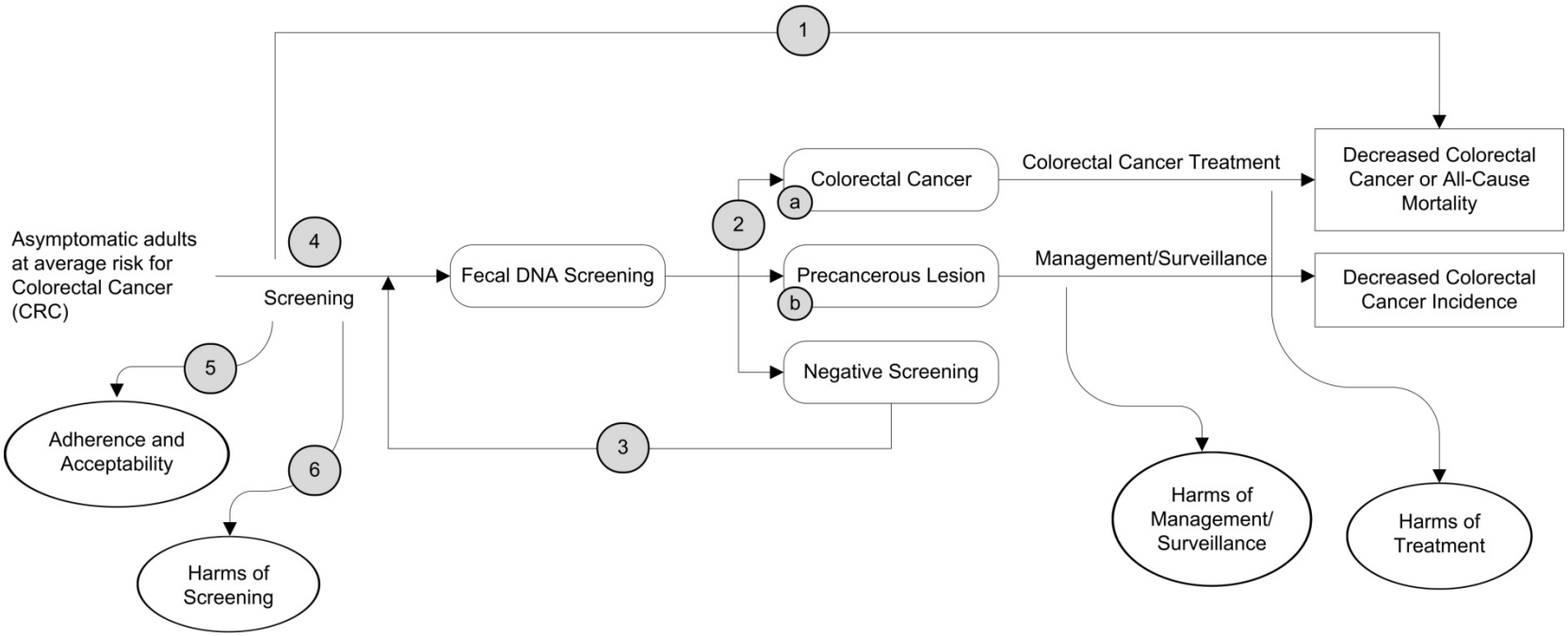
What is the acceptability and adherence of fecal DNA screening in comparison to other stool-based screening tests, or in comparison to more invasive modalities of screening?

Key Question 6. Harms.

What are the potential harms of fecal DNA testing?

Analytic Framework

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.

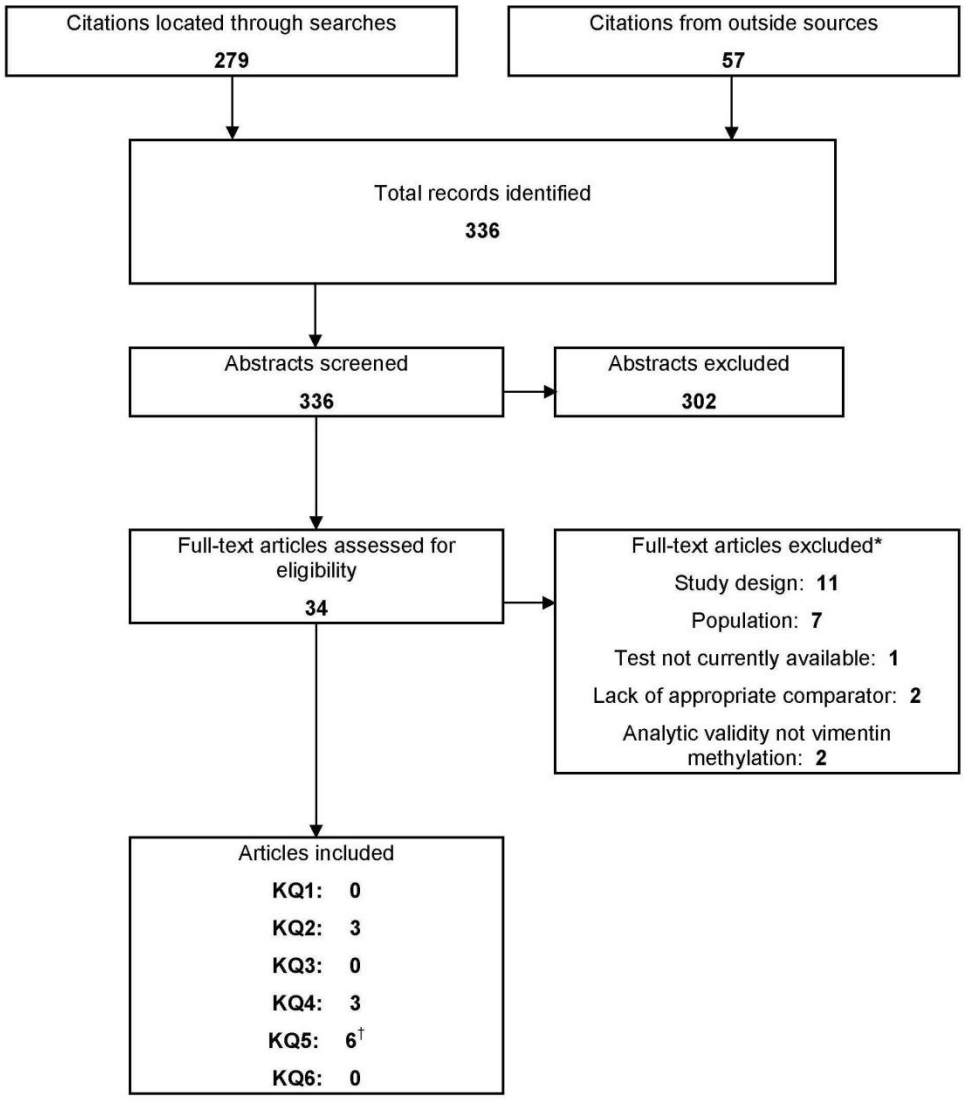
Data Synthesis and Analysis

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).

Figure B. Literature flow diagram



*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)

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=5004) in screening cohorts of average-risk patients undergoing colonoscopy evaluated a fecal DNA test (SDT-1) that was a prototype to a later 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 immunochemica 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 sDNA 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

Stool DNA (sDNA); Clinical Laboratory Improvement Amendments (CLIA); Laboratory-developed test (LDT); DNA integrity assay (DIA); Fecal immunochemical test (FIT)
*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, 200814	0.5% (19/3764)	SDT-1 (prototype sDNA version 1.0)	5.2% (129/2497)	98.2% (3766/3834)	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% (3766/3834)	CRC	58% (36-80%)*	NR
					Advanced adenomas	39% (26-52%)*	NR
					CRC + advanced adenomas	40% (32-49%)	NR
Haug, 200716	1.6% (NR)	KRAS testing	8% (70/875)	NR	CRC	0% (NR)	NR
					Advanced adenomas	0% (NR)	NR
Imperiale, 200415	0.7% (31/4404)	SDT-1 (prototype sDNA version 1.0)	8.2% (205/2505)	88.3% (4845/5486)	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 IITM	5.8% (146/2505)	92.2% (5060/5486)	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, 200814	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, 200716	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, 200415	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-1: sDNA version 1.0; SDT-2: sDNA version 2.0

Table D. Analytic validity of fecal DNA testing

Author, year	Experiment aim	Outcomes	Quality concerns	Applicability concerns
Li, 200917	To test methyl-BEAMing in the detection of methylated vimentin DNA in plasma and stool from CRC patients	Lower limit of detection: 0.1% (1/1000 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, 200718	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, 200519	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 1000-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, 200925	To compare patient and physician preferences about CRC screening tests	Cross-sectional survey N=1588 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, 200724	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 200720	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, 200723	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, 200622	To assess patients' screening experience with fecal DNA testing	Convenience survey N= 1211	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, 200521	To compare patients' perceptions of fecal DNA, FOBT, colonoscopy	Cross-sectional survey of participants in diagnostic accuracy study N= 4042	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

Introduction

Background

Prevalence and Disease Burden

Colorectal cancer (CRC) is the third most common cancer in men and women, with more than 141,000 new cases expected in the United States (US) in 2011. Approximately 50,000 deaths from CRC are expected to occur in 2011, making CRC the third leading cause of cancer deaths in the United States.[1] Survival largely depends on tumor stage at the time of diagnosis. Patients with localized disease at diagnosis have a 5-year survival rate of 90 percent. However, 5-year survival drops to 69 percent for those diagnosed with regionalized disease (cancer spread to regional lymph nodes) and to 12 percent for those with distantly metastasized disease.[2] Incidence and mortality are 35 to 40 percent higher in men than women, and are highest in African American men and women, who have 20 percent greater incidence and 45 percent greater mortality than White patients.[1]

Incidence and mortality rates for CRC have declined over the past two decades.[3,4] This decrease has been partially attributed to the use of CRC screening tests that allow for early detection and treatment of cancer or precancerous colorectal polyps. Individuals at increased risk for developing CRC include those over 50 years of age and those with a history of inflammatory bowel disease, family history of the disease, or inherited familial syndromes such as familial adenomatous polyposis or hereditary non-polyposis CRC.[1] Lifestyle factors have also been linked to an individual's risk of developing CRC, including a diet high in red or processed meats, lack of exercise, smoking, heavy alcohol use, being overweight, and having type 2 diabetes.[1]

Adenoma to Colorectal Cancer Progression

CRC usually develops over a period of 10 to 15 years with the cancer beginning as a precancerous lesion, most commonly a neoplastic polyp, although flat adenomas are increasingly recognized as an important precursor for CRC.[5,6] While neoplastic or adenomatous polyps can develop into cancers, fewer than 10 percent will eventually progress to cancer.[1] In general, larger adenomas and those with greater dysplasia are more likely to progress to cancer.[7] Advanced adenomas is a composite term used to describe precancerous lesions most likely to progress into cancer. Although there is some variation in the exact definition, advanced adenomas generally refer to adenomas 1 cm or greater, with villous components (tubulovillous or villous), or with high-grade or severe dysplasia.

Molecular events are involved in the initiation, promotion, and progression of CRC on many levels, including interactions between the patient's inherited (germ-line) genome and the tumor (somatic) genome.[8] Progressive genomic instability in colorectal tissues gives rise to cancer due to accumulating genetic alterations (including gene mutations and amplifications) and epigenetic alterations (including aberrant DNA methylation leading to gene inactivation) that transform healthy cells into carcinoma cells.[7] On the molecular level, there is a progression of specific genetic or epigenetic changes that lead to altered functions of proto-oncogenes and tumor suppressor genes that accompany cancer's progression from adenomatous polyp to invasive

cancer.[8] These genetic and epigenetic changes are the basis for the role of fecal DNA testing in the early detection of CRC.

