

Multitarget Stool DNA for Average Risk Colorectal Cancer Screening

Major Achievements and Future Directions



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KEYWORDS

- Colorectal neoplasms/prevention and control • Colorectal neoplasms/diagnosis
- DNA • Neoplasm/analysis • Early detection of cancer/methods
- Colonoscopy/trends • Proximal colorectal neoplasia
- Precancerous conditions/diagnosis

KEY POINTS

- Multitarget stool DNA testing has been endorsed by the US Preventative Services Task Force as a first-line colorectal cancer screening test.
- The number of providers prescribing multitarget stool DNA testing and the number of patients completing the test have increased exponentially since its approval by the US Food and Drug Administration and Centers for Medicare & Medicaid Services.
- Adherence to multitarget stool DNA testing is approximately 70%; patients with positive multitarget stool DNA test results have high diagnostic colonoscopy completion rates.
- Positive predictive value for colorectal neoplasia in postapproval studies is high.
- The US Food and Drug Administration expanded multitarget stool DNA approval for use in patients 45 to 49 years of age. Next-generation multitarget stool DNA test prototypes portend even higher specificity.

INTRODUCTION: COLORECTAL CANCER SCREENING SAVES LIVES BUT IS UNDERUSED

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States; an estimated 51,000 fatal cases occurred in 2019.¹ Both the incidence

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and mortality of this disease can be reduced by screening.¹ However, despite these demonstrated benefits, CRC screening remains underused; approximately one-third of the screen-eligible population report nonadherence with current guidelines.² To assist patients and clinicians toward greater CRC screening participation, several national organizations such as the US Preventive Services Task Force (USPSTF) and Multi-Society Task Force recommend endoscopic, radiologic, and stool-based CRC screening test strategies beginning at age 50.^{3,4} More recently, the American Cancer Society (ACS) has provided a recommendation to begin CRC screening at age 45.⁵

Of the endorsed screening tests, colonoscopy is the most often used in the United States²; reasons for preference include the simultaneous diagnosis and intervention for colorectal neoplasia (CRN). From an operator perspective, screening colonoscopy has several aspects actively targeted for improvement, including the need to increase sensitivity in the proximal colon^{6–12} and decrease interoperator variability in colonoscopy quality.^{13–16} From a patient perspective, test invasiveness, risk of complications, and the inconvenience of preparation, sedation, and time away from work are commonly cited as barriers to use.¹⁷ Noninvasive screening modalities are endorsed as an alternative to colonoscopy for use in average risk patients. Of noninvasive tests, only guaiac-based fecal occult blood testing has been shown in randomized controlled trials to reduce CRC-related mortality, with modest benefit.^{18–21} Dietary and medication interactions, poor performance in the proximal colon, and low adherence to the required annual screening program have reduced the use of this test.^{22–24} Fecal immunochemical testing (FIT) overcomes some but not all of these limitations, leading to its inclusion in screening guidelines.^{3,4} Relative to colonoscopy, FIT still has reduced sensitivity for right-sided and sessile serrated precursor lesions,^{22,23,25} and similar to fecal occult blood testing, there is persistent poor adherence to required annual testing.^{24,26–29} Other invasive (flexible sigmoidoscopy) and noninvasive (computed tomography colonography) are less widely used.

The multitarget stool DNA (mt-sDNA) assay (Cologuard, Exact Sciences, Madison, WI) is the most recently endorsed noninvasive option for average-risk CRC screening.^{3–5} The mt-sDNA screening is completed at home and requires no cathartic preparation, dietary modification, or medication restriction. Since receiving simultaneous US Food and Drug Administration (FDA) and Centers for Medicare and Medicaid Services (CMS) approval in August 2014, use of mt-sDNA testing has become widespread. Here, we describe the preclinical scientific development supporting mt-sDNA, highlight important achievements and real-world evidence since approval, and provide an outlook on future directions for mt-sDNA in clinical practice.

PRECLINICAL EVIDENCE AND BIOLOGICAL RATIONALE SUPPORT NONINVASIVE SCREENING BY STOOL DNA

The effectiveness of CRC screening as a whole is the product of test sensitivity, compliance, and access. Noninvasiveness and home testing enhances both compliance and access. Optimized sensitivity of noninvasive testing is centered on 2 important biologic observations. First, whereas tumor-associated bleeding can be used to detect CRC, such bleeding is intermittent. Ahlquist and colleagues³⁰ showed decades ago that asymptomatic persons with CRC had wide variability in stool hemoglobin concentrations (Fig. 1A). In contrast with hemoglobin, neoplastic colonocytes are exfoliated into the colon lumen at a more continuous and predictable rate. These cells show increased proliferation, decreased apoptosis, and altered intercellular adhesion. Consistent with these observations, cytokeratin staining was used to show that exfoliated colonocytes were much more abundant in the mucocellular layer overlying CRC

