Specificity of the Multi-target Stool DNA Test for Colorectal Cancer Screening in Average-risk 45-49 Year-Olds: A Cross-sectional Study

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Abstract

High-specificity colorectal cancer (CRC) screening is desirable to triage patients <50 years for colonoscopy; however, most endorsed CRC screening tests have not been rigorously evaluated in younger populations. This prospective cross-sectional study determined the specificity of the multi-target stool DNA (mt-sDNA) test in an average-risk screening population of 45-49 year-olds. Specificity was the primary outcome and was measured in participants without CRC or advanced precancerous lesions (APL– advanced adenomas [AA], and sessile serrated lesions ≥10 mm), and in the subgroup of participants with negative colonoscopic findings. APL sensitivity was a secondary outcome. The evaluable cohort included those who completed the study without protocol deviations and had a usable mt-sDNA test. Of 983 enrolled participants, 816 formed the evaluable cohort, with a mean age of 47.8 (SD 1.5) years; 47.7% were women. No participants had CRC, 49 had APL, 253 had nonadvanced adenomas (NAA), and 514 had negative colonoscopic findings. Mt-sDNA test specificity was 95.2% (95% CI, 93.4-96.6) in participants with NAA or negative findings, 96.3% (CI, 94.3-97.8%) in those with negative findings, and did not differ by sex (p=0.75) or race (p=0.36) in participants with NAA or negative findings. Sensitivity for APL was 32.7% (CI, 19.9-47.5%), with most APL (83.7%) measuring 10-19 mm and none having high-grade dysplasia. The area under the ROC curve for discriminating between APL and lesser findings was 0.72 (CI, 0.64-0.81). Mt-sDNA's high specificity would help minimize risk from unnecessary diagnostic procedures in this age group.

Introduction

Colorectal cancer (CRC) is the fourth most commonly diagnosed and second deadliest cancer in the United States in men and women combined [1]. In 2018, over 140,000 diagnoses and 50,000 fatalities from CRC were estimated [2]. While the incidence of CRC in individuals aged 55 years and older has been decreasing since the mid-1980s, it has been increasing in individuals 40 to 49 years old since the mid-1990s [3]. Between 2005 and 2014, CRC mortality increased in those 55 years old and younger [4], while colon cancer incidence rates increased 1.3% per year and rectal cancer rates increased 2.3% per year in adults ages 40 to 49 years between 2005 and 2013 [3]. Consequently, the current American Cancer Society guidelines recommend average-risk CRC screening beginning at age 45 years [5], although other major guidelines recommend initiating average-risk CRC screening at 50 years in most demographic groups [6-7].

While increasing, CRC in the 45-49 year old age group remains very uncommon, with a population prevalence that is half of that in persons aged 50-54 years (33.1 cases per 100,000 versus 59.5 cases per 100,000) [8]. The low population prevalence in this age group requires a screening test to have high specificity (along with good sensitivity) in order to minimize the number of false-positive tests, which would generate unnecessary and invasive diagnostic colonoscopy with its cost and risk that could outweigh the benefits. However, to date, most of the currently endorsed CRC screening tests have not been rigorously studied in younger age groups.

The multi-target stool DNA (mt-sDNA) test was approved by the Food and Drug Administration (FDA) in 2014 for screening average-risk individuals ≥50 years old for CRC. A

recent label expansion in 2019 approved the mt-sDNA test for average-risk individuals 45-49 years old. The mt-sDNA test is a noninvasive option recommended by the American Cancer Society, the United Services Preventive Services Task Force (USPSTF) and other guideline organizations for average-risk CRC screening [5-7]. The mt-sDNA test detects biomarkers associated with advanced colorectal neoplasia (CRC and advanced precancerous lesions). A positive test requires a colonoscopy. In a study of nearly 10,000 evaluable participants ages 50 and older at average risk of CRC, the mt-sDNA test was 92% sensitive for CRC and 87% specific among participants with nonadvanced adenomas (NAA) or negative findings on colonoscopy, while the sensitivity for advanced precancerous lesions (APL) was 42% [9].

