ANNUAL REVIEWS

Annual Review of Medicine

Fecal DNA Testing for Colorectal Cancer Screening

John M. Carethers

Division of Gastroenterology and Hepatology, Department of Internal Medicine and Department of Human Genetics and Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan 48109, USA; email: jcarethe@umich.edu

Annu. Rev. Med. 2020. 71:59–69

First published as a Review in Advance on August 26, 2019

The *Annual Review of Medicine* is online at med.annualreviews.org

https://doi.org/10.1146/annurev-med-103018-123125

Keywords

stool DNA tests, multitarget stool DNA test, fecal DNA tests, colorectal cancer screening

Abstract

Fecal (or stool) DNA examination is a noninvasive strategy recommended by several medical professional societies for colorectal cancer (CRC) screening in average-risk individuals. Fecal DNA tests assay stool for human DNA shed principally from the colon. Colonic lesions such as adenomatous and serrated polyps and cancers exfoliate cells containing neoplastically altered DNA that may be detected by sensitive assays that target specific genetic and epigenetic biomarkers to discriminate neoplastic lesions from nonneoplastic tissue. Cross-sectional validation studies confirmed initial case-control studies' assessment of performance of an optimized multitarget stool DNA (mt-sDNA) test, leading to approval by the US Food and Drug Administration in 2014. Compared to colonoscopy, mt-sDNA showed sensitivity of 92% for detection of CRC, much higher than the 74% sensitivity of another recommended noninvasive strategy, fecal immunochemical testing (FIT). Detections of advanced adenomas and sessile serrated polyps were higher with mt-sDNA than FIT (42% versus 24% and 42% versus 5%, respectively), but overall specificity for all lesions was lower (87% versus 95%). The mt-sDNA test increases patient life-years gained in CRC screening simulations, but its cost relative to other screening strategies needs to be reduced by 80–90% or its sensitivity for polyp detection enhanced to be cost effective. Noninvasive CRC screening strategies such as fecal DNA, however, have the potential to significantly increase national screening rates due to their noninvasive nature and convenience for patients.

INTRODUCTION

Colorectal cancer (CRC) is third in overall incidence (145,600 annual cases) and the second leading cause of cancer death in both men and women (51,020 annual deaths) in the United States as of 2019 (1). Screening the average-risk population for CRC has helped reduce the incidence and subsequent deaths, with application of screening tests showing durability for CRC prevention over time (2–4). Multiple professional medical societies recommend the application of one of several CRC screening strategies to continue to reduce the incidence for average-risk individuals (5, 6). These include first-tier tests such as colonoscopy and fecal immunochemical tests (FIT); second-tier tests such as CT colonography, stool (fecal) DNA (sDNA) tests, and flexible sigmoidoscopy; and third-tier tests such as capsule endoscopy (5). Other CRC screening tests, such as the fecal occult blood test (FOBT) and barium enema, have become relatively obsolete due to higher sensitivity and specificity of first- and second-tier tests (5).

Fecal DNA testing has recently come to the commercial market after multiple generations and prototypes. Each generation of sDNA testing improved upon prior generations in terms of test sensitivity in case-control studies due to technical innovations affecting sample preservation (e.g., improved buffers to prevent DNA degradation), improved discriminant biomarker target panels, and improved analytic platforms (7). Of six total versions of sDNA tests developed, two reached the status of a Clinical Laboratories Improvement Act–regulated laboratory diagnostic test prior to the latest version, which received approval by the US Food and Drug Administration (FDA) in August 2014 (8, 9).

Fecal DNA tests are considered second tier at present for CRC screening due to their sensitivity in detecting adenomatous polyps, as well as their cost, particularly compared to the benefit-to-risk ratios of colonoscopy and FIT. Continued improvements with new generations may increase sensitivity and/or enable a cost reduction that in the future will cause sDNA tests to be recommended as first-tier screening. The current FDA-approved commercial sDNA test is useful for patients who may refuse or are uncomfortable with colonoscopy, if colonoscopy is high risk due to medical morbidity, or if colonoscopy is not available. sDNA has higher sensitivity than FIT, and with additional studies, it could occupy niches of utility for interval screening and follow-up to negative colonoscopy.