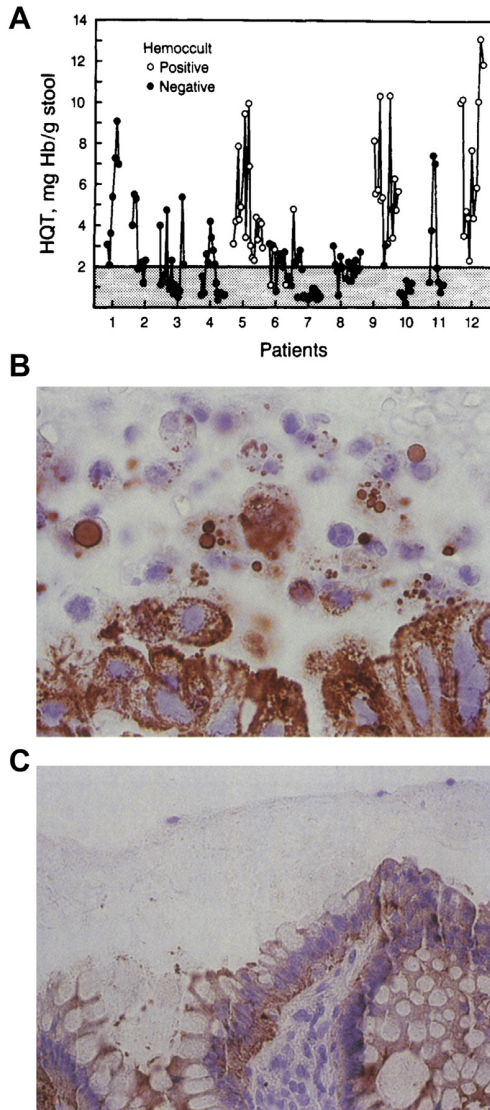


Fig. 1. Preclinical observations supporting the rationale to develop stool DNA testing. (A) Bleeding into stool from asymptomatic CRCs can be intermittent. (B, C, *cytokeratin immunostain*) Neoplastic cells and debris are continuously shed into the luminal mucocellular layer above (B) colon cancers and polyps, but not in (C) healthy control mucosae. HQT, HemoQuant. (Adapted from Ahlquist DA, McGill DB, Fleming JL, et al. Patterns of occult bleeding in asymptomatic colorectal cancer. *Cancer* 1989;63(9):1826-1830; and Ahlquist DA, Harrington JJ, Burgart LJ, et al. Morphometric analysis of the "mucocellular layer" overlying colorectal cancer and normal mucosa: relevance to exfoliation and stool screening. *Human pathology* 2000;31(1):51-57; with permission.)

tissue (Fig. 1B) compared with normal colorectal mucosa (Fig. 1C); tumor-exfoliated cells and cellular debris were also more consistently present in the mucocellular layer than erythrocytes.³¹ Tumor-specific molecular features of CRN have been widely studied and well-characterized over the last 4 decades. Of these, DNA abnormalities

seemed to be the most stable in the harsh stool environment and were thus specifically targeted for noninvasive test development. Early attempts to develop a stool DNA panel assaying primarily mutations were limited by the heterogeneity of CRC.³² However, DNA methylation-based panels were observed to be more broadly informative.³³

For the mt-sDNA assay, marker selection studies ultimately led to a final panel that included DNA methylation (*NDRG4*, *BMP3*), DNA mutation (*KRAS*), and hemoglobin (by immunoassay) as well as a measure of total human DNA (*β-actin*) for clinical assay development.^{34,35} To serve the needs of millions of screen-eligible persons, a high throughput automated assay system was designed. This system was tested in a multi-center case-control study of 93 CRC cases, 114 advanced precancerous lesions (APLs; adenomas with high-grade dysplasia, $\geq 25\%$ villous features, or ≥ 1 cm in diameter; sessile serrated polyps ≥ 1 cm in diameter), and 796 healthy controls.³⁶ Quantitative output from the automated analytical platform was used to derive a multimer logistic regression algorithm.