In this study (NCT03728348), we evaluated the performance of the mt-sDNA test in average-risk participants ages 45-49 years. The primary aim was to quantify the specificity of the mt-sDNA test. A secondary aim was to determine the sensitivity of the mt-sDNA test for CRC and advanced precancerous lesions (APL).

Methods

We conducted a prospective, cross-sectional study of average-risk 45-49 year olds who were interested in having a screening colonoscopy. Potential study participants were identified through advertisement. The study sponsor, Exact Sciences, provided advertising materials for utilization by the sites. Each site was also allowed to develop its own materials for dissemination, which included flyers/posters, radio, and social media to recruit participants. Additionally, Exact Sciences also conducted its own recruitment campaign through the clinical research organization (CRO) via online advertisement. Each mode of advertisement required

approval by both the sponsor and the Institutional Review Board. Although the sponsor

provided materials, sites were not required to advertise for the study.

Participants were enrolled at 31 sites in the United States from November 2018 through

June 2019. Institutional Review Board (IRB) approval was obtained from Copernicus Group

IRB, and all participants provided written informed consent prior to any study-related

procedures. The study was conducted in accordance with legal and regulatory requirements, the

general principles set forth in the International Ethical Guidelines for Biomedical Research

Involving Human Subjects (Council for International Organizations of Medical Sciences 2002),

the Declaration of Helsinki (World Medical Association), and applicable local regulatory

requirements and laws.

Study Population

Individuals aged 45 to 49 years who were average risk for CRC were eligible for

enrollment. Other than age, inclusion and exclusion criteria were identical to those of the mt-

sDNA pivotal study (DeeP-C; NCT01397747) [9]. To target average-risk persons, we excluded

from consideration persons with overt rectal bleeding; a positive fecal occult blood test (FOBT)

or fecal immunochemical test (FIT) within the 6 months prior to enrollment, previous

colonoscopy, or those who had undergone a double-contrast barium enema, CT colonography, or

flexible sigmoidoscopy within 5 years of enrollment; prior colorectal resection for any reason

other than sigmoid diverticular disease; a personal history of CRC or adenoma; a personal

history of familial adenomatous polyposis (FAP), Lynch Syndrome, or other hereditary cancer

syndromes; a history of aerodigestive tract cancer; 2 or more first-degree relatives diagnosed

with CRC; a first-degree relative diagnosed with CRC before age 60; a family history of FAP or

Lynch Syndrome; Cronkhite-Canada Syndrome; inflammatory bowel disease including chronic ulcerative colitis and Crohn's disease; any condition that, in the opinion of the investigator, precluded participation in the study; or who were unwilling or unable to provide informed consent.

Procedures

Participants completed the mt-sDNA test, followed by a screening colonoscopy within approximately 60 days of enrollment. Stool was collected prior to bowel preparation procedures for colonoscopy. Stool samples were shipped to Exact Sciences Laboratories (Madison, WI) for processing. Mt-sDNA test results were recorded as "positive," "negative," "sample could not be processed," or "no result obtained." For samples not collected according to the instructions for use or if there was no valid test result, a repeat sample was requested if collection could occur prior to the initiation of bowel preparation. A positive mt-sDNA test result was based on the FDA-approved logistic regression algorithm threshold score of ≥ 183; the algorithm is published as supplemental material [9]. The algorithm and threshold score were prospectively determined and locked prior to analyzing pivotal trial data and, since the mt-sDNA test is qualitative, the component values of the mt-sDNA test are not reported separately [10]. The test includes molecular assays for aberrantly-methylated *BMP3* and *NDRG4* promoter regions, mutant *KRAS*, and *ß-actin* (a control gene for DNA quantity), and an immunochemical assay for human hemoglobin, none of which have individual thresholds or cutoffs.