RATIONALE FOR FECAL DNA DETECTION

CRC is a genetic disease. The local colonic micro-environment influences the genetic make-up of colonocytes that are either primed (e.g., already containing a germline genetic or epigenetic alteration) or somatically-induced (causing subsequent somatic genetic and epigenetic alterations) to form neoplasias (10, 11). Sporadic CRCs result from the accumulation of somatic genetic and epigenetic events through clonal cell expansion. Each clone may acquire additional genetic mutations (also known as driver mutations) that may confer a selective growth advantage, further propelling the neoplasm toward malignancy (11). While each patient's CRC is genetically unique, most sporadic CRCs have several common mutational events that have accumulated since initiation (11). About 85% of all sporadic cancers are aneuploid and contain frequent mutations in *APC*, *TP53*, *KRAS*, *TTN*, *PIK3CA*, *FBXW7*, and *SMAD4* (12). About 15% of all sporadic cancers are diploid but hypermutated, accumulating hundreds of somatic mutations that are driven by hypermethylation of the DNA mismatch repair (MMR) gene *MLH1*. Hypermethylation prevents transcription and subsequent protein expression of *MLH1* and thus fully inactivates MMR. Because DNA MMR repairs post–DNA replication mistakes (single-nucleotide substitutions and microsatellite sequence slippages), this inactivation allows multiple mutations to accumulate in the

cell's genome. Additionally, this hypermutated group of CRCs shows a different complement of genes mutated somatically (these include *ACVR2*, *APC*, *TGFBR2*, *BRAF*, *MSH3*, *MSH6*, and others) largely due to intrinsic coding microsatellite frameshifts (11–14). In either genetic grouping, clones derived from parental cells may acquire additional driver mutations and passenger mutations (i.e., mutations that do not provide a selective growth advantage) at different rates, making the growing neoplasm heterogeneous (15). This has been observed through direct sampling of CRCs via colonoscopy or after surgical resection, as well as from CRC metastases (11, 15, 16). Epigenetic methylation of gene promoters is also acquired during the pathogenesis of CRC. In addition to some CRCs acquiring *MLH1* hypermethylation, other genes such as *SFRP*, *vimentin*, *MGMT*, *FBN1*, and *p16* can be characteristically methylated (17). The advancement of the genetic knowledge of the pathogenesis of CRC would inform approaches to potential noninvasive tests if one could sample a potential neoplasm without an invasive procedure and detect the presence of genetic alterations such as those indicated above as definitive biomarkers of the neoplastic state (11, 18).

Fecal material that is expelled was formed in the colon and had the longest dwell time there, and thus represents contents largely from the colon. Normal apoptotic cells are exfoliated into the colon lumen, and neoplastic cells with altered DNA proliferate at faster rates and have less adhesion to the basement membrane, so they are shed at faster rates into the lumen. Fecal abrasion of neoplastic lesions may further facilitate cell entry into the lumen for both CRCs and precursor adenomatous polyps (9, 11). Human DNA constitutes only 0.01% of total sDNA and must be separated from the much more abundant microbial DNA in feces (19). Human mutant DNA is an even smaller proportion within feces, and its integrity needs to be assessed to ensure an ability to detect genetic alterations. However, tumor DNA is more likely to remain intact than normal DNA once shed (20). Technological advances in sample preservation to prevent DNA degradation and newer analytic platforms affording higher sensitivity have made sDNA testing readily feasible (7).

With the advancing knowledge of the CRC mutational genome landscape, approaches to detect sDNA were initiated as a potential noninvasive test for CRC screening. Most sDNA evaluations were case-control studies for test development or initial validation. Early sDNA studies focused on detection of mutant *KRAS* due to its predictable mutations in codons 12, 13, and 61 (21–23). However, single-gene tests, such for mutant *KRAS*, would be expected to have some false positives (mutant *KRAS* is present in other benign and malignant conditions outside of the colon), as well as poor sensitivity as a single test (mutant *KRAS* is present in only about 50% of CRCs). Multiple genes and/or multiple targets assayed based on the knowledge of CRC neoplastic progression afforded a chance to improve the accuracy of CRC neoplasia detection. Mutant *APC* and *TP53*, although common in the pathogenesis of CRC, are more challenging to detect in a test because their mutations are distributed throughout each gene. More recently, the discovery of abnormal methylation of specific gene promoters has made hypermethylated genes better targets for sDNA assays, especially since abnormal methylation is an early event in CRC tumorigenesis (17, 24).

After multiple and heterogeneous proof-of-concept and case-control studies, only four cross-sectional population-based studies have examined sDNA performance while using colonoscopy as the gold standard for detection. Prior to the latest-generation sDNA test, discussed in the next section, a prototype 21-target sDNA test (that includes assays for mutant *KRAS*, *APC*, and the mononucleotide microsatellite marker *BAT26*, as well as assays for DNA integrity) was used on a single stool specimen to compare with FOBT (Hemoccult II) (25). This study, which examined 2,507 asymptomatic people over age 50, showed a CRC sensitivity rate of 51.6% for sDNA and 12.9% for FOBT, and an advanced adenoma sensitivity rate of 15.5% for sDNA and 10.6% for FOBT. Overall, sDNA detected 40.8% of CRCs/advanced adenomas, whereas FOBT detected 14.1% in this study (25). Specificity for sDNA was 94.4%, similar to FOBT at 95.2%. A study

done in parallel using the same 21-target assay on 4,482 average-risk persons showed an sDNA CRC sensitivity of 25% and specificity of 96% (23). Although this sDNA test detected more advanced adenomas and CRCs than FOBT in these two population-based studies, both sDNA and FOBT tests missed the majority of lesions detected on colonoscopy.