Locked-down and prespecified to the FDA before pivotal clinical testing, the mt-sDNA algorithm has 3 main components. The first is the logistic score, which is calculated from a logistic regression formula that uses group-level marker data to discriminate between patients with or without CRC or APLs. The second is the sum of scores, which combines the logistic score with individual marker scores to ensure that if the value from any DNA marker exceeds the 99.5th percentile among controls, the assay will yield a positive result. Third, there is a composite score, generated by subjecting the sum of scores to an exponential equation that generates an overall assay result ranging from 0 to 1000, with positive score threshold of 183 or greater. As designed and FDA approved, the mt-sDNA assay analysis platform does not provide independently interpretable marker data. This is because the algorithm yields higher sensitivity estimates than a single marker cutoff approach (positive assay result if ≥ 1 markers exceeds a set threshold) for both CRCs (98% vs 92%) and APLs (56% vs 49%).³⁶

HIGH SENSITIVITY AND SPECIFICITY IN SCREENING STUDY SUPPORT US FOOD AND DRUG ADMINISTRATION AND CENTERS FOR MEDICARE AND MEDICAID SERVICES APPROVAL

The “DeeP-C” pivotal study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01397747) identifier NCT01397747) was a cross-sectional investigation of mt-sDNA assay performance for the screening-setting detection of CRC and APLs in asymptomatic average risk persons, age 50 to 84, recruited from 90 sites in North America from June 2011 to November 2012.³⁷ Complete screening colonoscopy, mt-sDNA, and FIT data were available from 9989 participants. From these patients, 65 CRCs and 757 APLs were identified. Laboratory analyses and colonoscopy examinations were performed in blinded fashion, without prior knowledge of the comparator test results. Colonoscopy and associated histology were the reference standards for measurement of both mt-sDNA and FIT assay sensitivity and specificity. The primary outcome was to determine the sensitivity of mt-sDNA for CRC. The secondary outcomes included sensitivity for APL and noninferiority to FIT.

With respect to CRC detection, the sensitivity of the mt-sDNA assay both significantly exceeded the null hypothesis of 65% and was significantly higher than FIT (92.3% vs 73.8%; $P = .002$). Sensitivity for APLs was also higher in the mt-sDNA assay compared with FIT, both overall (42.4% vs 23.8%; $P < .001$) and in the APL subgroups of high-grade dysplasia (69.2% vs 46.2%; $P = .004$) and sessile serrated polyps greater than 1 cm in diameter (42.4% vs 5.1%; $P < .001$). Furthermore, mt-sDNA detection rates for highest risk polyp denominations by size increases proportionately

(Fig. 2A). Specificities for the mt-sDNA assay and FIT were 87% and 95%, respectively, when non-advanced adenomas (AA) were considered among neoplasia negative results. Test specificity estimates made among patients with negative colonoscopy results were higher at 90% and 96%, respectively. It is important to emphasize that a point-in-time specificity of 90% for mt-sDNA translates into an annualized specificity of approximately 97% because the test is recommended at 3-year intervals, and this finding compares favorably with FIT specificity when test recommended each year. Additional supporting data for nonmarker assay components, technical specifications, and experiments detailing low cross-reactivity in stool samples from patients on various medications and other diseases, including cancers, can be found in the FDA Premarket Approval application (P130017).³⁸

Based on these data and others, the FDA and CMS simultaneously approved the mt-sDNA in 2014; this medical device was the first to have completed the parallel approval process. As part of the FDA–CMS parallel review program, the CMS issued a National Coverage Determination, effective October 9, 2014, for mt-sDNA screening every 3 years in average-risk, asymptomatic, Medicare-eligible patients ages 50 to 85 years. The initial CMS reimbursement rate of \$492.72 was set by CMS using a method that compared mt-sDNA with 3 existing tests on the Clinical Laboratory Fee Schedule. Subsequent CMS reimbursement is based on the volume-weighted median of private payer rates in accordance with the Protecting Access to Medicare Act of 2014.

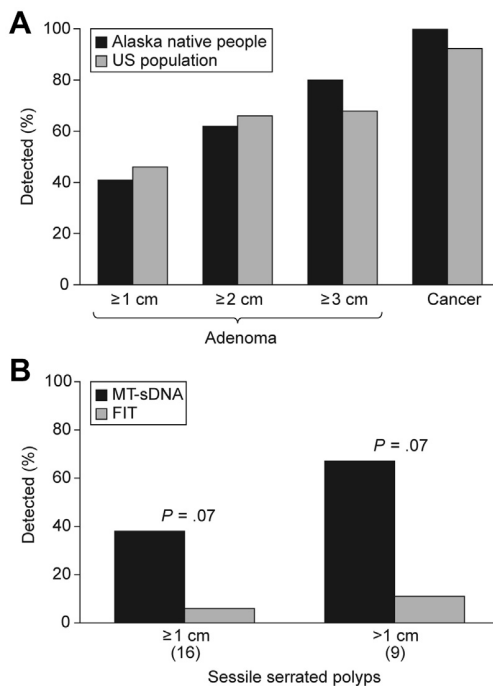


Fig. 2. Detection of CRN by mt-sDNA test in clinical studies. (A) mt-sDNA colorectal neoplasm detection rates by in the United States general and Alaska Native populations. (B) Comparison between mt-sDNA and FIT for sessile serrated polyp detection in Alaska Native people. (Adapted from Redwood DG, Asay ED, Blake ID, et al. Stool DNA Testing for Screening Detection of Colorectal Neoplasia in Alaska Native People. *Mayo Clin Proc* 2016;91(1):61-70; with permission.)