Screening of Colorectal Cancer

Rationale and current practice

Multiple tests are clinically used to screen for CRC, these include stool based tests (e.g., guaiac based or immunochemical based fecal occult blood testing), endoscopy (e.g., flexible sigmoidoscopy or colonoscopy), and imaging tests (e.g., double contrast barium enema or CT colonography). The decrease in CRC incidence and mortality over the past two decades in the United States corresponds to an increase in self-reported screening rates from less than 25 percent in the 1980s to about 52 percent in 2002 and about 65 percent in 2010.[1,3] Despite increases in CRC screening over time, screening rates remain below optimal. Multiple patient, clinician, and health-care delivery factors have been found to negatively influence CRC screening, including low socioeconomic or educational status, lack of physician recommendation, and lack of insurance or limited access to health care.[9]

Most organizations agree that any CRC screening is better than no screening, and that the age to begin screening in adults at average-risk for CRC is 50 years old. Currently, most U.S. guideline organizations, including the U.S. Preventive Services Task Force (USPSTF), agree that the recommended options in screening for CRC include: colonoscopy every 10 years; annual high-sensitivity guaiac fecal occult blood testing (FOBT) or fecal immunochemical testing (FIT); and flexible sigmoidoscopy every 5 years with or without fecal blood testing (FOBT or FIT).[10,11]

Some disagreement occurs between guideline organizations about screening interventions with less evidence to support their use. These tests include: computerized tomography (CT) colonography, double contrast barium enema (DCBE), and fecal or stool-based DNA testing.[10] In 2008, the USPSTF found that evidence was insufficient to recommend fecal DNA testing for CRC screening based on a systematic review of new and established CRC screening modalities.[5,11] 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. This discrepancy between recommendations appears to be due to differences in evidence considered. The ACS-MSTF-ACR recommendation was based a lower threshold of evidence than that of the USPSTF.[12] While the American College of Gastroenterology recognized that fecal DNA testing may offer an alternative form of CRC screening, they state that the preferred forms of screening include colonoscopy and FIT, noting the very limited evidence for fecal DNA testing.[13]

Evolution of fecal DNA testing for colorectal cancer screening

Unlike other stool-based screening tests that are designed to detect hemoglobin, fecal DNA tests are designed to detect molecular abnormalities in cancer or precancerous lesions that are shed into the stool. Molecular abnormalities in CRC that have served as the basis for screening tests have focused on three major genetic mechanisms: chromosomal instability due to abnormalities in mutational hotspots like *APC*, *KRAS*, and *TP53*; microsatellite instability (MSI) due to loss of function of mismatch repair genes that can result in accumulation of errors within the DNA sequence called microsatellites; and DNA methylation, an epigenetic alteration, in which promoter sites of genes are hypermethylated leading to suppression of gene transcription.[14] Although the presence of these alterations does not guarantee a progression to

cancer, it is thought that these molecular markers can identify the adenomas most likely to develop into cancer, in addition to detecting early stages of CRC.[7]

Fecal DNA testing to screen for CRC has evolved over time. Some of the most common (and well studied) DNA markers in stool include mutations in *APC, KRAS*, and *TP53;* methylation of vimentin, *SFRP2, MGMT*, *MLH1;* and measurements of long-DNA integrity and microsatellite instability.[15] The feasibility of stool DNA testing was originally demonstrated using a single marker assay for *KRAS* in the early 1990s. Later work in 2000 led to the development of a panel of markers intended to detect both advanced adenomas and colorectal tumors. Since that time several configurations of multi-marker stool tests have been evaluated. Earlier studies focused on the Wnt signaling pathway and microsatellite instability, but these initial studies proved less successful than anticipated. More recently, studies have incorporated the detection of methylated markers, in recognition that gene hypermethylation is a more common pathway in CRC than previously understood.[14] Based on our audit of initial validation studies, the most commonly evaluated methylated markers appear to be vimentin and *SFRP2*. Other potentially useful methylated gene targets for fecal DNA testing include *TFPI2* and *NDRG4*, which are both tumor suppressor genes.[16] Due to the molecular heterogeneity of CRC, potential screening tests have generally considered a panel of markers rather than a single marker in an attempt to maximize clinical sensitivity.[17] Industry now believes that they have designed panels of markers that cover 100 percent of the target lesions at the tissue level, as compared to only 67 percent of screen-relevant neoplasms when the tissue samples were examined with the first generation of available testing.[18]

In addition to improvements in relevant marker identification, there have also been significant technological advances in the past several years to allow for improved detection of molecular abnormalities in DNA.[16] However, only 0.01 percent of DNA in the feces is of human origin; most fecal DNA is acquired through outside sources including diet and microflora. Therefore, isolating and detecting target human DNA from the stool presents a challenge for fecal DNA test methodologies.[19] Assay development has had to focus on improving the analytic sensitivity (or lower limit of detection) of test methodology and technology. Techniques have been developed to better preserve stool DNA (e.g., buffer to stabilize DNA) and extract DNA from stool. In addition, techniques to enrich target DNA by selective capture from stool followed by digital or emulsion PCR have been developed (e.g., BEAMing and digital melt curve analysis) and seem promising in improving assay sensitivity.[16,18]

Thus far a single company, Exact Sciences, has been the major developer and licensor of fecal DNA testing in the United States. To date, only two fecal DNA tests have been commercially available. PreGen Plus™ was based on the first developed prototype (by Exact Sciences) and was available from 2003 to 2008 from LabCorp. Most recently, Exact Science has licensed technology to LabCorp, who manufactures and markets ColoSure™, the only commercially available test in the United States (Table 1a and b). The prototype and the previously available test PreGen Plus included the same 23 molecular markers, whereas the newer versions only include methylation of vimentin plus or minus an assay for DNA integrity. Thus, the currently available test ColoSure shares no markers with the previously available test. In addition to this evolution in the composition of the test there have also been advances in pre-analytic and analytic technologies. Pre-analytic factors include specimen collection, processing, handling, and delivery to testing site. Analytic factors include test methods and performance of procedures, and monitoring and verification of accuracy and reliability of test results. The major pre-analytic advance was the addition of DNA stabilization buffer at the time of stool collection,

which prevents the degradation of DNA while the stool is in transport and storage. The major analytic advances included the use of technologies to isolate human DNA targets that improve the analytic sensitivity or lower limit of detection of these assays.

Table 1a. Development of fecal DNA testing for colorectal cancer screening: Market availability and test details

Market Availability and 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 sDNA version 3.0†
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
Non-genetic measures	None	None	None	None	None	Fecal Immunochemical Test (FIT)
Advances in pre-analytic factors	Specimen homogenization for target uniformity in aliquots	Stabilizing buffer at time of stool collection	Stabilizing buffer at time of stool collection	Stabilizing buffer at time of stool collection	Stabilizing buffer at time of stool collection	Stabilizing buffer at time of stool collection
Advances in analytic factors	Bead based human DNA target hybrid capture from resolubilized, precipitated total DNA	Gel-capture method for isolating DNA targets from resolublized, precipitated total DNA	Bead based human DNA target hybrid capture from resolublized, precipitated total DNA	Bead based human DNA target hybrid capture from resolublized, precipitated total DNA	Bead based human DNA target hybrid capture from resolublized, precipitated total DNA	Direct bead based DNA target hybrid capture from stool homogenate supernatant QuARTS™ target and signal amplification method Multiplexed target assays Automated analytic steps, high through-put

Stool DNA=sDNA; Clinical Laboratory Improvement Amendments=CLIA; Laboratory-developed test= LDT; DNA integrity assay=DIA; Fecal immunochemical test =FIT

*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 pre-market approval or clearance planned for late 2012

Table 1b. Development of fecal DNA testing for colorectal cancer screening: Evidence

Evidence	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 sDNA version 3.0†
Test development and/or initial validation	Ahlquist 2000[20] Tagore 2003[21] Calistri 2003[22] Brand 2004[23] Syngal 2006[24]	Whitney 2004[25] Olson 2005[26]	Itzkowitz 2007[27]	Itzkowitz 2008[28]	Chen 2005[29] Itzkowitz 2007[27]§ Itzkowitz 2008[28]§ Baek 2009[30]§ Li 2009[31] Zou 2010[32]	Expected 2011-2012
Test validation in target population	Imperiale 2004[33] Ahlquist 2008[34]		Ahlquist 2008[34]			Expected 2013

Stool DNA =sDNA; Clinical Laboratory Improvement Amendments= CLIA; Laboratory-developed test=LDT; DNA integrity assay=DIA; Fecal immunochemical test =FIT

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

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

Only one fecal DNA test for the detection of adenomas and colorectal tumors is currently commercially available. This test, ColoSure is provided by LabCorp as laboratory-developed test (LDT) regulated by the Centers for Medicare and Medicaid Services under the Clinical Laboratory Improvement Amendments (CLIA) of 1988. 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).[35] ColoSure has not obtained clearance or approval from the U.S. Food and Drug Administration (FDA). Currently, there are no fecal DNA tests approved by the FDA for screening or diagnosing of CRC. The FDA has chosen to exercise "enforcement discretion" on LDT testing and historically, the FDA's oversight of genetic testing has been focused on commercial test kits. The FDA, however, is now engaged in dialogue with manufacturers and the public on how it should develop a consistent, reasonable, and fair approach for LDTs so as to ensure safety and promote innovation.[36]

A new fecal DNA test developed by Exact Sciences is projected to be available in 2012 (Table 1a and b). This test will include a combination of markers (methylation of *NDRG4*, *BMP3*; 7 point mutations in *KRAS* exon 2) and an immunochemical assay for hemoglobin.[37] The fecal immunochemical test (FIT), while optimized for this new assay, is similar to other currently available ELISA-based FITs.

Changes in markers and assay technology are extremely important to understand when evaluating the evidence base for fecal DNA testing because over time assays have included different markers and used different technologies, limiting the applicability of the existing evidence base to currently (and soon to be) available testing.

Scope and Purpose

This topic was nominated to the Agency for Healthcare Research and Quality for its Effective Healthcare (EHC) Program by an organization interested in using a review to develop an evidence-based recommendation statement. This review aims to synthesize the evidence on fecal DNA testing to screen for CRC in average-risk adults.

Key Questions

Six systematically reviewed questions are addressed in this report. These questions address the clinical utility (evidence for impact on patient health outcomes), clinical validity (diagnostic accuracy for CRC or precancerous lesions), analytic validity, acceptability (and adherence), and harms of fecal DNA testing to screen for CRC.

Question 1: Clinical utility

What is the effectiveness of fecal DNA testing (alone or in combination with other screening tests) to screen for colorectal cancer in reducing *morbidity* (colorectal cancer incidence) or *mortality* (all-cause or CRC-specific)?

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)?

Question 3: Interval of Screening

What is the test performance of fecal DNA testing across different screening interval(s)?

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?

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?

Question 6: Harms

What are the potential harms of fecal DNA testing?

Methods

The Agency for Healthcare Research and Quality (AHRQ) requested a comparative effectiveness review on the use of fecal DNA analysis in screening for CRC as part of its Effective Health Care (EHC) program. The Oregon Evidence-based Practice Center (EPC) was assigned this review and subsequently established a review team, developed a review protocol, conducted the review, and drafted a report summarizing the review's findings.

Topic Development and Refinement

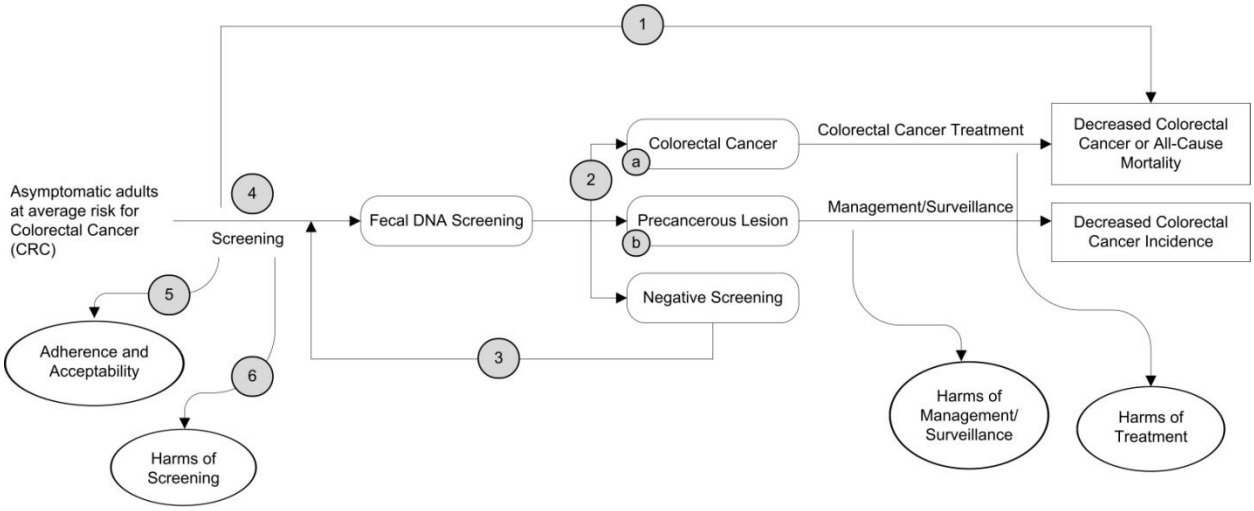
The Oregon EPC developed this topic for consideration for a systematic review based on a public nomination submitted to the AHRQ EHC program. For the topic development phase, we prepared a topic brief that provided a scan of the evidence and contextual details addressing the EHC program prioritization criteria. The topic prioritization group from the EHC program evaluated the appropriateness, importance, feasibility, desirability, and potential and relative value and recommended a Comparative Effectiveness Review.[38]

The proposed Key Questions and the Analytic Framework were posted for public comment on the EHC Program website from February 7 through March 7, 2011 before finalizing. During the public review of the proposed Key Questions, several individuals expressed concern about the optimal timing of this review due to the development and ongoing research on a new fecal DNA screening test. Despite these comments, it was felt that a review would still be helpful to stakeholders before the availability of newer testing. No changes to the KQs or Analytic Framework were made on the basis of the public comments. After developing the proposed review protocol, a Technical Expert Panel (TEP) was convened to help refine the draft protocol. The TEP consisted of experts in the areas of gastroenterology, cancer screening, stool-based testing (fecal DNA and FIT), genetic testing, and systematic review methods. Discussions among the EPC, AHRQ, and TEP members occurred during two teleconferences and via e-mail. In addition, input from the TEP during the conduct of the review was sought to help address content about the evolution of fecal DNA testing. The TEP was not otherwise involved in the conduct of the review or writing of the report.