KEY STUDIES THAT LED TO FDA APPROVAL OF THE LATEST-GENERATION FECAL DNA TEST

The disappointing results from earlier-generation sDNA population-based studies were greatly improved upon with the use of optimized technologies and newer DNA targets for the latest-generation test. These improvements ultimately led to its FDA approval and more acceptable prescriptive use by practitioners. The latest-generation sDNA test is an optimized multitarget stool DNA (mt-sDNA) panel that includes assays for mutant *KRAS*, aberrant methylation of *BMP3*, *NDGR4*, and *β-actin* as a reference gene for quantitative estimation of the total amount of human DNA in the sample (26, 27). These targets obtained from the fecal sample undergo a multiplexed QuARTS^{TM} step (quantitative allele-specific real-time target and signal amplification) (26, 27). This latest-generation mt-sDNA test also includes an assay for human hemoglobin (i.e., FIT) (26–28). Initial evaluation of this mt-sDNA test was performed on archived stool specimens from 252 CRC patients, 293 colonoscopy-negative controls, and 133 patients with adenomas of at least 1 cm in diameter (29). This study demonstrated a CRC sensitivity of 85% (specificity 90%) and advanced adenoma sensitivity of 54% (specificity 89%). The study did not detect any differences in sensitivity for location of adenomas or cancers, implying it sampled the entire colon without site discrimination.

As a result of the pilot study (29), a pivotal cross-sectional population-based study was performed that used a quantitative measure for each marker. These measures were input into a validated logistic regression equation with a value >182 being positive, and a cutoff value of >100 ng Hb/ml considered positive for FIT (28). This study of 9,989 asymptomatic persons 50–84 years of age compared one-time testing with the mt-sDNA versus FIT, using colonoscopy as the gold standard (**Table 1**). The CRC sensitivity rate (all stages) was 92.3% for the mt-sDNA test and 73.8% for FIT (with specificities of 86.6% and 94.9%, respectively) (28). The advanced adenoma sensitivity rate was 42.4% for the mt-sDNA test and 23.8% for FIT, and for serrated adenomas (a higher risk adenoma), the sensitivity was 42.4% for the mt-sDNA test and 5.1% for FIT. Site-specific sensitivities for both CRC and advanced adenomas are listed in **Table 1**. Overall,

Table 1 Sensitivity and specificity of neoplastic lesions detected in the colon when comparing mt-sDNA and FIT[a] (28)

	Colonoscopy	mt-sDNA test	FIT	P-value
Overall CRC sensitivity	65 (0.7%)	60 (92.3%)	48 (73.8%)	
Overall CRC specificity		86.6%	94.9%	
Proximal CRC sensitivity		90.0%	66.7%	
Distal CRC sensitivity		94.3%	80.0%	
Overall AAP sensitivity	757 (7.6%)	321 (42.4%)	180 (23.8%)	<0.001
Serrated polyps >1 cm		42.4%	5.1%	<0.001
Proximal AAP sensitivity		33.0%	15.5%	
Distal AAP sensitivity		54.6%	34.8%	
***n* needed to detect one CRC**	154 persons	166 persons	208 persons	

Abbreviations: AAP, adenoma of advanced pathology (≥1 cm, villous or adenocarcinoma component); CRC, colorectal cancer; FIT, fecal immunochemical testing; mt-sDNA, multitarget stool DNA.

[a] $n = 9,989$ persons; there were 90 sites.

the mt-sDNA test detected more advanced adenomas and CRCs than FIT but had more false positives. A second cross-sectional study performed in 661 native Alaskans (who have high rates of CRC) using the mt-sDNA test showed similar results (30). CRC sensitivities for white, black, Hispanic, and Asian patients were 96.4%, 62.5%, 88.9%, and 100%, respectively, and advanced adenoma sensitivities were 42.3%, 42.4%, 39.0%, and 43.8%, respectively (27, 28).

The FDA approved the mt-sDNA test (Cologuard®, Exact Sciences) in August 2014 for screening persons aged 50 years and older at average risk for CRC. Approval by the Centers for Medicare and Medicaid Services for reimbursement followed in October 2014.