The test price of mt-sDNA also includes additional services beyond the collection, processing, and reporting of the result. These include patient navigation support, which has been shown to improve participation rates.^{39,40} The patient navigation protocol begins with a telephone call from the support center describing mt-sDNA testing and sample collection. Next, a kit is mailed from Exact Sciences Laboratories (Madison, WI) to the patient. Until the kit is returned, telephone and mailed reminders are provided up to 3 times during the first 30 days after the test order. For incoming patient questions, telephone support from a human operator is available 24 hours per day, 365 days per year, with translation services available.

A SECOND SCREENING STUDY AND FAVORABLE BENEFITS TO HARMS SUPPORT US PREVENTATIVE SERVICES TASK FORCE ENDORSEMENT

The second major set of data on mt-sDNA performance in the screening setting was made available in early 2016. Redwood and colleagues⁴¹ conducted a prospective, cross-sectional study of asymptomatic Alaska Native adults aged 40 to 85 years undergoing screening or surveillance colonoscopy, the criterion standard; all received FIT and mt-sDNA. Among 435 patients in the screening group, CRC and APL detection rates were 50% for mt-sDNA and 31% for FIT ($P = .01$). Among all 661 patients, under either screening or surveillance, the sensitivity of mt-sDNA increased with adenoma size and risk for progression, and it exceeded FIT sensitivity at all adenoma sizes. For example, mt-sDNA detected 62% of adenomas 2 cm or larger but only 29% were detected by FIT ($P = .05$). The overall adenoma and cancer detection rates by mt-sDNA were nearly identical between the 2 prospectively done screening studies (see Fig. 2A). For the 9 sessile serrated polyps larger 1 cm or larger, detection by mt-sDNA was 67% but only 11% by FIT ($P = .07$) (Fig. 2B). The point specificity for mt-sDNA was 93% compared with 96% for FIT ($P = .03$).⁴¹

The USPSTF also commissioned a comparative effectiveness modeling report from the Cancer Intervention and Surveillance Modeling Network to inform their 2016 updated recommendation on CRC screening.⁴² Model outputs included the number of life-years gained and CRC deaths averted by screening participation for each modality. For the hypothetical screening cohort, the models also estimated total lifetime number of colonoscopies, the principal source of potential screening harm, and colonoscopy-specific complications related to gastrointestinal and cardiovascular events. Table 1 displays the Cancer Intervention and Surveillance Modeling Network model outputs and the ratios of benefits to harms of 3 screening strategies: colonoscopy performed every 10 years, FIT performed annually, and mt-sDNA performed every 3 years. Although colonoscopy resulted in the most life-years gained and CRC deaths averted, this strategy also resulted in the greatest potential harms. Noninvasive CRC screening with FIT or mt-sDNA produced substantial gains with fewer estimated harms than colonoscopy. Additionally, because mt-sDNA testing is performed less frequently, it resulted in fewer lifetime colonoscopies than FIT.³ Based on these data and others, the June 2016 USPSTF recommendation statement included mt-sDNA among several strategies for CRC screening.

CLINICAL MULTITARGET STOOL DNA TESTING USE IS RAPIDLY INCREASING AND ATTRACTING NEW COLORECTAL CANCER SCREENING PARTICIPATION

Since its commercial launch in late 2014, the number of tests completed has exceeded 3 million through September 2019, which closely parallels the rapid increase in providers enrolled to prescribe mt-sDNA (Fig. 3). Exact Sciences reported that 48% of mt-sDNA patients have not been screened for CRC before. This new-to-screening

Table 1
mt-sDNA screening results in fewer lifetime colonoscopies than FIT and has a superior benefit-to-harm ratio compared with colonoscopy

Modality	Life-Years Gained/ Complications (Ratio)	Life-Years Gained/ Colonoscopy (Ratio)	Deaths Averted ^a / Complication (Ratio)	Deaths Averted ^a / Colonoscopy (Ratio)
mt-sDNA	226/9 (25)	226/1714 (0.13)	20/9 (2.2)	20/1714 (0.01)
FIT	244/10 (24)	244/1757 (0.14)	22/10 (2.2)	22/1757 (0.01)
Colonoscopy	270/15 (18)	270/4049 (0.07)	24/15 (1.6)	24/4049 (0.006)

Values obtained by simulating a hypothetical cohort of 1000 persons participating in a screening program.