Analytic Framework

We developed an Analytic Framework to illustrate the relationship between the Key Questions addressed in our review (Figure 1).

Figure 1. Analytic framework of the benefits and harms of fecal DNA testing in screening for colorectal cancer



Literature Search Strategy

We performed comprehensive literature searches in the following databases: 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 were limited to English language studies published, from January 2000 through August 11, 2011. See Appendix A for complete search string. Searches of these databases were supplemented with manual searching of reference lists of relevant review articles and suggestions made by TEP members.

In addition to a search of the published literature, we also performed a focused search of the grey literature. For the purposes of this review, grey literature comprised information that was not controlled by commercial publishing, including: unpublished data from recent (2009–2011) conference abstracts (e.g., American Association for Cancer Research, American Association for Clinical Chemistry, American College of Gastroenterology, American Society of Clinical Oncology, Digestive Disease Week, Gastrointestinal Cancers Symposium), regulatory documents (e.g., FDA Medical and Statistical Reviews; Authorized Medicines for the European Union), proprietary data submitted via manufacturer, and information regarding ongoing and future research via clinical trial registry entries (e.g., ClinicalTrials.gov and WHO Clinical Trials). Additional unpublished literature was sought via a Scientific Information Packet (SIP) request to LabCorp. Literature from Exact Sciences were provided by TEP members.

The results of the searches were downloaded and imported into version 11.0.1 of Reference Manager (Thomson Reuters, New York, NY), a bibliographic management database.

Process for Study Selection

The results of the electronic literature searches, hand searches, and TEP-suggested literature were screened using a two-step process for study selection. Abstracts were screened

independently by two reviewers against a set of *a priori* inclusion criteria developed in consultation with the TEP (Table 2). We restricted included populations to persons at average-risk for CRC. Although we excluded case-control studies and cohorts in high-risk patients as this study design and distorted selection of patients has been shown to overestimate sensitivity,[39,40] because of a paucity of included studies to address Key Questions 1 and 2, we do acknowledge these excluded case-control or cohort studies in high-risk patients in a separate table. We included studies conducted in any setting, but limited the acceptable studies for analytic validity to tests currently available to patients. We also limited the literature to English language only published in 2000 or later, which our TEP confirmed would capture the relevant literature. Full-text articles of abstracts meeting inclusion criteria were retrieved and dual-reviewed against the inclusion criteria. Disagreements were resolved through consultation with a third reviewer. Reviewers were not masked to the study authors, institution, or journal. Excluded articles can be found with the reason for exclusion in Appendix B.

Table 2. Inclusion criteria

Category	Inclusion Criteria
Population	KQs 1–6: Adults ≥40 years old at average risk for CRC. We excluded populations exclusively in adults who are at high-risk for CRC and those diagnosed with CRC. Persons at high risk for CRC include persons with a strong family history of CRC including syndrome-related elevated risks (e.g., FAP, HNPCC) and persons referred to diagnostic colonoscopy for abnormal screening test results.
Interventions	KQs 1–6: Fecal assays intended to screen for CRC, through early cancer or precancerous lesions identification by DNA testing including genotyping, gene-expression measurement, and/or methylation detection. Fecal DNA tests may be performed alone or in combination with other CRC-screening tests. KQ 4: Tests will be limited to those that are currently available to patients, because the assay technology has changed significantly over time.
Comparator	KQ 1: No screening or another established CRC-screening modality (colonoscopy, FOBT [high-sensitivity or traditional], FIT, flexible sigmoidoscopy, barium enema, or CT colonography). KQ 2: For absolute test performance: colonoscopy alone or supplemented by another test. For relative test performance: any established CRC-screening modality. KQ 5: Any established CRC-screening modality.
Outcomes	KQ 1: CRC incidence (or advanced neoplasia incidence if CRC incidence is not reported), all-cause mortality, and CRC-specific mortality. KQs 2 & 3: Absolute or relative test-performance measures, including sensitivity, specificity, PPV, NPV, or relative detection rate: For detection of CRC (adenocarcinoma, carcinoma in situ). For adenomas (any histology). For advanced neoplasia, which is a composite outcome including adenocarcinoma, adenomas with high grade dysplasia or villous histology, and adenomas 1 cm or greater in diameter. KQ 4: Analytic sensitivity (lower limit of detection), analytic specificity, and reproducibility. KQ 5: Any self-reported or objective measures of patient acceptability of or patient adherence to fecal-DNA screening. KQ 6: Any reported harms, including test inaccuracy (i.e., false–positive or false–negative results), and negative psychological, ethical, legal, or social consequences.
Time Period	KQs 1–6: 2000–present.
Setting	KQs 1–6: All settings.
Study Geography	KQs 1–6: All locations.
Publication Language	KQs 1–6: English only.
Study Design	KQs 1–2: Systematic review, randomized or nonrandomized controlled trial, prospective or retrospective cohort, diagnostic accuracy studies (excluding case-control studies). KQs 3–6: Any study design.
Followup Duration	KQs 1–6: We did not exclude studies based on duration of followup. Timing of application of reference-standard testing was considered as part of the quality assessment.
Sample Size	KQs 1–6: We did not exclude studies based on sample size alone.

CRC=colorectal cancer; CT colonography=computed tomographic colonography; FAP=familial adenomatous polyposis; FIT= fecal immunochemical test; FOBT= fecal occult blood test; HNPCC=hereditary nonpolyposis colorectal cancer; NPV=negative predictive value; PPV=positive predictive value

Grey literature findings were initially reviewed by one reviewer and confirmed by a second reviewer. While grey literature sources were selected using the same eligibility criteria used for the published literature, the timeframe for conference abstracts was condensed to 2009-2011.

Data Abstraction and Data Management

Data from all included studies were abstracted into standardized evidence tables in Microsoft Excel by one reviewer and checked for accuracy and completeness by a second reviewer. Separate abstraction forms were created for key questions related to clinical utility/validity, analytic validity, and acceptability/adherence. The following information was extracted from each study, where applicable: author identification, year of publication, source of study funding, study design characteristics, recruitment setting/patient-inclusion criteria, sample size, and setting; important study population characteristics (e.g., age, race, sex); and fecal DNA test and comparator test (reference standard) characteristics, and all relevant outcomes. We recorded details relevant to the technical specification of the fecal DNA assay being conducted, including the gene mutations/expression analyzed, the assay characteristics and laboratory setting, and the technique used for sample analysis.

For excluded case-control or cohort studies in high-risk patients, we abstracted the markers examined, the study aim, the sample sizes, and outcomes. A higher-level summary of these studies is included in an excluded studies table.

Individual Study Quality Assessment

To assess the methodological quality of included studies, we applied the study design-specific quality criteria of the U.S. Preventive Services Task Force.[41] We supplemented these quality criteria with methods from the Evaluation of Genomic Applications in Practice and Prevention Working Group (specific to genetic testing),[42] the Newcastle Ottawa Scale (specific to cohort studies),[43] and the QUADAS criteria (specific to diagnostic accuracy studies).[44] Two independent reviewers assigned a quality rating of the internal validity for each study. Disagreements were resolved by discussion and consensus, or through consultation with a third reviewer. A rating of "good," "fair," or "poor" was assigned by using the predefined criteria for each study design. Good-quality studies generally met all of the study design-specific quality criteria. Fair-quality studies did not meet all the criteria, but did not have any fatal flaws in study design. Poor-quality studies had significant flaws or lack of reporting that imply bias, affecting interpretation of study results. No articles were excluded for quality reasons.

Data Synthesis

We conducted qualitative syntheses of study results for each key question with included studies. Due to limited number of studies included for each key question and clinical differences amongst studies, we did not conduct meta-analysis of results. For qualitative syntheses, we evaluated and summarize clinical and methodological characteristics of included studies, these included: number and characteristics of study participants, settings in which the study was conducted, specific fecal DNA test evaluated (and important test characteristics), outcomes assessed and statistical considerations of reported results. In our qualitative synthesis we also evaluated and summarize important internal (quality) and external (applicability) of studies, and how the validity affected confidence in interpretation of results. For details on quality and applicability please Individual Study Quality Assessment (above) and Applicability (below)

sections. Study results and limitations are also summarized in tables for easy comparison across studies.

Grading the Strength of Evidence

The strength of evidence for primary outcomes was graded using the standard process of the Evidence-based Practice Centers as outlined in the *Methods Guide for Effectiveness and Comparative Effectiveness Reviews*.[45] The grade was based on four major domains: risk of bias, consistency, directness, and precision of the evidence. Grades were assigned for the bodies of evidence pertaining to each primary outcome: high, moderate, low, and insufficient (Table 3).[45]

Table 3. Strength of evidence grades and definitions

Grade	Definition
High	High confidence that the evidence reflects the true effect. Further research is very unlikely to change our confidence in the estimate of effect.
Moderate	Moderate confidence that the evidence reflects the true effect. Further research may change our confidence in the estimate of effect and may change the estimate.
Low	Low confidence that the evidence reflects the true effect. Further research is likely to change the confidence in the estimate of effect and is likely to change the estimate.
Insufficient	Evidence either is unavailable or does not permit a conclusion.

Applicability

We also assessed the applicability of studies. Judgments of applicability for each outcome were performed separately from assessments of the other domains of strength of evidence, as recommended.[46] Factors in individual studies that might affect applicability were abstracted, particularly including factors related to the populations studied (e.g., how highly selected they were [what portion of those eligible were included], how they were recruited) and if the fecal DNA assay is currently available or not (or how similar is the assay to currently available fecal DNA assays).