The mt-sDNA test is available by prescription. Patients receive a kit containing a collection tub into which they can pass feces while sitting on a toilet at home. A portion of the fecal material is scraped for FIT analysis and tubed, while a DNA-preservation liquid is added to the remainder of the fecal material in the tub for subsequent molecular analysis (27). Both the tube and tub are shipped in one box by the patient to the commercial lab for processing and analysis within 72 h of collection, with the ensuing report sent to the patient. There are no known interfering substances for this test, and there is no bowel preparation. The generated report comes back as positive or negative; however, a positive report does not differentiate on whether the molecular analysis or the FIT portion of the test was positive. A survey of 434 men and women regarding a potential multi-organ sDNA test rated multi-cancer detection, absence of bowel preparation, and safety and noninvasiveness as the most attractive characteristics, and multi-organ sDNA testing was preferred over colorectal-only mt-sDNA testing, which in turn was preferred over FOBT, colonoscopy, sigmoidoscopy, and barium enema (in that order) (31). As of 2019, five years after FDA approval, over two million people have used the test, with originating prescriptions coming from many medical specialties, including family medicine, general medicine, gastroenterology, general surgery, obstetrics/gynecology, and others.

ISSUES REGARDING THE LATEST-GENERATION FECAL DNA TEST

The FDA-approved mt-sDNA test is to be used for average-risk patients over the age of 50 years for CRC screening, and can be repeated every three years for those with negative results (subject to an FDA-mandated follow-up longitudinal study to assess the interval; see **https://www.accessdata.fda.gov/cdrh_docs/pdf13/P130017A.pdf**). The mt-sDNA test is not intended for use outside of average-risk screening, such as in individuals with:

- Personal history of polyps
- Personal history of CRC
- Family history of polyps or CRC
- Risk for genetic diseases, such as familial adenomatous polyposis, Lynch syndrome, and others
- Inflammatory bowel disease (Crohn's disease or ulcerative colitis)

In the above situations, only colonoscopy should be used as the surveillance strategy (5, 9).

Patients and providers should not intermix CRC screening strategies, which can lead to inappropriate or excessive screening. For instance, if a person is getting screening colonoscopies at appropriate intervals (the gold standard), there is no need to order or perform the mt-sDNA test. If a person is getting FIT testing annually, there is no need for the mt-sDNA test unless the patient and provider are consciously upgrading from FIT alone as the long-term screening strategy.

Because of the reduced specificity in the pivotal study (28), there will be some false positives. In one study of 30 patients who had a positive mt-sDNA test with negative colonoscopy findings, 12 patients accepted an offer of repeat mt-sDNA testing 11–29 months later, when five patients

Table 2 Comparison of noninvasive colorectal cancer screening tests

Test	Sensitivity[a]	Specificity[a]	Advantages	Disadvantages
FOBT	+	+	Evidence for mortality reduction from randomized controlled trials	Must be done annually Indirect test (peroxidase)/false positives Dietary modification Three consecutive samples needed Poor sensitivity for advanced adenomas Detects more distal neoplasms
FIT	++	+++	Qualitative and quantitative Detects human hemoglobin Single sample needed Inferred mortality reduction from FOBT studies	Must be done annually Reliability declines with longer time from collection to analysis Sensitivity based on quantitative threshold of hemoglobin Poor sensitivity for advanced adenomas Detects more distal neoplasms
sDNA	+++	++	Detects human DNA Combined with FIT; inferred mortality reduction from FOBT studies Detects right and left neoplasia No dietary restrictions	High per-test cost Interval of three years selected with no data A portion of sensitivity based on quantitative threshold of hemoglobin Improved but still poor sensitivity for advanced adenomas

Abbreviations: FIT, fecal immunochemical test; FOBT, fecal occult blood test; mt-sDNA, multitarget stool DNA; sDNA, stool DNA.
[a]Sensitivity and specificity are low (+), medium (++), or high (+++).

were again positive, and in three of these five, neoplastic lesions were found on repeat colonoscopy (32). This suggests that persistently positive mt-sDNA tests might yield missed or new findings on colonoscopy. It should be noted that the mt-sDNA is a designed CRC screening test. It should not prompt workup for other, noncolonic sources of neoplasia.

Provider knowledge of the mt-sDNA results may affect yield at colonoscopy. One study had providers blinded to the results of 72 mt-sDNA tests and unblinded to 172 others (33). Colonoscopic neoplastic yields were higher in the unblinded group (total adenomas 70% versus 53% in the blinded group; advanced adenomas 28% versus 21%; flat right-sided lesions 40% versus 9%), and colonoscopy withdrawal times were longer (average 19 min versus 13 min in the blinded group) (33). This study suggests that providers of colonoscopy are more diligent in trying to identify neoplasia with a positive mt-sDNA test.