^a Deaths attributable to CRC.

population includes those entering programmatic screening at age 50 as well as those who seem to have been previously nonadherent.⁴³

Prince and colleagues⁴⁴ recently demonstrated increased use of mt-sDNA in previously nonadherent average-risk Medicare patients. Among a cohort of nearly 400 persons, 51% were found to have APLs at diagnostic colonoscopy after a positive mt-sDNA test. Notably, the rate of negative colonoscopy in the mt-sDNA-positive patients in this study was only 20%.⁴⁴ Moreover, in a detailed chart review at Mayo Clinic of nearly 2000 mt-sDNA-positive patients in the first 3 years of test availability, approximately 25% both had never received prior CRC screening and were over age 60, suggesting that mt-sDNA may be changing behaviors and attitudes toward participation in CRC screening.⁴⁵ Although it is difficult to imply causality, these

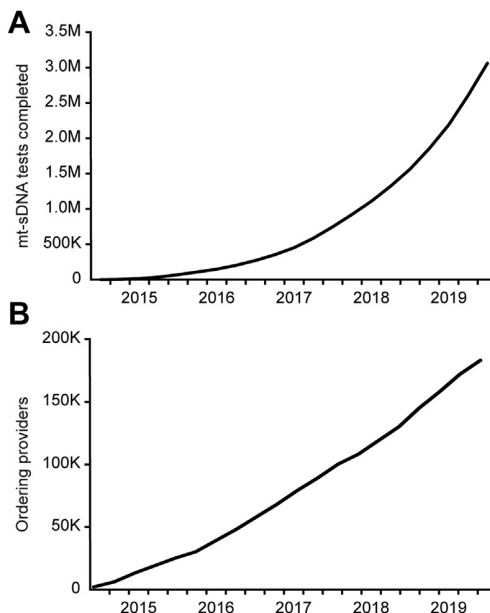


Fig. 3. Use of mt-sDNA testing in clinical practice. (A) Cumulative mt-sDNA tests completed over time and (B) providers who have enrolled to prescribe mt-sDNA. (Courtesy of Exact Sciences Corporation, Madison, WI.)

findings suggest that mt-sDNA is attracting previously nonadherent patients, a goal of great importance to public health efforts to reduce CRC-attributable mortality.

MULTITARGET STOOL DNA TESTING HAS A HIGH POSITIVE PREDICTIVE VALUE IN CLINICAL PRACTICE

In the largest cohorts of patients screened with mt-sDNA, 14% to 16% will have positive results.^{37,45,46} For these patients, a diagnostic colonoscopy is required to exclude CRN. The adherence to diagnostic colonoscopy is thus critically important to the effectiveness of mt-sDNA testing. Estimates of adherence to diagnostic colonoscopy have to date ranged from 75% to 96%^{44,46}; our analysis showed a diagnostic colonoscopy completion rate of approximately 90% in a large multipractice setting.⁴⁵ Nonadherence to recommended diagnostic colonoscopy is multifactorial and commonly related to patient aversion and medical comorbidities that might increase procedural risks, commonly from sedation or procedural intervention.⁴⁶ It is therefore critical that ordering providers provide education to patients before completing mt-sDNA testing to ensure the appropriate patients are selected. Regardless of the initial testing strategy, CRC screening should be discontinued in persons for whom colonoscopy poses an increased risk of harm.⁴⁷

In a recent series, approximately two-thirds of patients were found to have at least 1 neoplastic lesion at diagnostic colonoscopy after positive mt-sDNA.⁴⁵ Among patients with CRN, 40% had at least 1 APL, and approximately 1% had CRC. Sessile serrated adenomas/polyps were detected in about one-half of patients with positive tests. Importantly, mt-sDNA testing in clinical practice seems to recapitulate clinical trial performance in detecting right-sided (proximal) neoplasia. In our multisetting practice, more than 50% of patients overall (and 80% of those with any neoplasm) were found to have proximal CRN at colonoscopy to evaluate positive mt-sDNA.⁴⁵ This high yield of proximal neoplasms is likely due to the infrequency of hemorrhage among these flat lesions⁴⁸ and the more continuous exfoliation of DNA markers during ongoing cellular turnover.³⁵ These data are consistent with a prior head-to-head observation that mt-sDNA is more sensitive than FIT for the detection of screen-relevant neoplasms proximal to the splenic flexure.³⁷