Bowel preparation and colonoscopy were performed according to usual practice at each clinical site. All colonoscopies were performed blinded to mt-sDNA results. Source documentation included the quality of bowel preparation, cecal intubation, colonoscope withdrawal time [11], and colonoscopy findings including histopathology results for any excised

or biopsied lesion. Lesion location (proximal, distal, or rectal) and size (mm) were recorded for all CRCs and APLs. Participants were characterized based on the histopathologic diagnosis of their most clinically significant lesion (the index lesion). The proximal colon was defined as the cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, right colon not otherwise specified, or an insertion depth >60 cm. The distal colon was defined as the descending colon, sigmoid colon, recto-sigmoid colon, left colon not otherwise specified, or an insertion depth of 16 to 60 cm, inclusive. The rectum was defined as the rectum or an insertion depth of 0 to 15 cm, inclusive.

Histopathology was analyzed from biopsy and surgical specimens according to each site's local surgical pathologist. Index lesions were categorized as CRC, APL (high-grade dysplasia/carcinoma in situ of any size, villous growth pattern [≥25%] of any size, adenomas ≥10 mm, and serrated lesion ≥10 mm), nonadvanced adenoma (NAA), non-neoplastic findings (hyperplastic polyps, lymphoid aggregates, others), and negative (no colorectal neoplasia, no findings on colonoscopy, no biopsy taken). Diagnoses of advanced colorectal neoplasia (CRC or APL) required confirmation a central pathologist, with discrepant findings adjudicated through review and interpretation by a second central pathologist. All pathologists were blinded to mt-sDNA test results.

Outcomes and Measures

The primary outcome was the specificity of the mt-sDNA test with colonoscopy as the reference standard. Specificity for advanced colorectal neoplasia was the primary endpoint. Specificity for any precancerous lesion, advanced or nonadvanced, was also measured. We report APL sensitivity as a secondary outcome, despite the study's low prevalence for a precise

estimate. We compared the area under the receiver operating characteristic (ROC) curve (AUC) [12] and 95% CI for APL in 45-49 year-olds to the historical AUC and 95% CI for APL in ≥50 year-olds from the mt-sDNA pivotal study [9].

Statistical Analysis

The primary analysis required a one-sided 97.5% lower bound for specificity of the mt-sDNA test to exceed 85.0%, which was the lower bound acceptability criteria for specificity from the mt-sDNA test pivotal study [9]. The minimum number of non-advanced neoplasia and negative subjects needed to rule out an 85.0% lower bound on the specificity with 90% power for a one-sided test with 2.5% Type 1 error according to an exact binomial distribution was determined to be 225. A minimum sample size of 225 evaluable subjects (age 45-49 inclusive) was required to adequately power the primary analysis. This minimum sample size requirement was adjusted to 731 non-advanced neoplasia and negative subjects to more precisely estimate the specificity by narrowing the CI.

We included participants in the primary analysis if they completed the study without major protocol deviations, had a usable mt-sDNA test, and had a complete colonoscopy (the evaluable cohort). We also report analyses for participants who had a valid or usable mt-sDNA test and a reportable or complete colonoscopy (the intent-to-screen cohort). Participants with stool samples received outside of the 72-hour processing window for the mt-sDNA test and major protocol violations (did not meet inclusion/exclusion criteria) were excluded from the evaluable cohort but included in the intent-to-screen cohort.

Mt-sDNA test specificity analysis confidence intervals were calculated using the exact binomial (Clopper-Pearson) method to compute the one-sided 97.5% confidence lower bound.

Test performance by race, ethnicity, age, and gender subgroups were analyzed using chi-square tests or Fisher's exact test if the sample size for the categories being compared was <5. All CIs are reported as two-sided 95.0% CI. For the specificity primary endpoint, the lower bound of the two-sided 95.0% CI corresponds to a lower one-sided 97.5% CI.