Some studies challenge the conclusion that mt-sDNA is superior to FIT. One study evaluated 3,494 patients with FIT and used the specificities from the mt-sDNA pivotal trial; it was not a head-to-head comparison. This study showed a CRC sensitivity rate of 96.7% and advanced adenoma sensitivity rate of 54.3% for FIT (34). However, these rates appear markedly higher than in multiple previous studies regarding FIT and/or FOBT (5, 9). General comparisons of FOBT, FIT, and sDNA are listed in **Table 2**.

A meta-analysis regarding the performance of sDNA tests examined 53 studies inclusive of 7,524 patients (35). Its conclusion suggests that assays using multiple genes (multiple targets), compared with single-gene assays, did not increase the sensitivity or specificity of sDNA testing for CRC (35). This meta-analysis did not address sensitivity and specificity for high-risk adenomas, where a multitarget sDNA assay may have the advantage. One study evaluated the prevalence of genetic alterations used in the mt-sDNA test and additional markers, comparing CRC patients who were FIT-negative versus FIT-positive (36). The authors noted no difference in the genetic changes between the FIT-negative and FIT-positive groups, meaning the CRCs are expressing

Table 3 Archimedes modeling of effect of screening strategies on CRC incidence, mortality, and costs (39)

CRC screening strategy	Decrease in CRC incidence (%)	Decrease in CRC mortality (%)	Quality-adjusted life-years (QALY) gained	Cost-effectiveness[a] ratios ($/QALY)
No screening	0	0	0	$0
Colonoscopy every 10 years	65	73	0.1330	—
mt-sDNA annually	63	72	0.1290	$20,178
mt-sDNA every 3 years	57	67	0.1160	$11,313
mt-sDNA every 5 years	52	62	0.1050	$7,388

Abbreviations: CRC, colorectal cancer; mt-sDNA, multitarget stool DNA.
[a]Cost used for the mt-sDNA was $600 per test, and colonoscopy was $1,500 per test.

biomarkers independent of FIT positivity (36). A blinded prospective study of 456 asymptomatic adults yielded 29 sessile serrated polyps (SSPs) with a median diameter of 1.2 cm, of which 93% were located in the proximal colon (37). The aberrant methylation of *BMP3* in the mt-sDNA assay proved to be a discriminant factor for detecting SSPs whereas other genetic markers were indiscriminant. By means of assay for aberrant methylation of *BMP3* alone, the detection of SSPs was 66% versus 0% for FIT at the 100-ng-Hb/ml cutoff level (37).

Data from the mt-sDNA pivotal trial were largely limited to a predominantly white population, and other races and ethnicities demonstrated with small numbers relatively similar CRC sensitivity for detection (except black patients) and advanced adenoma detection (27, 28). One study evaluated the mt-sDNA test in 760 patients, of whom 34.9% were black, and evaluated detection sensitivities for adenomas between black and white patients (38). The prevalences of total adenomas as well as advanced adenomas were similar between black and white patients (38.9% and 6.8% for black patients and 33.9% and 6.7% for white patients). The overall mt-sDNA advanced adenoma detection sensitivity was 43% with no differences by race (38). Thus, the mt-sDNA test appears to detect lesions similarly between races.

As mentioned above, the mt-sDNA test reports a positive result when either the FIT portion or molecular portion hits its threshold for positivity, and the report does not distinguish which is positive. With much of the focus for development of the test based on the pathogenesis, technological advances, and science of the molecular portion, it would be interesting to see how either the FIT or molecular assays correspond with the actual finding of neoplasia. At present, this aspect has not been reported.

The interval between mt-sDNA tests was approved by the FDA at three years, and the approval stipulated a follow-up study. One study utilized an Archimedes model to simulate a five-arm virtual clinical screening study comparing the mt-sDNA test (at a $600 per-test cost) at 1-, 3-, and 5-year intervals and colonoscopy at 10-year intervals versus no screening (39). Colonoscopy yielded the largest reduction in CRC incidence and mortality and the largest gain in quality-adjusted life-years, followed by the mt-sDNA tests at 1, 3, and 5 years (**Table 3**). The respective cost-effectiveness ratios for the 1-, 3-, and 5-year mt-sDNA intervals compared favorably to cervical cancer screening and mammography screening cost-effectiveness ratios. The study concludes that a 3-year mt-sDNA test interval provides a decrease in CRC incidence and mortality that is clinically acceptable and cost effective at a $25,000 quality-adjusted life-year willingness-to-pay threshold (39).