These high point estimates for positive predictive value in clinical practice compared with clinical trials may reflect underlying differences in the populations being studied, or in differences in those opting to prescribe or complete the test. However, 2 additional mechanisms must be considered. Although mt-sDNA and FIT both increase post-test probability when the results are positive, data have emerged to show that endoscopist knowledge of a positive mt-sDNA test significantly increases withdrawal time and adenoma detection rate at diagnostic colonoscopy compared with blinded endoscopists from the same practice.⁴⁹ Moreover, recent data suggest that, although the yield of diagnostic colonoscopy is observed in all endoscopists in a large academic practice, the greatest improvement in polyp detection was seen in providers with the lowest baseline serrated polyp and adenoma detection rates.⁵⁰ This observation indicates that the improvement in the adenoma detection rate is not solely attributable to the increase in post-mt-sDNA probability, implying a change in colonoscopist behavior.

To date, real-world mt-sDNA performance and impact has largely been measured in single-center experiences. New data on mt-sDNA predictive value, screening adherence, and long-term health outcomes are anticipated to come from the prospective Voyage: Real-World Impact of the Multi-target Stool DNA Test on CRC Screening and Mortality study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04124406) identifier NCT04124406). The Voyage study is

currently recruiting toward a target of 150,000 adults prescribed mt-sDNA for routine CRC screening by their health care provider.

DISCORDANT MULTITARGET STOOL DNA TESTING AND COLONOSCOPY: AVOID FURTHER TESTING

Between 7% and 13% of patients tested with mt-sDNA will have a positive mt-sDNA test followed by a negative colonoscopy. There are recent data that provide clarity for patients and providers on the management of a false-positive mt-sDNA test result. In this situation, negative colonoscopy is defined according to the same standards used in the pivotal clinical trial of mt-sDNA. FDA labeling, therefore, defines true-positive mt-sDNA tests only when subsequent screening colonoscopy detected APL or CRC.³⁷ When positive mt-sDNA is followed by a diagnostic colonoscopy showing nonadvanced precursor lesions, mt-sDNA has most likely detected exfoliated DNA from a neoplasm falling below size-based criteria defined by epidemiology rather than biology. Next, providers must ensure that the diagnostic colonoscopy was high quality. Returning to the mt-sDNA clinical trials, only those with high-quality colonoscopy, defined as having good or excellent bowel preparation, photographic evidence of cecal intubation, and a withdrawal time of 6 or more minutes,¹⁵ were included in primary analyses.^{37,41}

The long-term follow-up of such patients found no increased risk of CRC in long-term follow-up of clinical study participants with negative colonoscopy after a positive mt-sDNA test result. A retrospective analysis of approximately 1000 patients with either false-positive or true-negative MT-sDNA tests found no increased rate of aerodigestive (lung and gastrointestinal tract) cancers after a median follow-up of 4 years.⁴¹ Another prospective study evaluated 30 patients with initial false positive mt-sDNA testing after both colonoscopy and esophagogastroduodenoscopy (EGD), who underwent follow-up mt-sDNA, colonoscopy, and EGD 11 to 29 months later.⁵¹ No neoplasms were found on EGD. APLs were found in 2 patients with persistently positive mt-sDNA, both in the proximal colon, and were found by endoscopists aware of the mt-sDNA test result. Although bowel preparation conditions from the index colonoscopy were not reported, the authors concluded that a more careful inspection at colonoscopy would likely have avoided missed colonic lesions.⁵¹

More recently, a multisite retrospective cohort study performed by Berger and colleagues⁵² followed approximately 1200 DeeP-C study patients with negative colonoscopy for a median of 5 years, stratified by negative versus positive mt-sDNA test results. Although 16 aerodigestive cancer were diagnosed by individual chart review and cancer registry query, no increased incidence of aerodigestive cancers was observed in the positive mt-sDNA group ($n = 5$) versus the negative mt-sDNA group ($n = 11$) ($P = .151$). Aerodigestive cancer rates in those with positive mt-sDNA and negative colonoscopy were equivalent to the general population based on Surveillance, Epidemiology, and End Results Program data.⁵² Therefore, based on the currently available evidence, guidelines advise that clinicians should not recommend repeat colonoscopy, EGD, or other further testing in patients with a positive mt-sDNA who have undergone a negative high-quality diagnostic colonoscopy and have no localizing signs or symptoms that would mandate evaluation.⁴