Peer Review and Public Comments

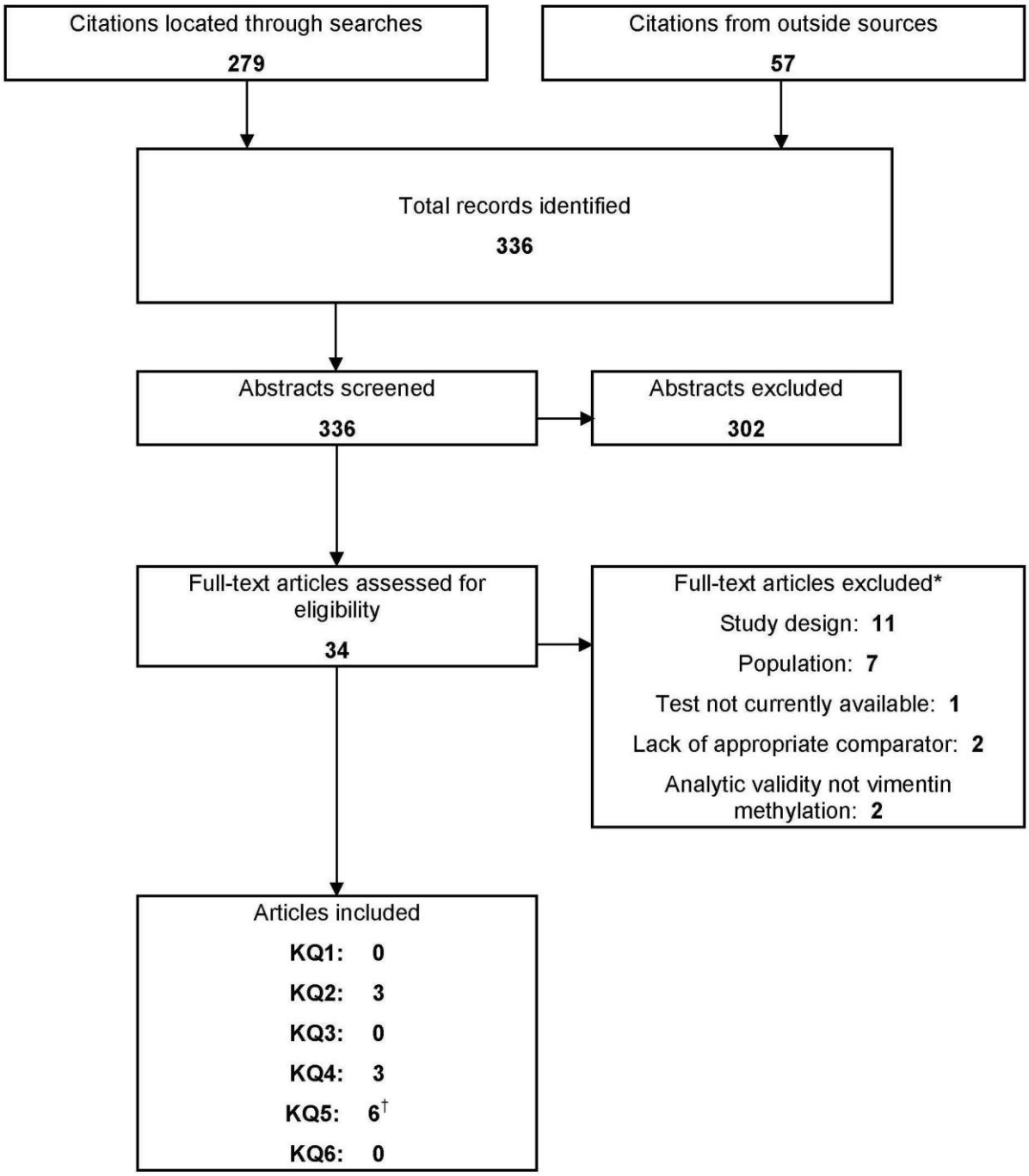
A draft of this review was circulated to eight expert reviewers on August 1, 2011. In addition to this formal expert review process, a draft of this report was posted for public comment from August 1 to August 29, 2011. Members of the TEP were invited to review this draft and submit comments during public posting of this report. We subsequently made revisions based on reviewer comments and an updated literature search.

Results

Literature Yield

The literature search yielded a total of 336 citations from electronic database searches and outside sources (Figure 2). Based on the review of title and abstracts, we subsequently reviewed 34 full-text articles for their eligibility. We included 12 articles in total, three diagnostic accuracy studies 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. Two studies for Key Question 2 also reported adherence to testing and therefore 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).

Figure 2. Literature flow diagram



*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)

Results of Included Studies

Key questions 1 to 3. Benefits of fecal DNA testing

We found no studies that evaluated the effectiveness of fecal DNA screening on CRC incidence or mortality, alone or in combination with other screening tests (Key question 1). We found three fair- to poor-quality studies in screening populations (n=5662) that evaluated the absolute test performance of fecal DNA testing for CRC screening, compared to colonoscopy (reference standard).[33,34,47] Two of these studies also concomitantly evaluated guaiac-based fecal occult blood testing (FOBT) (Key Question 2).[33,34] We found no studies that evaluated the test performance of fecal DNA testing across different screening intervals (Key Question 3). We excluded 65 initial test-validation studies that were not conducted in screening populations, the majority of which were case-control studies in patients with CRC and healthy controls. Only three of these 65 studies were prospective cohort studies conducted in high-risk patients (Table 4a and b). Six of the excluded case-control studies evaluated vimentin methylation independently, which is the basis for the only commercial fecal DNA test currently available (Table 1a and b).

Despite the availability of numerous initial validation studies of fecal DNA testing, we found only three studies that examined the test accuracy of fecal DNA testing in screening populations.[33,34,47] In summary, two fair- to poor-quality diagnostic accuracy studies in screening cohorts of average-risk patients undergoing colonoscopy have evaluated multi-marker fecal DNA testing (two different versions) by Exact Sciences.[33,34] The third study was rated as poor-quality and was a smaller cohort study evaluating the test accuracy of *KRAS* mutations.[47] Due to the evolution of fecal DNA tests, none of these studies evaluated tests currently on the market. Two studies (n analyzed=5004) evaluated a multi-marker fecal DNA test that was a prototype to a later version that was clinically available as PreGen Plus™.[33,34] These two studies found somewhat different estimates of sensitivities to detect CRC (25 percent [95% CI, 5 to 57 percent] versus 51.6 percent [95% CI, 34.8 to 68.0]). There were some differences in study populations, but it is unclear if these differences should affect test performance. Sensitivities for advanced adenomas were very low in both studies. A smaller (n=217) subset analysis evaluating a different multi-marker fecal DNA test and a smaller (n=441) study evaluating a single marker (*KRAS*) were both poor quality.

Table 4a. DNA markers evaluated in excluded studies focusing on test development and/or initial validation of fecal DNA testing for colorectal cancer: Case control studies

Author	Year	Mutations†	Methylation†	Other†‡
Tang[48]	2011		SFRP2	
Zhang[49]	2011	KRAS		
Zhang[50]	2011		TFPI2	DIA
Ahlquist[51]*	2010	KRAS	BMP3, TFPI2, NDRG4, vimentin	
Azuara[52]	2010		RARB, CDKN2A, MGMT, APC	
Chang[53]	2010		ITGA4, SFRP2, CDKN2A	
Hussain[54]*	2010	BRAF		
Kalimutho[55]	2010	APC		DIA
Kisiel[56]*	2010	TP53, APC, KRAS, BRAF, PIK3CA	*EYA4,* vimentin	
Zou[32]*	2010		Vimentin	
Baek[30]	2009		MGMT, MLH1, vimentin	
Bosch[57]*	2009		PHACTR3	
Calistri[58]	2009			DIA
Cretella[59]*	2009	APC, KRAS, BRAF, TP53		DIA
Glockner[60]	2009		TFPI2	
Hellebrekers[61]	2009		GATA4, GATA5	
Kim[62]	2009		OSMR, SFRP1, B4GALT1	
Li[31]	2009		Vimentin	
Mayor[63]	2009		*EN1* CpG island	
Melotte[64]	2009		NDRG4	
Nagasaka[65]	2009		RASSF2, SFRP2	
Zou[66]*	2009			DIA
Zou[67]*	2009	TP53, *KRAS*, APC, CDH1, *CTNNB1*, BRAF, SMAD4, *CDKN2A*		
Zou[68]	2009	*KRAS*, APC, BRAF, *TP53*		
Zou[69]*	2009	*KRAS*, APC	BMP3	
Itkowitz[28]	2008		Vimentin	DIA

Table 4a. DNA markers evaluated in excluded studies focusing on test development and/or initial validation of fecal DNA testing for colorectal cancer: Case control studies (continued)

Author	Year	Mutations†	Methylation†	Other†‡
Oberwalder[70]	2008		SFRP2	
Onouchi[71]	2008	APC, KRAS, TP53		
Wang[72]	2008		SFRP2	
Abbaszadegan[73]	2007		CDKN2A	DIA, MSI (*BAT-26*)
Abbaszadegan[74]	2007	TP53		
Huang[75]	2007		SFRP2	
Huang[76]	2007		SFRP2, TMEFF2, MGMT	
Itkowitz[27]	2007	KRAS, TP53, APC	vimentin, *HLTF*	DIA
Leung[77]	2007		APC, ATM, MLH1, SFRP2, HLTF, MGMT, GSTP1	
Onouchi[78]	2007	TP53, KRAS, APC		
Rennert[79]	2007	KRAS		
Zou[80]	2007		Vimentin	
Zhang[81]	2007		SFRP1	
Jin[82]	2006	BRAF, KRAS		MSI (*BAT-26*)
Zou[83]	2006			DIA
Chen[29]	2005	APC		DIA, MSI (*BAT-26*)
Lenhard[84]	2005	HIC1		
Matsushita[85]	2005	APC, KRAS, TP53		
Petko[86]	2005		MGMT, CDKN2A. MLH1	
Belshaw[87]	2004		ESR1, MGMT, TMEFF2, CDKN2A, APC, MLH1	
Calistri[88]	2004			DIA
Leung[89]	2004		APC, ATM, HLTF, MGMT, MLH1, GSTP1	
Muller[90]	2004		SFRP2	
Wan[91]	2004	KRAS		
Whiney[25]	2004	KRAS, TP53, APC		DIA, MSI (*BAT-26*)

Table 4a. DNA markers evaluated in excluded studies focusing on test development and/or initial validation of fecal DNA testing for colorectal cancer: Case control studies (continued)

Author	Year	Mutations†	Methylation†	Other†‡
Boynton[92]	2003			DIA
Calistri[22]	2003	TP53, KRAS, APC		MSI (D2S123, D5S346, D17S250, BAT-25, BAT-26)
Tagore[21]	2003	APC, TP53, KRAS		DIA, MSI (*BAT-26*)
Ito[93]	2002	KRAS		
Koshiji[94]	2002			LOH (APC, TP53, DCC, MLH1, D9S162, D9S171, IFNA)
Nishikawa[95]	2002	KRAS		
Traverso[96]	2002	APC		
Traverso[97]	2002			MSI (*BAT-26*)
Doolittle[98]	2001	KRAS		
Rengucci[99]	2001	TP53, KRAS		MSI (D2S123, D5S346, D17S250, BAT-25, BAT-26)
Ahlquist[20]	2000	KRAS, TP53, APC		MSI (*BAT-26*), DIA

DNA integrity assay =DIA; Loss of heterozygosity LOH; Microsatellite instability=MSI

*Conference abstract only

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

‡Full information on microsatellite markers can be found at www.genome.ucsc.edu

Table 4b. DNA markers evaluated in excluded studies focusing on test development and/or initial validation of fecal DNA testing for colorectal cancer: Cohort studies in high risk patients

Author	Year	Mutations†	Methylation†	Other†‡
Kalimutho[100]	2011			DIA
Calistri[101]	2010			DIA
Puig[102]	2000	KRAS		

DNA integrity assay =DIA; Loss of heterozygosity =LOH; Microsatellite instability=MSI

*Conference abstract only

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

‡Full information on microsatellite markers can be found at www.genome.ucsc.edu

The most recent study by Ahlquist and colleagues published in 2008 was an NCI and industry-funded diagnostic accuracy study conducted in a large cohort (n enrolled=4482) of 50 to 80 year olds at average-risk for CRC (Table 5).[34] Due to cancellations, protocol violations, incomplete colonoscopy or incomplete stool samples, 718 persons (16 percent) were excluded, thus 3764 participants were included in the analysis. Based on limited characteristics (i.e., age, sex, and race/ethnicity), included participants did not appear to be different from the overall enrolled study population. The study evaluated a pre-commercial stool DNA test (SDT-1, pre-commercial version of PreGen Plus), and a second, different multi-marker panel (SDT-2) assay, that evolved after the study had started. The SDT-1 evaluation ended after reviewing interim results on the first 2497 participants, which, per the author, was precipitated by a decision to add Hemoccult SENSA™ as a comparator. The evaluation of SDT-2 was a smaller nested case-control study in which the SDT-2 test was run on a subset of patients (n analyzed=217). This subset included all cancers (n=19), high-grade dysplasia (n=20), adenomas ≥ 2 cm (n=53), a random subset of 1-2 cm adenomas (n=50), and normal controls (n=75). All participants received a colonoscopy for validation of CRC-related findings. SDT-1 was a multi-target fecal DNA test that included 21 mutations in the *KRAS, APC,* and *TP53* genes, along with markers for microsatellite-instability (MSI) and long DNA. SDT-2 was a different multi-target fecal DNA test that included mutations in the *KRAS, APC* genes as well as vimentin gene methylation. Guaiac-based FOBTs, both Hemoccult II™ and Hemoccult SENSA, were concomitantly evaluated. Only a subset of patients, however, were advised about dietary and medication restrictions. Therefore, results reported for FOBT (n=3764) are problematic and not discussed further in the results. Test performance outcomes for SDT-1 were rated fair quality despite reporting of an unplanned interim analysis as the final results. However, test performance outcomes for SDT-2 were rated as poor quality (see below).