COST ANALYSIS AND COMPARATIVE EFFECTIVENESS OF FECAL DNA FOR POPULATION-BASED COLORECTAL CANCER SCREENING

Even prior to the latest-generation mt-sDNA test, simulation models had assessed the cost effectiveness of sDNA tests, utilizing published detection sensitivities (40, 41). Using a cost of $350

for the sDNA test (prior generation), microsimulation modeling indicated that compared to no screening, the gain in life-years by patients using the sDNA test was lower than the gain associated with FOBT. This was true unless the sDNA test was given at an interval of at least three years; however, the overall costs in this case were higher than all other screening strategies (including FOBT, FIT, sigmoidoscopy, and colonoscopy). Screening with the sDNA test at three-year intervals would be cost effective at a per-test cost of $40–$60. Based on the microsimulation modeling, there was no level of sensitivity and specificity for which earlier-generation sDNA testing would be cost effective at $350 per test (40, 41).

The latest-generation mt-sDNA test has also been evaluated for cost and clinical effectiveness. Using a Markov model comparing no screening to FIT every two years, FIT yearly, the mt-sDNA test every three years (at a per-test cost of $260), and colonoscopy every 10 years, Ladabaum & Mannalithana (42) demonstrated that FIT and colonoscopy were more effective (increasing mean quality-adjusted life-years gained per person) and less costly than the mt-sDNA test. Another study using a Markov model compared screening strategies of persons aged 50–75 years based on colonoscopy, sigmoidoscopy, computed tomographic (CT) colonography, FIT, FOBT, and mt-sDNA testing, and demonstrated that colonoscopy was the most effective screening strategy, with 0.022 life-years gained, 1,068 CRCs prevented, and the lowest total costs at $2,861 (43). For comparison, FOBT screening added 0.012 life-years with 547 CRCs prevented at a total cost of $3,164, and mt-sDNA screening added 0.011 life-years with 647 CRCs prevented at a total cost of $4,296. Improved sensitivity or specificity of the mt-sDNA test was not sufficient to close the outcomes gap compared to colonoscopy. This study also demonstrated that unless the cost of mt-sDNA testing was reduced to $29 or less per test and adenoma detection was included in its performance, mt-sDNA testing remained more expensive and less effective than colonoscopy (43).

The US Preventive Services Task Force has done extensive modeling to compare mt-sDNA annually, mt-sDNA every three years, and six other CRC screening strategies: FOBT annually, sigmoidoscopy every five years, FIT annually, CT colonography every five years, sigmoidoscopy every 10 years with annual FIT, and colonoscopy every 10 years (44, 45). Among stool-based tests, FIT strategies predominated on efficiency ratios (defined as the incremental number of lifetime colonoscopies divided by the incremental number of quality life-years gained), whereas FOBT was consistently below efficiency ratios (45). The mt-sDNA performed annually was not a recommended strategy due to efficiency ratios being larger than the benchmark colonoscopy strategy. The mt-sDNA performed every three years added fewer life-years than did the benchmark colonoscopy strategy, and was dominated by other stool-based strategies (45). Among the eight strategies assessed, models of outcomes showed that mt-sDNA testing every three years ranked seventh for life-years gained, CRC deaths averted, complications, and lifetime number of colonoscopies (44).

SUMMARY AND FUTURE OF FECAL DNA TESTING

The latest-generation mt-sDNA test is an achievement based on knowledge of the pathogenesis of colorectal neoplasia coupled with advances in technology that allow detection of minute amounts of human DNA assayed from fecal material. Population-based clinical trials show that its detection of CRCs and adenomas is superior to FIT with slightly lower specificity. The mt-sDNA test does increase patient life-years with every-three-year testing, but at greater cost than other strategies. Significant reductions in cost and/or increases in sensitivity are necessary to make mt-sDNA screening cost effective relative to other testing methods like colonoscopy.

Despite this, the best test for screening average-risk individuals is the test that gets done, and colonoscopy, the gold standard in the United States, is not uniformly utilized for a variety of

reasons. In 2014, the mt-sDNA test was approved by the FDA and its cost covered by the Centers for Medicare and Medicaid Services, and it has been utilized more than two million times over five years. The test is only approved for average-risk CRC screening in those aged 50 years or older. As a second-tier test for CRC screening, the mt-sDNA test can be used as a primary strategy for CRC screening, useful (*a*) if patients refuse or are uncomfortable with colonoscopy as the initial screening strategy, (*b*) if colonoscopy is high risk due to medical morbidity, and (*c*) if colonoscopy is not locally available.

Further technological advances in mutation detection along with additional DNA target selection will likely improve upon the current mt-sDNA test. In one study, *TWIST1* methylation was detected by a combined restriction digital polymerase chain reaction assay, which, in combination with FIT, showed a sensitivity for advanced adenomas of 82.4% (46). This would greatly improve upon the 42.4% advanced adenoma sensitivity of the latest-generation mt-sDNA test. Increased sensitivity in newer-generation tests, particularly for adenomas, would likely increase the clinical efficiency ratio for sDNA tests compared to other screening strategies.