MULTITARGET STOOL DNA TESTING IS A COST-EFFECTIVE TEST FOR COLORECTAL CANCER PREVENTION

Triennial mtSDNA screening is a cost-effective strategy for reducing CRC incidence and mortality.^{53,54} However, we challenge summative statements that mt-sDNA

screening is an inefficient option compared with other strategies on the basis that existing model inputs for relative adherence to CRC screening for any modality and cost for FIT and colonoscopy are inconsistent with real-world observations. Nevertheless, there are existing data that suggest that the required thresholds for efficiency of mt-sDNA are already met. Naber and colleagues⁵³ proposed a threshold of greater than 30% adherence needed for mt-sDNA to be deemed cost effective relative to FIT adherence. A recent study of cross-sectional adherence in a large, national sample ($n = 368,494$) of Medicare beneficiaries reported an mt-sDNA test completion rate of 71%.⁵⁵ This rate exceeds by 58% the modeled FIT screening adherence rate of 45%,⁵⁴ and is well above the proposed cost-effectiveness threshold from Naber and colleagues.⁵³

The cost inputs applied in the Naber study also do not allow for direct comparisons across CRC screening strategies, nor do they account for the required programmatic support and delivery of evidence-based interventions to achieve the desired high levels of CRC screening participation.^{56,57} As previously described, mt-sDNA is the only CRC screening option that includes nationwide patient navigation support to improve the overall experience and facilitate increased adherence. In our opinion, it would be more financially informative and operationally applicable to better model the combined clinical and nonclinical costs associated with each test. For example, Ladabaum and Mannalithara⁵⁴ include the addition of \$153 per cycle to each round of FIT screening for patient support costs in their modeling analyses.

Existing modeling strategies are further limited by incomplete simulation of serrated neoplasia and adenoma progression,⁵³ nor do current input assumptions account for variability in colonoscopy performance, such as operator dependency in the adenoma detection rate, differential risk reduction for proximal versus distal neoplasia, and other patient, polyp, and provider characteristics associated with missed and/or interval lesions^{58,59} that can substantially affect the simulated outcomes of lifetime CRC screening and surveillance.

US FOOD AND DRUG ADMINISTRATION APPROVAL OF MULTITARGET STOOL DNA TESTING HAS BEEN EXPANDED TO YOUNGER PATIENTS

The 2018 ACS Colorectal Cancer Screening Guideline recommended that average-risk CRC screening begin at age 45.⁵ It included the use of mt-sDNA among other stool-based noninvasive tests and structural examination approaches, depending on patient preference and test availability.⁵ The ACS based their recommendation on increasing CRC incidence and mortality rates among patients under age 50,⁶⁰ and the results from microsimulation modeling, which suggested substantial gains in quality-adjusted life-years from CRC screening beginning at age 45.⁵

The ACS also expected that screening tests would perform similarly in adults aged 45 to 49 compared with adults aged 50 or older. Data to support this assumption have been lacking until recently. Through September 2018, there had been 2241 completed mt-sDNA tests (through Exact Sciences Laboratories) in individuals aged 45 to 49. Although it was unknown if these patients were at average risk, only 7.4% (165/2241) had a positive result and 92.6% (2076/2241) had a negative result. Although colonoscopy results are not available for either mt-sDNA–positive or –negative patients, these proportions indicate that the specificity of mt-sDNA in this age group cannot be less than 92.6%, which is comparable with the specificity of patients aged 50 to 59 from the DeepP-C study.³⁷ This observation was included in the FDA decision in September 2019 to expand approval for mt-sDNA use in patients aged 45 to 84.⁶¹

After the label expansion, new data are beginning to address marker representation and specificity of mt-sDNA in patients aged 45 to 49. First, we conducted a retrospective study of DNA markers (*KRAS*, *BMP3*, *NDRG4*) included in the mt-sDNA assay to quantify and compare tissue marker levels in sporadic CRC cases and normal colon controls from patients in 45- to 49-year-old and 50- to 64-year-old age groups. DNA extracted from CRC tissue in the 45 to 49 ($n = 90$) and 50 to 64 ($n = 96$) age groups were compared with normal colon samples from adults aged 45 to 49 ($n = 76$) and 50 to 64 ($n = 92$). These samples were amplified by the Quantitative Allele-specific Real-time Target and Signal amplification assay, which is FDA approved for mt-sDNA. The Quantitative Allele-specific Real-time Target and Signal amplification products with *KRAS* mutations or *BMP3* and *NDRG4* methylation were not statistically different between cases of the younger versus older groups or between cases and controls.⁶² A clinical study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03728348) identifier NCT03728348) has also recently completed enrollment of more than 983 patients aged 45 to 49 at average risk for CRC. The primary end point—the specificity of the mt-sDNA test in the 45 to 49 age group in an average-risk population using colonoscopy with histopathology as the reference method—is anticipated to be reported in early 2020.