Table 5. Patient characteristics for studies of diagnostic accuracy of fecal DNA testing (KQ2)

Author, year	Population	n	Mean age (years)	% male	% non-White	SES	Risk factors
Ahlquist, 2008[34]	Enrolled	4482	63.7	47.8	6.4	NR	NR
	Analyzed for SDT-1	2497	60.4	46.0	7.3	NR	NR
	Analyzed for SDT-2	217	66.4	50.2	7.4	NR	NR
Haug, 2007[47]	Included	894	NR	47	NR	NR	% with 1st-degree relative with CRC: 12.0
	Analyzed	441	NR*	NR*	NR*	NR*	NR†
Imperiale, 2004[33]	Included	4404	68.6	44.6	12.7	NR	% with family history of CRC: 14.0
	Analyzed	2507	69.5	44.5	13.0	NR	% with family history of CRC: 13.9

CRC: Colorectal cancer; NR: not reported; SDT-1: sDNA version 1.0; SDT-2: sDNA version 2.0; SES: socioeconomic status
*authors state that patients analyzed did not differ from included patients
†authors state that more patients analyzed reported a 1st degree relative with CRC

The cohort for SDT-1 evaluation (n analyzed=2497) had a mean age of 60.4 years, were 46.0 percent male, and 92.7 percent White (Table 5). Overall test positivity was 5.2 percent. The sensitivity for one-time fecal DNA testing with SDT-1 was 25 percent (95% CI, 5 to 57) for CRC, 19 percent (95% CI, 5 to 42) for advanced adenomas and 20 percent (95% CI, 14 to 26) for CRC and advanced adenomas. Specificity for any CRC or advanced adenomas was 96 percent (95% CI, 95 to 97) (Table 6). The subset who were included in the SDT-2 evaluation (n analyzed=217) were generally older than those evaluated in the overall cohort (mean age 66.4), 50.2 percent male and 92.6 percent White (Table 5). Because only a selected subset of persons had SDT-2 testing, overall test positivity was artificially elevated at 35 percent. Weighted

sensitivity for one-time fecal DNA testing with SDT-2 was 40 percent (95% CI, 32 to 49) for detection of CRC and advanced adenomas, which was twice the sensitivity of SDT-1 (Table 6). Sensitivity for detection of CRC was 58 percent (95% CI, 36 to 80). Although specificity was not reported, 16 percent of patients with normal colonoscopy had an abnormal SDT-2 result, and 26 percent of patients 65 years or older with a normal colonoscopy had an abnormal SDT-2 result. Thus, enhanced sensitivity with SDT-2 likely had a cost in terms of decreased specificity.

Table 6. 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[34]	0.5% (19/3764)	SDT-1 (prototype sDNA version 1.0)	5.2% (129/2497)	98.2% (3766/3834)	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% (3766/3834)	CRC	58% (36-80%)*	NR
					Advanced adenomas	39% (26-52%)*	NR
					CRC + advanced adenomas	40% (32-49%)	NR
Haug, 2007[47]	1.6% (NR)	*KRAS* testing	8% (70/875)	NR	CRC	0% (NR)	NR
					Advanced adenomas	0% (NR)	NR
Imperiale, 2004[33]	0.7% (31/4404)	SDT-1 (prototype sDNA version 1.0)	8.2% (205/2505)	88.3% (4845/5486)	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% (146/2505)	92.2% (5060/5486)	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

In general, the strength of this study was that it was a large cohort in an average-risk screening population, which recruited from 22 academic and regional health care systems. However, important study limitations impacted our ability to interpret the results for SDT-2 test performance. These include the small sample size, exclusion of all patients with protocol violations, inadequate samples, or colonoscopy, limited sampling of controls despite weighting sensitivity for proportion of screen relevant neoplasia in the entire population, and inability to accurately estimate test specificity (Table 7). Although the lack of adherence to dietary and medication restrictions should, in theory, not decrease sensitivity of FOBT, the sensitivities for Hemoccult II and Hemoccult SENSA reported in this study were much lower than more generally accepted estimates for sensitivities.[103] In addition to concerns about the generalizability

of the cohort studied (i.e., inclusion of a mostly White population, n eligible not reported), the single most important limitation is that neither SDT-1 or SDT-2 were ever available for clinical use and are both different from the currently available test ColoSure™.

Table 7. Limitations and quality concerns for diagnostic accuracy studies of fecal DNA testing (KQ2)

Author, year	Quality rating	Quality concerns	Applicability concerns
Ahlquist, 2008[34]	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 US 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[47]	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[33]	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 Patients received financial compensation 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-1: sDNA version 1.0; SDT-2: sDNA version 2.0

The study by Imperiale and colleagues was another industry-funded fair-quality large cohort study (n included=4404) evaluating the same pre-commercial stool DNA test (SDT-1, pre-commercial version of PreGen Plus) as was evaluated in the study by Ahlquist and colleagues (Table 5).[33] In this study, researchers compared SDT-1 with 3-card non-rehydrated Hemoccult II average-risk, asymptomatic patients who all underwent colonoscopy. Of the 5486 enrolled participants, 1082 (20 percent) were excluded due to incomplete testing. Baseline characteristics for enrolled participants were not reported in comparison with included participants. About 50 percent more patients (641 vs. 426) did not provide an adequate sample for fecal DNA testing as compared to Hemoccult II, which may signal differences in feasibility or acceptability to patients. Although all included patients had colonoscopy and Hemoccult II testing, only a subset

received SDT-1 testing. A subset (n=2507) of the 4404 that completed testing were analyzed. This subset included all subjects with an invasive cancer (n=31) or advanced adenoma (n=403), along with a random subgroup with minor polyps (n=648) and normal findings (n=1423). The analyzed subgroup was similar with respect to age, sex, race/ethnicity, and family history of CRC, as compared to the overall cohort (Table 5). As a group, this cohort included slightly older participants (69.5 yrs), slightly more men (44.5% male), and slightly more non-White individuals (13%) than the cohort studied by Ahlquist and colleagues. Two patients (one rectal carcinoid tumor, one cloacogenic tumor) were excluded from analyses and reported results. As compared to the study by Ahlquist and colleagues, the comparative results for Hemoccult II performance are of fair (as opposed to poor) quality. In this study, investigators reported that subjects were given proper dietary and medication instructions, cards were returned for non-rehydrated analysis consistent with manufacturer's instructions, and results for Hemoccult II are given for the same subgroup of persons who received fecal DNA testing.

Of those tested with SDT-1, 8.2 percent were test-positive on the fecal DNA panel and 5.8 percent had a positive Hemoccult II. One-time fecal DNA testing was more sensitive for CRC than Hemoccult II (51.6 percent, [95% CI, 34.8, 68.0] and 12.9 percent [95% CI, 5.1, 28.9], respectively). This estimate of sensitivity is much higher than the sensitivity for CRC reported for SDT-1 in the study by Ahlquist and colleagues (Table 6). Sensitivity for advanced adenomas was similarly poor for fecal DNA testing (15.1 percent, [95% CI, 12.0 to 19.0] and for Hemoccult II (10.7 percent, [95% CI, 8.0 to 14.1]). While specificity for CRC or CRC and advanced adenomas did not differ significantly between fecal DNA and Hemoccult II, power to detect a difference was limited since the full sample was not tested (Table 6).

In general, this was a fair-quality study conducted in a large, average-risk screening population. This population was drawn from 81 private-practice and university-based settings. Study investigators were blinded, except for the gastroenterologists who had access to FOBT results. This study had several limitations, however, that impacted both its internal and external validity (Table 7). These limitations include: poor precision in the estimates of test performance characteristics due to sample size issues, exclusion of 20 percent of the study population for incomplete testing data (and unknown comparability of patient characteristics for excluded participants), inclusion of a mostly older population (in which three-quarters of the study population was over 65 years of age), inclusion of a mostly White population, number of eligible participants not reported, conduct of the fecal DNA testing centrally at a single lab, and an unusually low estimate for Hemoccult II sensitivity (compared to conventional understanding of Hemoccult II performance). Most importantly, the version of the test evaluated in this study was never commercially available, and the included markers for evaluation are different (no overlap) from the currently available test ColoSure.

The third included diagnostic accuracy study was rated as poor quality and was an analysis from a population-based cohort study that examined baseline stool samples for a single mutation of the *KRAS* gene in 441 older adults (aged 50 to 75 years) within a larger cohort study (n=9953) (Table 5).[47] This subgroup represented those who opportunistically received their reference colonoscopy within two years of the DNA testing. The included subgroup was similar to the overall study population, except that more participants in this group reported a first-degree relative with CRC. The fecal test had zero percent sensitivity, testing positive in none of the 31 participants with advanced colorectal neoplasia (seven patients with invasive CRC) (Table 6). The highest rate of mutant *KRAS* was reported in participants with a negative colonoscopy (7.5 percent). Important study limitations included bias in the spectrum of patients self-selecting for

colonoscopy, and the lag-time between stool collection and clinical diagnosis that could have affected test performance (Table 7).

Key Question 6. Harms of fecal DNA testing

We found no studies that specifically evaluated the harms of fecal DNA testing. Hypothesized harms other than harms from diagnostic inaccuracy (false positives and false negatives) include psychological harms (anxiety or worry) around testing. It is unclear if the psychological harms around genetic based testing are qualitatively different than psychological harms of other stool based testing like guaiac-based or immunochemical fecal occult blood testing.

The downstream effects of false positive results primarily include the harms of unnecessary diagnostic interventions (i.e., colonoscopies). Therefore, the harms of fecal DNA testing inaccuracy would be relative to comparative specificity of other stool based testing, but less than colonoscopies itself as a screening option. Based on the included studies, the specificity of SDT-1 and FOBT were not statistically significantly different, although the studies had limited power to detect a difference.[33] Although the specificity of SDT-2 was not reported, SDT-2 had a positivity rate of 16 percent (95% CI, 8 to 24) in persons with normal colonoscopies, and the positivity rate increase with age.[34,104] This positivity rate is much higher than that reported for FOBT test positivity, but FOBT test performance data from this study is of poor quality (for the quality reasons discussed above). As stated above, these data have poor applicability given that SDT-1 and SDT-2 are not clinically available and are very different from currently available testing.

Key Question 4. Analytic validity of fecal DNA testing

We found three poor-quality studies that evaluated the analytic validity of currently available fecal DNA assays, specifically a single-marker test for methylated vimentin.[29,31,80] We did not identify any additional information by searching grey literature or from requesting non-published literature from LabCorp via the Scientific Information Packet. For analytic validity, we specifically looked for analytic accuracy of the test, repeatability or reproducibility, analytic sensitivity or lower limit of detection of the test, or analytic specificity (Table 8).

Table 8. Definitions for analytic validity[105]

Accuracy	Ability of assay to measure what it purports to measure determined independently by a reference method
Repeatability or precision	Replication of results when the assay is performed multiple times on a single specimen
Reproducibility	Replication of results when assay is performed on different specimens, or on different days, or by different operators
Analytic sensitivity or lower limit of detection	Ability of assay to detect all true positive specimens, for quantitative tests this is the defined as the smallest quantity of a substance that can be reliably detected or quantified
Analytic specificity	Ability of assay to measure the target substance when potentially interfering or cross-reacting substances are present in the sample

In summary, these three studies show that technological advances in analytic factors (i.e., test methods and performance of procedures) can improve the analytic sensitivity of assays to detect methylated vimentin in stool samples (Table 9). None of the studies evaluated the repeatability, reproducibility, or analytic specificity of testing. These three studies were generally of poor

quality, and it is unclear if the exact technological advances evaluated in these studies are applicable to the currently available test for methylated vimentin (ColoSure).

Table 9. Analytic validity of fecal DNA testing (KQ4)

Author, year	Experiment aim	Outcomes	Quality concerns	Applicability concerns
Li, 2009[31]	To test methyl-BEAMing in the detection of methylated vimentin DNA in plasma and stool from CRC patients	Lower limit of detection: 0.1% (1/1000 copies) methylated DNA detected using methyl-BEAMing versus no detection <6.2% without methyl-BEAMing	Poor: Small sample size (n=1 series of dilution) and poor reporting, unclear if experiments were repeated and results replicated	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
		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 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	
Zou, 2007[80]	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	Poor: Small sample size (n=1 series of dilution), unclear if experiments were repeated and results replicated	Unknown if MBD column is used in assay studied (KQ2) or currently available testing
		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=8)	
Chen, 2005[29]	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 1000- 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

Two studies evaluated technological advances aimed at improving the analytic sensitivity of testing. The most recent study by Li and colleagues was aimed at testing methyl-BEAMing to methylated vimentin in plasma and stool from colon cancer patients.[31] Methyl-BEAMing (Beads, Emulsion, Amplification, and Magnetics) is a method for performing methylation specific PCR in compartments created by the emulsion that contain individual strands of DNA to allow digital enumeration of the PCR products. This technique's accuracy was compared with next generation sequencing (sequencing by synthesis) in stool samples from patients with CRC (n=5) and the analytic sensitivity was compared to methylation specific PCR without methyl-BEAMing. The study found that enumeration of methylation by either next generation sequencing or methyl-BEAMing produced essentially the same result in both samples with low and high fractions of

methylated vimentin (Table 9). Another experiment within this study showed that methyl-BEAMing enhanced overall technical sensitivity for detecting methylated vimentin DNA by at least 62-fold in artificial samples created by mixing DNA from peripheral blood lymphocytes (unmethylated) with DNA from CRC cell lines (methylated). The overall quality of both experiments was poor given the small sample sizes and incomplete reporting of results. Accuracy was only assessed in five samples, and only reported for four of the five samples. It is unclear if the experiment for the analytic sensitivity was replicated. The applicability of this experiment is also poor given that the accuracy study was conducted in plasma samples, rather than stool samples, and methyl-BEAMing does not appear to be used in the assay evaluated by Ahlquist and colleagues (included in Key Question 2), or in the currently available methylated vimentin test.