Results

Study Population

From 31 sites, 983 non-consecutive participants were enrolled after providing informed consent; 876 participants underwent colonoscopy and submitted a stool sample, with 842 participants included in the intent-to-screen cohort and 816 participants included in the evaluable cohort (Figure 1). Fifty-three participants submitted a stool sample but were excluded because they did not undergo colonoscopy, while 4 participants were excluded who underwent colonoscopy but did not submit a stool sample. The evaluable cohort had a mean age of 47.8 (SD 1.5) years and was 47.7% female. All enrolled participants, the intent-to-screen cohort, and the evaluable cohort were similar in age, sex, and race/ethnicity (Table 1). Colonoscopy was completed in 89.6% of participants, with 742 (90.9%) having "good" or "excellent" quality bowel preparation. The mean time from the first mt-sDNA stool sample collection to first colonoscopy was 27 (SD 19.4) days (Supplemental Table S1). Among 947 enrolled subjects who received a mt-sDNA test, 19 had an uninterpretable result, for a test failure rate of 2.0%.

Among all evaluable participants, 53 (6.5%) participants had a positive mt-sDNA test. No participants in the evaluable cohort had CRC, while 49 (6.0%) had APL, and 767 (94.0%)

had either nonadvanced neoplasia (n=253) or negative findings on colonoscopy (n=514) (Figure 1). Of the APL, none had high-grade dysplasia/carcinoma in situ, 20.4% were adenomas characterized as villous or tubulovillous, 65.3% were adenomas ≥10 mm in size without other advanced features, and 14.3% were serrated lesions ≥10 mm in size. Index lesion distributions were similar in the intent-to-screen cohort and all enrolled participants (Supplemental Table S2). The majority (83.7%) of APL were 10-19 mm in size, with only 1 APL <10 mm. APL were distributed among the proximal colon (40.8%), distal colon (42.9%), and rectum (16.3%) (Supplemental Table S3).

Multi-target Stool DNA Test Performance

Among the 767 participants with either NAA or negative findings, mt-sDNA test specificity was 95.2% (CI, 93.4-96.6%) (Table 2). Specificity did not differ by sex (94.9% male vs 95.4% female; p=0.75) or race (94.5% white vs 98.9% black or African American; p=0.36 across all races). When considering only the 514 participants with negative findings, specificity was 96.3% (CI, 94.3-97.8%) (Table 2). Due to the narrow age range examined in this study, variations in specificity by age were not analyzed.

Mt-sDNA test sensitivity for APL was 32.7% (95% CI, 19.9-47.5%), detecting 16 of 49 APL (Table 2). The lack of CRC and low prevalence of APL did not permit further estimation of sensitivity by APL size or location. Based on APL test characteristics and 6.0% prevalence, other relevant study sample metrics include a positive predictive value of 30.2% (CI, 18.3%-44.3%), a negative predictive value of 95.7% (CI, 94.0%-97.0%), a positive likelihood ratio of 6.77 (CI, 4.06-11.28), and a negative likelihood ratio of 0.71 (CI, 0.58-0.86). The AUC was 0.72 (95% CI 0.64-0.81) for discrimination between APL and lesser findings (NAA or negative findings) in

participants 45-49 years (Figure 2), nominally comparable to the historical AUC of 0.73 (95% CI 0.69-0.74) for participants 50 years and older [9]. The mt-sDNA collection kit incurred no adverse effects among the study participants.

Discussion

Performance characteristics of CRC screening tests in average-risk individuals ages 45-49 years are largely unknown. In this prospective, cross-sectional study of 983 participants, we examined the performance of the guideline-endorsed [5-7] mt-sDNA test in average risk participants aged 45-49 years with all participants undergoing colonoscopy as the reference standard. The mt-sDNA test demonstrated a high specificity (95.2%) in this age group, which is higher than in persons who are 50 years and older. The higher specificity in this younger age group is consistent with the expected lower prevalence of any colorectal neoplasia, lesions that cause bleeding, and lower background methylation in stool samples [13].