The future may hold other innovations using sDNA tests, particularly once they improve sensitivity for CRC screening. Application toward interval cancer screening (between screening colonoscopies) and follow-up to suboptimal bowel preparation for colonoscopy could be envisioned. Expanded applications in theory with improved sensitivity and specificity of future tests could involve surveillance of CRC in inflammatory bowel disease patients and screening for pan-digestive or aero-digestive tract tumors in specific populations. These potential applications will need to be adequately tested in appropriate clinical trials.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Work on this review was supported by the US Public Health Service (NIH grant CA206010) and the A. Alfred Taubman Medical Research Institute of the University of Michigan (to J.M.C.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

LITERATURE CITED

1. Siegel RL, Miller KD, Jemal A. 2019. Cancer statistics, 2019. *CA Cancer J. Clin.* 69:7–34
2. Nishihara R, Wu K, Lochhead P, et al. 2013. Long-term colorectal-cancer incidence and mortality after lower endoscopy. *N. Engl. J. Med.* 369:1095–105
3. Zauber A, Winawer SJ, O'Brien MJ, et al. 2012. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N. Engl. J. Med.* 366:687–96
4. Shaukat A, Mongin SJ, Geisser MS, et al. 2013. Long-term mortality after screening for colorectal cancer. *N. Engl. J. Med.* 369:1106–14
5. Rex DK, Boland CR, Dominitz JA, et al. 2017. Colorectal cancer screening: recommendations for physicians and patients from the U.S. Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 153:307–23
6. Carethers JM. 2015. Screening for colorectal cancer in African Americans: determinants and rationale for an earlier age to commence screening. *Digest. Dis. Sci.* 60:711–21
7. Ahlquist DA. 2015. Multi-target stool DNA test: a new high bar for noninvasive screening. *Digest. Dis. Sci.* 60:623–33

8. Lin JS, Webber EM, Beil TL, et al. 2012. *Fecal DNA testing in screening for colorectal cancer in average-risk adults: comparative effectiveness review*. Executive summary no. 52. AHRQ Pub. No. 12-EHC022-1. Rockville, MD: Agency for Healthcare Research and Quality. **https://effectivehealthcare.ahrq.gov/sites/default/files/related_files/colorectal-cancer-screening_executive.pdf**. Accessed June 1, 2019
9. Carethers JM. 2014. DNA testing and molecular screening for colon cancer. *Clin. Gastroenterol. Hepatol.* 12:377–81
10. Grady WM, Carethers JM. 2008. Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology* 135:1079–99
11. Carethers JM, Jung BH. 2015. Genetics and genetic biomarkers in sporadic colorectal cancer. *Gastroenterology* 149:1177–90
12. Cancer Genome Atlas Network. 2012. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 487:330–37
13. Carethers JM. 2016. Hereditary, sporadic and metastatic colorectal cancers are commonly driven by specific spectrums of defective DNA mismatch repair components. *Trans. Am. Clin. Climatol. Assoc.* 127:81–97
14. Carethers JM. 2017. Microsatellite instability pathway and EMAST in colorectal cancer. *Curr. Colorectal Cancer Rep.* 13:73–80
15. Carethers JM, Fearon ER. 2015. Molecular subtyping of colorectal cancer: time to explore both intertumoral and intratumoral heterogeneity to evaluate patient outcome. *Gastroenterology* 148:10–13
16. Koi M, Garcia M, Choi C, et al. 2016. Microsatellite alterations with allelic loss on 9p24.2 signify less aggressive colorectal cancer metastasis. *Gastroenterology* 150:944–55
17. Coppede F, Lopomo A, Spisni R, Migliore L. 2014. Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer. *World J. Gastroenterol.* 20:943–56
18. Carethers JM. 2015. Biomarker-directed targeted therapy in colorectal cancer. *J. Digest. Cancer Rep.* 3:5–10
19. Klaassen CH, Jeunink MA, Prinsen CR, et al. 2003. Quantification of human DNA in feces as a diagnostic test for the presence of colorectal cancer. *Clin. Chem.* 49:1185–87
20. Anderson BW, Ahlquist DA. 2016. Molecular detection of gastrointestinal neoplasia. *Gastroenterol. Clin. N. Am.* 45:529–42
21. Sidransky D, Tokino T, Hamilton SR, et al. 1992. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 256:102–5
22. Zou H, Taylor WR, Harrington JJ, et al. 2009. High detection rates of colorectal neoplasia by stool DNA testing with a novel digital melt curve assay. *Gastroenterology* 136:459–70
23. Ahlquist DA, Sargent DJ, Loprinzi CL, et al. 2008. Stool DNA and occult blood testing for screen detection of colorectal neoplasia. *Ann. Intern. Med.* 149:441–50
24. Shen L, Toyota M, Kondo Y, et al. 2007. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. *Proc. Natl. Acad. Sci. USA* 104:18654–59
25. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. 2004. Fecal DNA versus fecal occult blood for colorectal cancer screening in an average-risk population. *N. Engl. J. Med.* 35:2704–14
26. Bailey JR, Aggarwal A, Imperiale TF. 2016. Colorectal cancer screening: stool DNA and other noninvasive modalities. *Gut Liver* 10:204–11
27. Exact Sciences Corp. Cologuard® physician brochure. Madison, WI: Exact Sciences Corp. **https://cdn2.hubspot.net/hub/377740/file-1412311339-pdf/Document__LBL-0260[1].pdf?t=1534449532931**. Accessed June 1, 2019
28. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. 2014. Multitarget stool DNA testing for colorectal-cancer screening. *N. Engl. J. Med.* 370:1287–97
29. Ahlquist DA, Zou H, Domanico M, et al. 2012. Next generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology* 142:248–56
30. Redwood DG, Asay ED, Blake ID, et al. 2016. Stool DNA testing for screening detection of colorectal neoplasia in Alaska native people. *Mayo Clin. Proc.* 91:61–70
31. Yang D, Hillman SL, Harris AM, et al. 2014. Patient perceptions of a stool DNA testing for pan-digestive cancer screening: a survey questionnaire. *World J. Gastroenterol.* 20:4972–79