NEXT-GENERATION MULTITARGET STOOL DNA TESTING SHOWS HIGH SPECIFICITY

Next-generation mt-sDNA testing is also anticipated to have high specificity. Since the development of the mt-sDNA assay, next-generation sequencing has led to the discovery and development of hundreds of novel methylated DNA targets with strong association to CRC and precursor lesions.⁶³ To further improve CRC screening effectiveness, we conducted a blinded case-control study evaluating the accuracy of a mt-sDNA panel of novel, highly discriminant methylated DNA markers for CRC detection. Using a panel of 3 novel methylated DNA markers and FIT in archival stool samples from 117 CRC, 120 AA, 161 non-AA, and 327 controls, the cross-validated area under the curve was 0.97 for CRC and 0.84 for AA. At 92% (95% confidence interval, 88%–94%) specificity, the sensitivity of the panel was 92% (95% confidence interval, 86%–96%) for CRC.⁶⁴ These provocative results from next-generation mt-sDNA testing (mt-sDNA 2.0) will be prospectively validated in a large cross-sectional study, now open to enrollment. Clinical Validation of An Optimized Multi-Target Stool DNA (mt-sDNA 2.0) Test for Colorectal Cancer Screening "BLUE-C" ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04144738) identifier NCT04144738) will enroll up to 12,500 patients 40 years of age and older, who will complete the mt-sDNA 2.0 test and the commercially available FIT, followed by completion of a screening colonoscopy. Like the DeeP-C study, the results of the mt-sDNA screening test and FIT will not be provided to investigators,

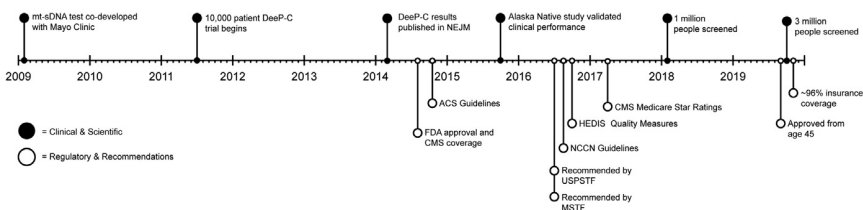


Fig. 4. Timeline of major milestones in mt-sDNA testing for CRC screening. FOBT, fecal occult blood test; HEDIS, Healthcare Effectiveness Data and Information Set; MSTF, US Multi-Society Task Force; NCCN, National Comprehensive Cancer Network; NEJM, *New England Journal of Medicine*.

and personnel performing the colonoscopy will also remain blinded to the results of the mt-sDNA 2.0 test results. Primary outcomes include the sensitivity and specificity of mt-sDNA 2.0 for CRC and secondary end points include sensitivity for AA, comparison with FIT, and specificity calculated from patients with non-neoplastic colonoscopy results.

SUMMARY

Although the scientific underpinnings of DNA-based CRC detection are now decades old, the last 10 years have seen rapid advancement of mt-sDNA from preclinical laboratory benchwork to wide dissemination in clinical practice ([Fig. 4](#)). Screen-setting studies have shown high sensitivity for CRC and APL by mt-sDNA, which is now included in major society guidelines and endorsed by USPSTF as a first-line CRC screening test. Uptake of mt-sDNA testing has increased exponentially since approval by the FDA and CMS. Emerging real-world data are encouraging. Adherence to mt-sDNA testing is approximately 70% and patients with positive mt-sDNA test results have high diagnostic colonoscopy completion rates in single-center studies. The positive predictive value for CRN in postapproval studies is high, possibly owing to increased endoscopist attention during diagnostic colonoscopy, as measured by withdrawal times and improvements in lesion detection rates. Patient with negative high-quality diagnostic colonoscopy after positive mt-sDNA do not seem to be at an increased risk for cancers of the gastrointestinal tract and do not require additional testing beyond what is directed by symptoms. The mt-sDNA test is cost effective in comparison with no screening and we anticipate that modeling studies that use more accurate inputs for CRC screening test costs and more accurately reflect the known variability in colonoscopy performance will show that mt-sDNA is efficient compared with other options. Based on high specificity of mt-sDNA in patients aged 45 to 49, the FDA recently expanded mt-sDNA approval to include this age range. A large cross-sectional study is now underway to validate the mt-sDNA 2.0 test, which is anticipated to maintain high sensitivity for CRC and APL and show even greater specificity than the first-generation mt-sDNA test.

DISCLOSURE

Mayo Clinic and Exact Sciences Corporation (Madison, WI) own intellectual property under which Dr J.B. Kisiel is listed as an inventor and may receive royalties in accordance with Mayo Clinic policy. Dr P.J. Limburg serves as Chief Medical Officer for Exact Sciences through a contracted services agreement with Mayo Clinic. Dr P.J. Limburg and Mayo Clinic have contractual rights to receive royalties through this agreement. Dr J.D. Eckmann has no conflicts to disclose.

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