Test specificity, rather than sensitivity, was the primary outcome of this study for 2 reasons. First, the expected prevalence of CRC and APLs is lower in the 45-49-year-old age group. For a screening test to be viable in a low prevalence setting, it requires high specificity to minimize the costs and burdens resulting from false-positive test results. Our findings of no CRC in more than 800 evaluable participants, along with a low prevalence of APLs, supports this contention. Such low prevalence of advanced neoplasia precludes the feasibility of estimating mt-sDNA test sensitivity as the primary endpoint, since the required sample size needed to quantify sensitivity for CRC and APLs with reasonable precision would be nearly 20 times that of the current study. Second, there is no reason to expect a difference in CRC sensitivity for

persons aged 45-49 years from that in older persons, especially when the majority of early-onset CRC are located in the distal colon and rectum [14-15]. Further, in a recent study comparing tissue markers in CRC cases aged 40-44, 45-49, and 50-64 years, there were no statistically significant age-related differences in tissue distributions of *NDRG4*, *BMP3*, and *KRAS* [16]. Last, the 32.7% sensitivity for APLs is consistent with that of the pivotal study, although the relatively lower prevalence reduces the precision for this comparison.

With the ACS guidelines recommending initiation of average risk CRC screening at age 45 [5], a noninvasive test with high specificity, such as the mt-sDNA test, is required to optimize resource utilization and to minimize the risk from more invasive procedures. Using noninvasive screening in this age group would identify individuals most likely to benefit from colonoscopy, thereby mitigating the impact of potential diversion of colonoscopy resources from higher risk patients to screening younger individuals with lower risks of CRC and APL (as evidenced by the current data showing no CRCs and low prevalence of APL in this study population), as the latter are less likely to benefit from colonoscopy. Further, patients under age 50 may be resistant to an invasive screening procedure but more amenable to a noninvasive test.

The mt-sDNA test, guaiac-based fecal occult blood test (gFOBT), and fecal immunochemical test (FIT) represent the noninvasive CRC screening options currently endorsed by major guidelines [5-7]. In a large study comparing the mt-sDNA test to FIT in average risk individuals 50 years and older, the mt-sDNA test detected significantly more CRC and APL than FIT, while FIT had fewer false positive results [9]. In the referenced study, however, mt-sDNA test specificity in 45-49 year-olds was 95.2%, which is similar to that of FIT in individuals ages 50-85 years (96.4%) [9] and 40-49 years (97.4%) [17]. While CRC screening with the mt-sDNA test is recommended every 3 years, [5,7,18] gFOBT/FIT screening requires annual testing [5-6].

Increased test frequency may be burdensome to patients and negatively impact longitudinal adherence. Over 3 to 5 years, adherence to annual gFOBT ranges between 14% and 48% [19-22]. A high-performing, noninvasive test with a longer re-screening interval may be preferred for screening younger, average-risk individuals [23], as it balances the need to utilize colonoscopy resources efficiently with the potential for a higher degree of adherence with guideline recommendations.

We observed an APL prevalence of 6.0% in average-risk participants 45-49 years of age, which is consistent with a recent study that found an APL prevalence of 6.4% [24]. Karsenti et al, found that APL prevalence in average-risk 45-49 year-olds was almost twice that in 40-44 year-olds.

Based on the test characteristics observed in this study population, we can speculate on how the mt-sDNA test would perform clinically in average-risk 45-49 year-olds. We observed a mt-sDNA test sensitivity of 32.7% for APL. As no participants with CRC were identified in the study, consistent with the low reported prevalence of CRC in this age group [25], the sensitivity of the mt-sDNA test for CRC could not be determined. Given a 6% prevalence of APL, a negative mt-sDNA test result reduces the risk of APL to 4.3% while a positive result increases APL risk 5-fold to 30.2%. If we assume a prevalence of CRC of 0.11% (Prevalence = incidence × duration; (31.4 cases/100,000 annually [5]) × (average 3-4 year duration [26]) yields a prevalence between 0.09%-0.13%, or an average of 0.11%.) in this age group and further assume CRC sensitivity of 92.3% based on performance in persons 50 to 85 years old [9], then a positive mt-sDNA test increases CRC risk from 0.11% to 1.5% (a greater than 10-fold increase), while a negative result reduces CRC risk to 0.01% (a 10-fold reduction). While the assumption of CRC sensitivity requires validation, these calculations suggest that the mt-sDNA test potentially has a