The second study by Zou and colleagues tested a method known as methyl-binding domain (MBD) enrichment to see if it could increase the analytic sensitivity for detecting tumor-specific methylated markers in patient stools.[80] Two sets of experiments showed that the assay with MBD enrichment had a lower limit of detection of methylated vimentin in normal stool aliquots with added DNA from CRC cell lines, and in stool samples from patients (n=8) with known CRC with tissue positive for methylated vimentin (Table 9). The lower limit of detection of methylated vimentin with MBD enrichment was 10 ng from the normal stool with added DNA from cell lines, and 4 ng from stool samples from patients with CRC. This study, however, was rated as poor quality because it had very small sample sizes. We cannot assess the applicability of this study because it is unclear if MBD enrichment was used in the assay evaluated by Ahlquist and colleagues (included in Key Question 2), or is used in the currently available methylated vimentin test.

The earliest study by Chen and colleagues is essentially a proof-of-concept study aimed at testing the technical limits of the sensitivity of detecting DNA methylation.[29] It showed that methylation-specific PCR could detect as little as 25-50 pg of input methylated DNA, which corresponds to a detection limit for the assay of approximately 15 methylated cells (Table 9). This study, however, was rated as poor quality mainly because the experimental results were only reported for one sample. More importantly, this study has poor applicability to current tests as it was conducted in tumor tissue and not conducted in stool samples. This lower limit of detection is an order of magnitude lower than the Zou study conducted in stool samples.

Key Question 5. Acceptability and adherence of testing

We found six fair- to poor-quality studies that evaluated the acceptability,[27,106-110] and two diagnostic accuracy studies (from Key Question 2) that reported the adherence to fecal DNA testing.[33,34] From very limited evidence, it appears that fecal DNA testing, in the form of a single whole-stool sample, is generally acceptable, although an important test attribute for acceptability appears to be the test's accuracy. In one fair-quality diagnostic accuracy study fecal DNA adherence was lower than adherence to Hemoccult II. No studies have evaluated the relative acceptability or adherence of fecal DNA tests to FIT tests. This is an unfortunate omission, as FIT is the most similar to fecal DNA testing in that it is a non-invasive stool based test that does not require any dietary or medication restrictions. Unlike other stool-based testing, however, fecal DNA testing is currently a single whole-stool sample that theoretically may be preferable to serial card based testing.

It is likely that future fecal DNA testing will be sufficiently different both in the format/collection (no longer a single whole stool sample) and in test accuracy, such that this currently available evidence on acceptability and adherence to fecal DNA testing will no longer be relevant.

Acceptability of testing. We found five fair- to poor-quality studies that evaluated the acceptability of fecal DNA testing (Table 10). Two fair- to poor-quality studies were conducted by Marshall and colleagues.109,110 In these two studies, authors used a cross-sectional survey and modeling to measure Canadian preferences for CRC screening tests109 or to measure patient and physician preferences for CRC screening tests in both Canada and the United States.110 These two studies were rated as fair quality in assessing patient preferences on attributes of testing, but poor quality in assessing patient or physician preference (or willingness to pay) for type of testing. In both studies, adults without a history of CRC (n=2135) were surveyed to elicit preferences on key attributes of available screening tests (e.g., process, preparation, pain, sensitivity, specificity, frequency, followup, complication risk and cost). The studies found that patient's preferred tests that were non-invasive, required no pain or preparation, were highly accurate, did not require repeated measurements over time, and caused no complications. One study used modeling to rank the importance of attributes and found that the relative importance was (in order of most to least importance): sensitivity, specificity, preparation, process, pain.109 The second study also surveyed 100 United States and 100 Canadian practicing primary care physicians.110 The most preferred attribute for primary care physicians was high sensitivity, other important attributes included the pain, specificity, complication risk, preparation, and testing frequency. The major limitations of the survey portion of the study include was a 52 percent response rate in one study 109 and no reported response rate in the second study.110 Both studies used modeling to rank the types of tests (in order of most preferred to least preferred), however, the results from these models are not reliable due to substantial modeling limitations (Table 10).

One fair-quality test manufacturer-funded study by Schroy and colleagues was designed to compare the perceptions of fecal DNA, Hemoccult II, and colonoscopy in a screening population (Table 10).106 In this study, participants (n=4840) in the diagnostic accuracy study by Imperiale and colleagues received a 25-item questionnaire on the three different screening tests that they completed as part of the study. Eighty-four percent (4042/4840) of all participants who completed all three screening tests returned the mailed questionnaire. This study evaluated the

prototype to PreGen Plus a multi-marker panel requiring one single whole stool sample (mailed into laboratory by patient). Overall, 45 percent of respondents preferred fecal DNA testing, 32 percent preferred Hemoccult II, 15 percent preferred colonoscopy, and eight percent had no preference ($p<0.001$). However, on the individual measures (ease of instruction, simplicity of collection, comfort, invasiveness, embarrassment, anxiety of prep, anxiety of test, accuracy, and likeliness to repeat test) there was no meaningful difference between fecal DNA testing and Hemoccult II. The authors concluded that fecal DNA testing was rated more favorably (for simplicity of collection, comfort, anxiety of prep, accuracy, and likeliness to repeat) based on statistical significance alone. However, the difference was a fraction of a point on a five-point scale. Although colonoscopy was perceived to be more accurate than fecal DNA testing, respondents rated colonoscopy as less favorable in terms of invasiveness, anxiety (around prep and test), and less likely to repeat the test. Although this study was rated as fair quality, a few limitations of this study make it difficult to generalize study findings—no mention of missing data, participants received financial compensation for the study, and participants had to adequately complete all three screening tests to be included in the study.

The other three poor-quality studies provide little additional information given their methodological weaknesses (Table 10). One case-control diagnostic accuracy study (n=162) by Itzkowitz also evaluated patient preferences in a survey, however, no response rate for this survey was reported and patients likely knew if they had CRC or a normal colonoscopy prior to doing the fecal DNA test. Another study by Schroy and colleagues was a cross-sectional survey (n=263) of participants at average-risk for CRC designed to assess patient preferences for CRC screening methods.108 The survey was conducted as a structured interview after receiving patient education about the different screening modalities, including fecal DNA testing, FOBT, colonoscopy, flexible sigmoidoscopy with or without FOBT, and double contrast barium enema (DCBE). Unfortunately, as stated by the authors, during the education component the accuracy of fecal DNA testing was incorrect (it stated that the ability to predict precancerous polyps was medium to high). While the study also reported on participants' willingness to pay out of pocket for testing, these patients were not presented with the costs of each test to inform their decision. The last study was a convenience sampling survey designed to examine patients' screening experiences with fecal DNA testing. Berger and colleagues analyzed returned questionnaires that were included with the PreGen Plus collection kit for the first 2 years it was commercially available. However, only 18 percent of persons who received the PreGen Plus test kit returned the survey.

Table 10. Patient preferences and acceptability of fecal DNA testing (KQ5)

Author, year	Study aim	Study design N participants	Outcomes	Quality concerns	Applicability concerns
Marshall, 2009[110]	To compare patient and physician preferences about CRC screening tests	Cross-sectional survey N=1588 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[109]	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 US 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	

Table 10. Patient preferences and acceptability of fecal DNA testing (KQ5) (continued)

Author, year	Study aim	Study design N participants	Outcomes	Quality concerns	Applicability concerns
Itzkowitz 2007[27]	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
Schroy, 2007[108]	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 followup 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[107]	To assess patients' screening experience with fecal DNA testing	Convenience survey N= 1211	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[106]	To compare patients' perceptions of fecal DNA, FOBT, colonoscopy	Cross-sectional survey of participants in diagnostic accuracy study N= 4042	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 immunochemicaltest; FOBT= fecal occult blood test; SDT-2= sDNA version 2.0

Adherence to testing. Two studies included from Key Question 2 examining the diagnostic accuracy of fecal DNA testing reported completion of fecal DNA testing in comparison to FOBT and colonoscopy.[33,34] We found no additional studies that specifically examined adherence to fecal DNA testing. Both of the studies addressing adherence evaluated fecal DNA testing requiring a single whole stool sample mailed to the laboratory. In the fair-quality study by Imperiale and colleagues (see Key Question 2 results), 11.7 percent (641/5486) did not complete the fecal DNA test versus 7.8 percent (426/5486) did not complete Hemoccult II, and 14 percent (770/5486) did not complete colonoscopy.[33] In the other fair-to poor-quality study by Ahlquist and colleagues (see Key Question 2 results), the adherence to testing was much higher, only 1.8 percent (68/3834) did not complete the stool testing (assumed both fecal DNA and FOBT) within the allotted time (120 days).[34] Authors report that 4.3 percent (171/4005) did not have an adequate colonoscopy (did not reach cecum or view over 90 percent of the colorectum). It is unlikely that these completion rates can be generalized to practice given that participants were given financial compensation for participating in both studies. Additionally, completion of the fecal DNA and FOBT testing were done simultaneously in one study (patients collected three whole-stool specimens in a plastic bucket and promptly smeared stool onto both window of the Hemoccult II and Hemoccult SENSA cards and mailed all specimens into the lab).[34]

Summary and Discussion

Strength and Applicability of Evidence

Despite considerable media attention and expert-based clinical recommendations that include fecal DNA testing as an option for CRC screening, there is currently insufficient evidence regarding the clinical accuracy (or clinical validity) for fecal DNA tests in patients at average-risk for CRC (Table 11). Few studies use appropriate study designs to determine screening test accuracy in asymptomatic populations, and these studies do not evaluate clinically available fecal DNA tests.

We found only three studies that evaluated the performance of fecal DNA tests in asymptomatic persons. The best evidence is from two studies (n analyzed=5004) that evaluated a multi-marker fecal DNA test that was a prototype to a later version that was clinically available as PreGen Plus™.[33,34] The sensitivity to detect CRC for this prototype was discordant between two studies (25 percent [95% CI, 5 to 57] versus 51.6 percent, [95% CI, 34.8 to 68.0]), although the confidence intervals overlapped. Sensitivity for advanced adenomas was similarly poor in both studies (19 percent [95% CI, 5 to 42] and 15.1 percent, [95% CI, 12.0 to 19.0]). Between-study differences, such as differences in study populations, do not clearly account for differences in test sensitivities. Specificity for any screen-relevant neoplasm (CRC and advanced adenoma) ranged from 93.6 percent (95% CI, 92.9 to 94.3) to 96 percent (95% CI, 95 to 97). Hemoccult II™ performed unusually poorly in both of these studies, with sensitivity for CRC of 12.9 percent (95% CI, 5.1 to 28.9) and 11 percent (95% CI, 6 to 16). Best, and generally accepted, estimates for sensitivity of Hemoccult II for CRC ranges from 25 to 38 percent, with 98 to 99 percent specificity for CRC.[103] Accurate interpretation of Hemoccult II requires training and supervision, especially when interpreting borderline results, and therefore the lower sensitivities of Hemoccult II in these two studies and real-world settings may be indicative of suboptimal (poor) oversight and standardization of the lab development of Hemoccult II.[111-113] Other available stool-based tests have been shown to have much higher sensitivities (but with lower specificity) for CRC. Best estimates for Hemoccult SENSA™ for CRC are approximately 64 to 80 percent sensitivity and 87 to 90 percent specificity.[5] Although FITs represent a very heterogeneous group of tests, sensitivity for CRC appears to range from about 61 to 91 percent, and specificity ranges from 91 to 98 percent.[5] Hemoccult SENSA and the various FITs have not been as well studied as Hemocccult II, but it is clear that they also have similar issues around quality assurance (e.g., accurate interpretation, number of samples) and therefore real-world performance estimates may be lower than those previously reported.[5,10] Furthermore, these estimates of sensitivity and specificity are based on a single test application. The overall test performance of these stool-based tests in a program of screening will depend on the interval between repeated screening (and adherence to testing), which varies between the different types of stool-based testing. Thus far, only Hemoccult II has randomized controlled trial evidence to show that a program of (bi-) annual screening can reduce mortality.[5,114]

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 (and continues to develop), reflecting advances in the understanding of molecular markers that reflect neoplastic change and ongoing advances in technologies to stabilize, extract, and amplify/detect low levels of human target DNA in stool samples. Therefore the only three

studies on test performance 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 also have insufficient evidence (Table 11) and are largely not applicable to currently available fecal DNA tests. Because test preference is influenced by test performance, acceptability, and adherence to testing will need to be reexamined once test performance is known. In addition, acceptability and adherence should be examined in comparison to other stool-based screening methods, specifically FITs, which do not require any dietary or medication restrictions. Subtleties in stool collection (e.g., number of samples; cards versus vials versus whole stool) may also affect acceptability and adherence, and therefore may change if future fecal DNA testing no longer requires a single whole stool specimen. Currently, there is no evidence that directly addresses health benefit (or clinical utility) or intervals of screening for fecal DNA testing. Experts suggest that new diagnostic tests may be substituted for an established test based on diagnostic accuracy studies alone if the new test is clinically superior to existing tests (e.g., safer or more specific than, but of similar sensitivity to, the established test).[115] After test performance in screening populations is established, and if the test is not obviously clinically superior to existing tests, modeling using robust CRC screening models (MISCAN, SimCRC) could inform net health benefit (e.g., tradeoffs from improved sensitivity and reduced specificity) and optimal intervals of screening using fecal DNA testing.