32. Cooper GS, Markowitz SD, Chen Z, et al. 2018. Evaluation of patients with an apparent false positive stool DNA test: the role of repeat stool testing. *Digest. Dis. Sci.* 63:1449–53
33. Johnson DH, Kisiel JB, Burger KN, et al. 2017. Multitarget stool DNA test: clinical performance and impact on yield and quality of colonoscopy for colorectal cancer screening. *Gastrointest. Endosc.* 85:657–65
34. Brenner H, Chen H. 2017. Fecal occult blood versus DNA testing: indirect comparison in a colorectal cancer screening population. *Clin. Epidemiol.* 9:377–84
35. Zhai R-L, Xu F, Zhang P, et al. 2016. The diagnostic performance of stool DNA testing for colorectal cancer. *Medicine* 95:e2129
36. Levin TR, Corley DA, Jensen CD, et al. 2017. Genetic biomarker prevalence is similar in fecal immunochemical test positive and negative colorectal cancer tissue. *Digest. Dis. Sci.* 62:678–88
37. Heigh RI, Yab TC, Taylor WR, et al. 2014. Detection of colorectal serrated polyps by stool DNA testing: comparison with fecal immunochemical testing for occult blood (FIT). *PLOS ONE* 9:e85659
38. Cooper GS, Markowitz SD, Chen Z, et al. 2018. Performance of multitarget stool DNA testing in African American patients. *Cancer* 124:3876–80
39. Berger BM, Schroy PC III, Dinh TA. 2016. Screening for colorectal cancer using a multitarget stool DNA test: modeling the effect of the interest interval on clinical effectiveness. *Clin. Colorectal Cancer* 15:e65–74
40. Zauber AG, Lansdorp-Vogelaar I, Wilschut J, et al. 2007. *Cost-effectiveness of DNA stool testing to screen for colorectal cancer*. Technology Assessment Report. Rockville, MD: Agency for Healthcare Research and Quality. **https://www.ncbi.nlm.nih.gov/books/NBK285164/**. Accessed June 1, 2019
41. Landsdorp-Vogelaar I, Kuntz KM, Knudsen AB, et al. 2010. Stool DNA testing to screen for colorectal cancer in the Medicare population: a cost-effectiveness analysis. *Ann. Intern. Med.* 153:368–77
42. Ladabaum U, Mannalithana A. 2016. Comparative effectiveness and cost effectiveness of a multitarget stool DNA test to screen for colorectal neoplasia. *Gastroenterology* 151:427–39
43. Barzi A, Lenz HJ, Quinn DI, Sadeghi S. 2017. Comparative effectiveness of screening strategies for colorectal cancer. *Cancer* 123:1516–27
44. Bibbins-Domingo K, Grossman DC, Curry SJ, et al. 2016. Screening for colorectal cancer: US Preventive Services Task Force recommendation statement. *JAMA* 315:2564–75
45. Knudsen AB, Zauber AG, Rutter CM, et al. 2016. Estimation of benefits, burden, and harms of colorectal cancer screening strategies: modeling study for the U.S. Preventive Services Task Force. *JAMA* 315:2595–609
46. Suehiro Y, Zhang Y, Hashimoto S, et al. 2018. Highly sensitive faecal DNA testing of TWIST1 methylation in combination with faecal immunochemical test for haemoglobin is a promising marker for detection of colorectal neoplasia. *Ann. Clin. Biochem.* 55:59–68