clinically important post-test effect on both CRC and APL risk when positive and on CRC risk when negative. Further, as compared with screening this age group with colonoscopy, mt-sDNA would reduce this need by (1-0.065= 0.935) 93.5%, while potentially detecting nearly all CRC in a single application if CRC sensitivity of mt-sDNA is no different in 45-49 year olds.

Limitations of this study warrant comment. One limitation is the low prevalence of advanced neoplasia (CRC and APL) observed in this age group, which precludes a precise estimate of mt-sDNA sensitivity. No study participants were found to have CRC or high-grade dysplasia. As adenomas with high-grade dysplasia are the category of APL most likely to develop into CRC, the lack of this finding limits the interpretation of the APL sensitivity reported here; no other mt-sDNA sensitivity data in 45-49 year-olds are reported. Nevertheless, the AUC for discrimination between APL and lesser findings is consistent with the AUC reported in participants 50 years and older [9], suggesting that mt-sDNA test performance is similar in 45-49 year-olds. A second potential study limitation is selection bias, as persons enrolled in this study self-selected to complete screening colonoscopy. We do not know how representative this population is and whether it represents the average-risk spectrum of adults 45-49 years old. CRC screening guidelines have not previously included this age group; therefore, characteristics associated with CRC screening adherence in younger patients have not been well defined. While no data indicate that mt-sDNA test performance is affected by patient health, we cannot fully dismiss the possibility that the participants who agreed to enroll in this study may not represent the larger average-risk 45-49 year-old population.

Beyond study-specific limitations, those specific to the mt-sDNA test itself require consideration. Although cost has not been formally considered by the U.S. Preventive Services Task Force in its guideline recommendations, the cost difference between FIT ($25-35) and mt-

sDNA ($595-$695) is substantial. Compared to no screening, mt-sDNA is considered to be cost-effective [18, 27]; however, when compared to any other screening test, incremental cost-effectiveness ratios exceed thresholds for cost-effectiveness under conditions of high screening adherence [28]. A second concern of mt-sDNA use has been the scenario where colonoscopy is "negative" (i.e., no polyps), but the mt-sDNA test is positive. Such discordant findings raise the question of whether an evaluation for other aerodigestive cancers is required. Among 1,216 persons with a negative colonoscopy 205 (16.7%) of whom had discordant test results, there was no difference in the incidence of aerodigestive cancers between discordant and concordant groups with a 5.3-5.4 year median follow-up, nor were the numbers of observed aerodigestive cancers different from the numbers expected based on SEER data [29]. Lastly, this study does not consider the important issues of patient preference, uptake, adherence, and cost, all of which are required to understand real-world performance of this (and any) screening test. Studies of mt-sDNA test uptake and its CRC sensitivity, in particular, are warranted in persons under 50 years to better characterize test performance in this age group.