Table 11. Strength of evidence for fecal DNA testing

Outcome (key question)	# studies (n pts)	Study design	Risk of bias	Consistency	Directness	Precision	Strength of evidence
Morbidity or mortality (KQ1)	None	-	-	-	-	-	Insufficient
Test performance (KQ2)	3 (n=5662)	Diagnostic accuracy studies	Medium	Inconsistent (2 studies)	Indirect	Imprecise	Insufficient
Test performance, interval (KQ3)	None	-	-	-	-	-	Insufficient
Analytic validity (KQ4)	3 (not applicable)	In vitro laboratory studies	High	Not applicable (no replication)	Indirect	Imprecise	Insufficient
Acceptability Adherence (KQ5)	6 (n=8013) 2 (n=9968)	Cohort, cross-sectional	High	Consistent	Indirect	Imprecise	Insufficient
Harms-diagnostic inaccuracy (KQ6)	3 (n=5662)	Diagnostic accuracy studies	Medium	Consistent	Indirect	Imprecise	Insufficient

Evidence Gaps

In order to understand the clinical utility of fecal DNA testing, clinicians and decision-makers need evidence to inform the net benefit and comparative effectiveness of fecal DNA testing in screening asymptomatic persons for CRC. Table 12 outlines the development of research for fecal DNA screening tests. While different decision-makers may have different thresholds for the types of evidence required to consider a test for clinical use (e.g., may not require evidence of clinical utility), most would agree that robust evidence on the clinical test validation in the intended population would be the minimum evidence required for clinical decision making. Presently, there is no such evidence on test performance (diagnostic accuracy and inaccuracy) in a screening population for either currently available testing or soon-to-be-available testing. Evidence about optimal screening intervals, analytic validity, and acceptability of adherence (helpful in understanding the implementation of screening and the real world effectiveness of screening) are also generally lacking. Evidence thus far has primarily focused on initial test validation of an evolving series of tests (Table 12). However, clinical decision making cannot rely on initial test validation results alone, especially when viable alternative tests exist (other CRC screening tests including stool based testing).[115] Empiric evidence shows that distorted selection of participants (including non-representative patients) and use of case-control study designs overestimate overall test accuracy due to both variation and spectrum bias.[39,40]

Our review is consistent with these findings. When we compare the results from the three included studies with the initial validation studies identified, but excluded from this review, we found exaggerated sensitivities in these studies excluded for their high potential bias. 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).[15] In addition, independent validation of tests in a larger and (more) representative cohort can reveal "implementation" issues that can also affect test performance in a clinical setting. For example, authors suggest that the diagnostic accuracy study by Imperiale and colleagues had a much lower sensitivity than anticipated because of DNA degradation during the transport and storage of the stool sample, which led to the subsequent development and addition of a buffer to stabilize the target DNA in stool samples.[17] Although CRC is not uncommon, prospectively designed diagnostic accuracy studies for colorectal cancer screening need to be quite large to accrue enough cancer outcomes, especially in average-risk adults. Well conducted nested case-control designs similar to the included Ahlquist and Imperiale studies can provide valuable diagnostic accuracy information. The cases and controls should be nested in a screening population and the index test (fecal DNA test) should be conducted on stool samples acquired before the colonoscopy, as the colonoscopy is often therapeutic in removing suspicious lesions.

Table 12. Evidence landscape for fecal DNA testing

Phases of test development	Description	Available evidence
Marker identification and assay development	To identify molecular alterations associated with colorectal cancer To develop assays that can detect these alterations in tumors and in stool samples	Likely numerous studies, often unpublished, our review only included studies on analytic validity of currently available testing (n=1) and not other aspects of assay development
Initial test validation and assay refinement	To determine ability and accuracy of test in persons with CRC or advanced adenomas To develop technological improvements to assays	Numerous studies (N>60) on different assays/markers Limited evidence for analytic validity of currently available testing (n=2), often unpublished
Test validation in intended population and generalizability	To determine diagnostic accuracy for detection of CRC or advanced adenomas of test in screening population To determine factors affecting application and test performance in real-world setting (including test feasibility and acceptability)	Limited studies (n=3) evaluating test performance of different tests, none of which are applicable to currently available testing Limited studies (n=5) evaluating acceptability of fecal DNA tests (no studies compared fecal DNA to FIT acceptability)
Clinical test performance and health impact	To compare the effectiveness and harms of fecal DNA testing versus established screening alternatives on health outcomes	No direct evidence (modeling exercises can be valuable, if good estimates of diagnostic accuracy are available)
Net benefit of testing, cost-effectiveness	To determine population net benefit or cost-effectiveness of fecal DNA screening in a program of repeated screening (compared to relevant screening options)	No direct evidence, cost-effectiveness not addressed in this review (modeling exercises can be valuable, if good estimates of diagnostic accuracy are available)

Therefore, 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. While we found no evidence to specifically address harms of testing, we do not expect any clinically significant harms other than the (unnecessary) downstream effects of testing and complications from testing resulting from false positives, or clinically significant sequelae from missed diagnosis resulting from false negatives. Potential harms due to diagnostic inaccuracy should therefore be weighed in comparison to the diagnostic inaccuracy from other non-invasive stool based testing. While some degree of worry could be engendered by being offered fecal DNA testing, evidence to support clinically meaningful negative psychological impacts from stool based screening or the negative impacts of fecal DNA testing compared to other stool based tests does not exist.116 Ultimately, the issue of considering the net benefit of fecal DNA testing compared to the best CRC screening alternative(s) may require some degree of modeling, especially without clearly superior new testing115 or applicable comparative effectiveness trials reporting health outcomes (which may never happen given the rapid evolution of fecal DNA testing).

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 (NCI) Cancer Intervention and Surveillance Modeling Network (CISNET) for evaluating CRC screening to estimate net benefit of testing and optimal intervals of testing, compared to other currently used or promising screening

modalities. In 2007, the Centers for Medicare and Medicaid Services (CMS) commissioned to CISNET to do a cost-effectiveness analysis of screening with fecal DNA testing in support of a National Coverage Determination process to determine whether PreGen Plus, version 1.1 should be covered for Medicare enrollees.117 Based on two independently conducted microsimulation models (MISCAN and SimCRC), the cost-effectiveness of fecal DNA testing was modeled for fecal DNA test every 3 or 5 years to screen for a cohort of patients at average-risk for CRC aged 65 years compared to other screening modalities (Hemoccult II, Hemoccult SENSA, FIT, flexible sigmoidoscopy, and colonoscopy) enrollees.117 Based on these modeling exercises, the investigators found that at $350 per test, fecal DNA testing would not be cost-effective regardless of sensitivity and specificity at any level of test accuracy. They found that that fecal DNA testing would be cost-effective at a per-test cost of $40 to $60 (depending on the model used), if used every 3 years. Fecal DNA testing, however, could be effective at current cost if the relative adherence to fecal DNA testing was at least 50 percent better than that with other screening tests.

Although modeling exercises can use sensitivity analyses around test adherence to see if the comparative effectiveness between different screening tests is robust to variation and differential test adherence, understanding issues around patient acceptability and actual adherence to fecal DNA testing is still quite important. The most important driver of poor early detection of CRC is not the less-than-perfect sensitivities of different screening tests, but the suboptimal uptake of screening tests. Advocates in the field have stated that the best screening test is the test that is completed. Therefore, if fecal DNA testing can improve uptake of CRC screening because it appeals to persons who are unscreened or under screened, it could have a significant clinical and public health benefit. Existing studies, albeit limited, seem to consistently identify factors that influence patient preferences around testing. Patients prefer tests that are non-invasive, require no pain or preparation, are highly accurate, do not require (frequently) repeated testing, and cause no complications. Stool-based tests that require little to no preparation, like fecal DNA testing or FITs, may be preferable to patients, although both would require repeated testing. Currently there is no evidence about the relative preference and adherence between fecal DNA testing and FITs. In addition to understanding the comparative test performance between these two types of testing, any differences in preference and adherence between these tests will also be important. Subtleties in stool collection may also affect acceptability and adherence, and therefore preference and adherence studies must be specific to particular tests because the test accuracy and method/details of stool collection may even vary between different fecal DNA and FIT tests.

Finally, issues around the fecal DNA test's analytic validity are important to address since these issues/factors can help inform understanding of clinical validity of a test as well as inform what may be needed for post implementation monitoring and quality control. Analytic validity is the assay's ability to accurately and reliably measure the genotype.105 Diagnostic tests subject to FDA approval must provide evidence on the accuracy in, and reliability of, measuring the analyte (or genotype) of interest.118 Our review of analytic validity focused on the accuracy, lower limit of detection, analytic specificity, repeatability and reproducibility of fecal DNA assays for methylated vimentin (the basis of ColoSureTM). We located only three relevant studies despite searching non-published and grey literature. We found no evidence on the overall analytic validity of methylated vimentin fecal DNA testing. We found one early proof-of-concept paper focusing on experiments showing that testing for methylated vimentin as a marker for CRC was possible (in tumor tissue), and two studies with experiments evaluating specific

technologies (methyl-BEAMing, and methyl-binding domain enrichment) and their ability to improve the lower limit of detection for target DNA in stool. We found no evidence on the repeatability or reproducibility of the currently available fecal DNA test on methylated vimentin. These two dimensions of analytic validity are clinically relevant to larger-scale implementation of testing and quality assurance of testing once implemented, especially as fecal DNA testing becomes more complicated. This may result from including multiple molecular markers and more advanced or combinations of technologic components because there may be more opportunities for variations in important pre-analytic and analytic factors. Reporting of potentially important details that may affect analytic validity of assays should be routinely reported in clinical evaluation studies, so it is transparent from the studies differences in the conduct of the test between studies and between research and clinical practice (implementation). 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

Based on this systematic review, we know that fecal DNA test research and development is a continuously evolving field. The limitations in this review primarily stem from the limitations in the primary research and the moving target nature of fecal DNA testing (resulting in a mismatch between primary research and available testing). However, there are a few important limitations in 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 (i.e., in a diagnostic algorithm for individuals at high risk for developing CRC, monitoring or surveillance of individuals with previously abnormal screening results, or as a prognostic test or test for treatment prediction once cancer is diagnosed). Our review also did not address the specific role of fecal DNA screening to detect clinically significant lesions traditionally missed by colonoscopy, i.e., flat lesions; or the clinical significance of a positive fecal DNA test result in light of a negative colonoscopy. In addition, our review did not include stool-based RNA testing 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. Given the rapidly evolving nature of fecal DNA testing, this review will likely be out of date in the near future, as new tests and evidence supporting them become available in the next 1 to 2 years. However, many of the issues laid out in the report will be helpful framing the evaluation of the evidence based in the future.

Future Research

Future research for fecal DNA testing to screen for CRC should focus on identified evidence gaps. Research to facilitate clinical adoption of fecal DNA testing should focus on the diagnostic accuracy of testing of those tests that are widely available at present or in the near future, as well as the comparative effectiveness of fecal DNA testing versus other best alternative stool-based screening tests (e.g., FIT). Future research to inform the net clinical benefit and optimal intervals for repeated screening will likely be from CISNET's microsimulation models. Analytic validity studies, especially on accuracy and repeatability/reproducibility, should be published or made publically available. More upstream test development should focus on defining the optimum combination of markers and simplifying and automating test procedure without loss of analytic validity to improve throughput and reduce cost.