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Legends for Figures

Figure 1. Flowchart for Evaluable Cohort

Figure 2. Receiver Operating Characteristic Curves for Multi-target Stool DNA Test Specificity. **A**, ROC for the evaluable cohort discriminating between advanced precancerous lesions and lesser findings (nonadvanced adenoma and negative colonoscopy). **B**, ROC for the evaluable cohort discriminating between advanced precancerous lesions and negative colonoscopy. AUC, area under the receiver operating characteristic; ROC, receiver operating characteristic.
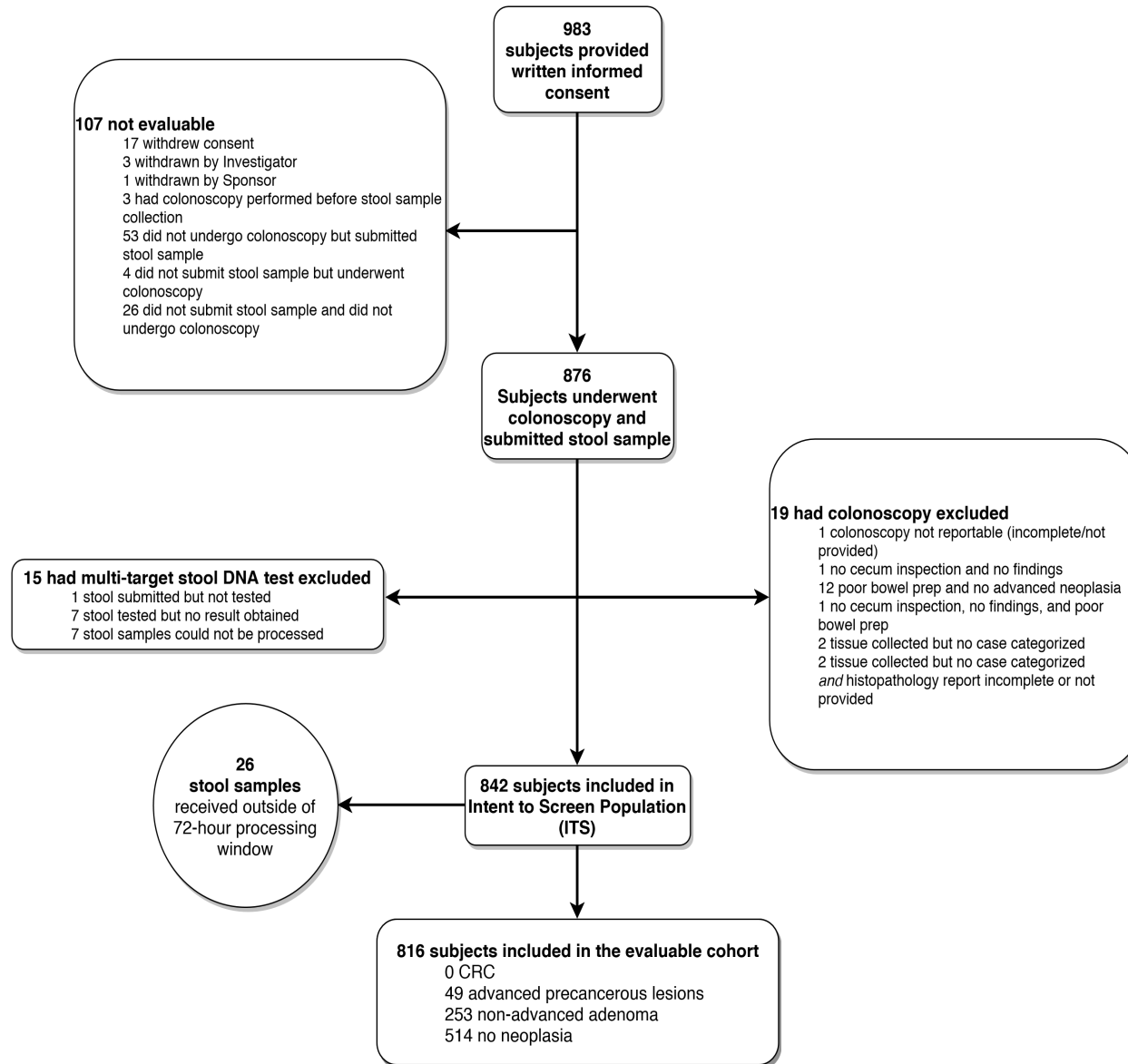
Tables