Upcoming Studies

Through conversations with our TEP, conference presentations, and information from investment conference telephone calls, we understand that Exact Science is currently developing a new assay, a multi-marker fecal DNA test plus FIT, Cologuard[SM]. This test is expected to be available within the next couple of years. To our knowledge it includes a combination of different markers (two methylation markers, seven mutations in *KRAS* exon 2) and a FIT, and uses new proprietary technology QuARTS [TM] (Table 1).[119] Initial validation results which have been presented suggest improved sensitivity for both CRC and large adenomas (85 percent and 64 percent, respectively).[120] Initial validation studies are expected to be published in later 2011 and 2012. A large prospective study (DeeP-C) to evaluate Cologuard test performance in average-risk patients for CRC cancer has started to enroll patients as of June 30, 2011 (clinicaltrials.gov identifier: NCT01397747). Results from this prospective study are expected in 2013 to coincide with an application for FDA approval and review for CMS National Coverage Decision of Cologuard to screen for CRC. Other than Cologuard, we did not identify any other forthcoming (widely or commercially available) fecal DNA testing in North America.

Although fecal DNA test development and validation continues to be an active area of research, we found only two upcoming studies, in addition to the Cologuard study, that appear to evaluate tests in clinically relevant populations (asymptomatic screening populations). From our review of relevant conferences, we found numerous abstracts (without full publication of results) on the initial validation of different tests (and different combinations of markers) (Table 4a and 4b). However, we identified one potential study in abstract form that evaluated fecal DNA testing in an asymptomatic screening population.[121] In addition to conference abstracts, we searched for upcoming studies in study registries and found an additional study (currently in progress) evaluating the test performance of a fecal DNA test for methylated DNA, Colohybritest.[122] This test evaluation, however, is being conducted in asymptomatic persons with a positive FOBT.[122]

Other Emerging Genomic Tests

Genetic or genomic tests other than fecal DNA testing were not included in this review. The use of RNA markers in stool has not been as extensively studied as DNA markers, but is a new

area of active research. Multiple forms of RNA have been studies as potential cancer biomarkers in fecal samples. These include both messenger RNA (mRNA), which is protein coding, as well as microRNA which are noncoding sequences that affect gene expression. Recent preliminary research presented as meeting abstracts indicates that the most promising mRNA markers may be COX2, MMP7, B2M, CKB, and Snail mRNAs.[123-125] Candidate markers for microRNA (miRNA) that been identified in preliminary exploratory research include miR-17-92, miR-18a, miR-20a, miR-21, miR-92, miR-106a, miR-135, and miR-221.[126-129]

In addition to genetic testing in stool samples, the use of DNA or RNA in plasma and serum has also been examined using PCR-based assays that can detect small amount of genetic and epigenetic alterations in circulating tumor DNA.[130] The most recent efforts have focused on the detection of methylated DNA , the most widely studied marker is currently *SEPT9,* this marker is one of the few that has had multiple initial test validation studies.[131] Test accuracy based on initial validation studies have varied across independent case-control studies. Preliminary results from a large prospective study (n=7,914) in asymptomatic patients focused on *SEPT9* plasma screening are expected to be published in the Summer of 2011.[132] Research is ongoing to identify additional markers that may add to the sensitivity of detection in plasma and serum samples.[130]

Finally, the detection of nucleic acid biomarkers of CRC in urine is a new approach with very limited data at this time. For markers to be present in urine some degree of metabolism would be required to clear glomerular filtration and be excreted. Levels of nucleosides (small-fragment metabolic products of DNA) characteristic of CRC and adenomas have been found in urine and have been proposed as a mechanism to discriminate patients with CRC from controls.[17,133]

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Abbreviations

95% CI—95 percent confidence interval
ACR—American College of Radiology
ACS—American Cancer Society
AHRQ—Agency for Healthcare Research and Quality
CISNET—Cancer Intervention and Surveillance Modeling Network
CLIA—Clinical Laboratory Improvement Amendments
CMS—Centers for Medicare & Medicaid Services
CRC—Colorectal cancer
CT colonography—Computed tomographic colonography
DCBE—Double contrast barium enema
DIA—DNA integrity assay
DNA—Deoxyribonucleic acid
EHC Program—Effective Health Care Program
ELISA—Enzyme-linked immunosorbent assay
EPC—Evidence-based Practice Center
FAP—Familial adenomatous polyposis
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)
FPC—Familial polyposis coli
HNPCC—Hereditary nonpolyposis colorectal cancer
KQ—Key question
LDT—Laboratory-developed test
MBD—Methyl-binding domain
miRNA—MicroRNA
mRNA—Messenger RNA
MSTF—Multi-Society Task Force
NCI—National Cancer Institute
NPV—Negative predictive value
NR—Not reported
PCR—Polymerase chain reaction
PPV—Positive predictive value
RNA—Ribonucleic acid
sDNA—Stool DNA test
SES—Socioeconomic status
SIP—Scientific Information Packet
TEP—Technical Expert Panel
USPSTF—U.S. Preventive Services Task Force
WHO—World Health Organization

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 non-cancer cells.

Enzyme-linked immunosorbent assay (ELISA)—A biochemical technique used to detect the presence of an antibody or an antigen in a sample

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

Germ-line—Genetic material passed from parents to offspring

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.

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

Methylation specific PCR—A method of methylation analysis that uses bisulfite-treated DNA but does not require sequencing

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

Oncogenes—Genes that have the potential to cause cancer

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

Proto-oncogenes—A normal gene that may become an oncogene due to mutations or increased expression

Reagent—A substance or mixture for use in chemical analysis or other reactions

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

Somatic cells—cells of the body excluding reproductive (germ-line) cells

Transcription—the copying of DNA into mRNA in gene expression

Tumor suppressor genes—a gene that protects a cell on the path to cancer

Wnt signaling pathway—a network of proteins in which alterations are associated with carcinogenesis

Appendix A. Search Strategy

Database:
Ovid MEDLINE(R) without Revisions <1996 to August 11, 2011>

1. ((fecal or faecal or stool) adj5 (DNA or deoxyribonucleic acid)).ti,ab.
2. (((fecal or faecal or stool) adj5 (genetic$ or genomic$)) and screen$).ti,ab.
3. ((fecal or faecal or stool) adj5 molecular).ti,ab.
4. (f-dna or fdna).ti,ab.
5. (sdna or s-dna).ti,ab.
6. colosure$.ti,ab.
7. cologuard$.ti,ab.
8. DNA
9. DNA Methylation
10. DNA Mutational Analysis
11. DNA, Neoplasm
12. 8 or 9 or 10 or 11
13. Feces
14. 12 and 13
15. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 14
16. Colorectal Neoplasms
17. Colonic Polyps
18. Colonic Neoplasms
19. Sigmoid Neoplasms
20. Rectal Neoplasms
21. Anus Neoplasms
22. Anal Gland Neoplasms
23. Intestinal Polyps
24. (Colon$ adj5 cancer$).ti,ab.
25. (Colorectal adj5 cancer$).ti,ab.
26. (Colon$ adj5 neoplas$).ti,ab.
27. (Colorectal adj5 neoplas$).ti,ab.
28. or 16-27
29. 15 and 28
30. limit 29 to humans
31. limit 29 to animals
32. 31 not 30
33. 29 not 32
34. limit 33 to english language
35. limit 34 to yr="2000 -Current"

Appendix B. List of Excluded Studies

1. Anderson WF, Guyton KZ, Hiatt RA, et al. Colorectal cancer screening for persons at average risk. J Natl Cancer Inst 2002 Aug 7;94(15):1126-33. PMID: 12165637. **Study design.**

2. Belshaw NJ, Elliott GO, Williams EA, et al. Use of DNA from human stools to detect aberrant CpG island methylation of genes implicated in colorectal cancer. Cancer Epidemiol Biomarkers Prev 2004 Sep;13(9):1495-501. PMID: 15342451. **Population.**

3. Calistri D, Rengucci C, Casadei GA, et al. Fecal DNA for noninvasive diagnosis of colorectal cancer in immunochemical fecal occult blood test-positive individuals. Cancer Epidemiol Biomarkers Prev 2010 Oct;19(10):2647-54. PMID: 20929882. **Population.**

4. Capella G. Is fecal DNA testing superior to fecal occult-blood testing for colorectal cancer screening? Nat Clin Pract Oncol 2005 May;2(5):234-35. PMID: 16264954. **Study design.**

5. Ferretti G, Bria E, Carlini P, et al. Is stool DNA multitarget testing an unreliable strategy for colorectal cancer screening? Gut 2005 Jun;54(6):891. PMID: 15888808. **Study design.**

6. Frossard JL, de Peyer R. Fecal DNA for colorectal-cancer screening. N Engl J Med 2011 Mar 31;352(13):1384-85. PMID: 15800239. **Study design.**

7. Hardison DM, Shuber AP. Stool DNA: a viable option for colorectal cancer screening. Gastroenterology 2005 Dec 31;129(6):2128-9. PMID: 16344091. **Study design.**

8. Itzkowitz S, Brand R, Jandorf L, et al. A simplified, noninvasive stool DNA test for colorectal cancer detection. Am J Gastroenterol 2008 Nov;103(11):2862-70. PMID: 18759824. **Lack of appropriate comparator.**

9. Kalimutho M, Del Vecchio BG, Cretella M, et al. A simplified, non-invasive fecal-based DNA integrity assay and iFOBT for colorectal cancer detection. Int J Colorectal Dis 2011 Jan 12 PMID: 21225430. **Population.**

10. Kato I, Badsha KZ, Land S, et al. DNA/RNA markers for colorectal cancer risk in preserved stool specimens: a pilot study. Tumori 2009 Nov;95(6):753-61. PMID: 20210241. **Lack of appropriate comparator, Analytic validity not vimentin methylation.**

11. Laboratory Corporation of America. Technical Review: ColoSure™, A Stoll DNA-based Colorectal Cancer Screeninng Test. Laboratory Corporation of America. 2008. **Study design.**

12. Leung WK, To KF, Man EP, et al. Detection of epigenetic changes in fecal DNA as a molecular screening test for colorectal cancer: a feasibility study. Clin Chem 2004 Nov;50(11):2179-82. PMID: 15502094. **Population.**

13. Muller HM, Oberwalder M, Fiegl H, et al. Methylation changes in faecal DNA: a marker for colorectal cancer screening? Lancet 2004 Apr 17;363(9417):1283-85. PMID: 15094274. **Population.**

14. Nechvatal JM, Ram JL, Basson MD, et al. Fecal collection, ambient preservation, and DNA extraction for PCR amplification of bacterial and human markers from human feces. J Microbiol Methods 2008 Feb;72(2):124-32. PMID: 18162191. **Analytic validity not vimentin methylation.**

15. Ned R, Melillo S, Marrone M. Fecal DNA testing for Colorectal Cancer Screening: the ColoSure™ test. PLoS Currents: Evidence on Genomic Tests. 2011. PMID: 21487548. **Study design.**

16. Olson J, Whitney DH, Durkee K, et al. DNA stabilization is critical for maximizing performance of fecal DNA-based colorectal cancer tests. Diagn Mol Pathol 2005 Sep;14(3):183-91. PMID: 16106201. **Test not currently available.**

17. Puig P, Urgell E, Capella G, et al. A highly sensitive method for K-ras mutation detection is useful in diagnosis of gastrointestinal cancer. Int J Cancer 2000 Jan 1;85(1):73-77. PMID: 10585586. **Population.**

18. Ransohoff DF. Fecal DNA tests for colorectal cancer. N Engl J Med 2003 Jun 13;346(24):1912-13. PMID: 12063379. **Study design.**

19. Rennert G, Kislitsin D, Brenner DE, et al. Detecting K-ras mutations in stool from fecal occult blood test cards in multiphasic screening for colorectal cancer. Cancer Letters 2007 Aug 18;253(2):258-64. PMID: 17349741. **Population.**

20. Stephenson J. Experimental colon cancer test appears to hold promise. JAMA 2000 Nov 22;284(20):2584-29. PMID: 11086349. **Study design.**

21. Worthley DL, Cole SR, Young GP. Fecal DNA for colorectal-cancer screening. N Engl J Med 2000 Nov 22;352(13):1384-85. PMID: 15803528. **Study design.**

22. Young GP, Worthley DL. Screening with a fecal multitarget DNA test. Gastroenterology 2005 Aug;129(2):757-59. PMID: 16083732. **Study design.**