Table 1. Patient Demographics for Analysis Cohorts

Demographic Feature	All Enrolled Participants N=983	Intent-to-Screen Cohort[a] N=842	Evaluable Cohort[b] N=816
Age (years)			
Mean (SD)	47.8 (1.5)	47.9 (1.5)	47.8 (1.5)
Sex, n (%)			
Male	518 (52.7%)	440 (52.3%)	427 (52.3%)
Female	465 (47.3%)	402 (47.7%)	389 (47.7%)
Race, n (%)			
White	803 (81.7%)	706 (83.8%)	685 (83.9%)
Black or African American	127 (12.9%)	95 (11.3%)	90 (11.0%)
Asian	41 (4.2%)	31 (3.7%)	31 (3.8%)
American Indian or Alaska Native	1 (0.1%)	1 (0.1%)	1 (0.1%)
Native Hawaiian or Other Pacific Islander	1 (0.1%)	1 (0.1%)	1 (0.1%)
Other	10 (1.0%)	8 (1.0%)	8 (1.0%)
Ethnicity, n (%)			
Hispanic or Latino	67 (6.8%)	48 (5.7%)	47 (5.8%)
Not Hispanic or Latino	916 (93.2%)	794 (4.3%)	769 (94.2%)

a. The intent-to-screen cohort included participants with a valid or usable mt-sDNA and a reportable or complete colonoscopy.
b. The evaluable cohort included only participants with a usable mt-sDNA and complete colonoscopy. Participants with stool samples received outside of the 72-hour mt-sDNA processing window, incomplete/not reportable colonoscopies, and other major protocol violations (inclusion/exclusion criteria not met) were excluded.
mt-sDNA, multi-target stool DNA test; NAA, nonadvanced adenomas; SD, standard deviation.

Table 2. Test Performance in the Evaluable Cohort

Most Advanced Finding	Colonoscopy (N=816)		Multi-target Stool DNA Test (N=816)	
	No.		Positive Results, no.	Specificity, % (95% CI)
All nonadvanced adenomas, non-neoplastic findings, and negative results on colonoscopy	767		37	95.2 (93.4-96.6)
Negative results on colonoscopy	514		19	96.3 (94.3-97.8)
		Prevalence, % (N=816)		Sensitivity, % (95% CI)
Colorectal cancer	0	NA	NA	NA
Advanced precancerous lesions[a]	49	6.0	16	32.7 (19.9-47.5)
High-grade dysplasia	0	<0.1	NA	NA
Adenoma, villous growth pattern	10	1.2	6	60.0 (26.2-87.8)
Adenoma ≥10 mm	32	3.9	7	28.1 (13.7-46.7)
Serrated lesion ≥10 mm	7	0.9	1	14.3 (0.4-57.9)
Nonadvanced adenoma	253	31.0	18	7.1 (4.3-11.0)

a. Based on most advanced lesion; therefore, prevalence estimates for less advanced lesions are biased downward.
CI, confidence interval; NA, not applicable; no., number.

Figure 1. Flowchart for Evaluable Cohort

983
subjects provided written informed consent

107 not evaluable
17 withdrew consent
3 withdrawn by Investigator
1 withdrawn by Sponsor
3 had colonoscopy performed before stool sample collection
53 did not undergo colonoscopy but submitted stool sample
4 did not submit stool sample but underwent colonoscopy
26 did not submit stool sample and did not undergo colonoscopy

876
Subjects underwent colonoscopy and submitted stool sample

19 had colonoscopy excluded
1 colonoscopy not reportable (incomplete/not provided)
1 no cecum inspection and no findings
12 poor bowel prep and no advanced neoplasia
1 no cecum inspection, no findings, and poor bowel prep
2 tissue collected but no case categorized
2 tissue collected but no case categorized *and* histopathology report incomplete or not provided

15 had multi-target stool DNA test excluded
1 stool submitted but not tested
7 stool tested but no result obtained
7 stool samples could not be processed

842 subjects included in Intent to Screen Population (ITS)

26 stool samples received outside of 72-hour processing window

816 subjects included in the evaluable cohort
0 CRC
49 advanced precancerous lesions
253 non-advanced adenoma
514 no neoplasia

Figure 2. Receiver Operating Characteristic Curves for Multi-target Stool DNA Test Specificity