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Stool DNA and Occult Blood Testing for Screen Detection of Colorectal Neoplasia

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Background: Stool DNA testing is a new approach to colorectal cancer detection. Few data are available from the screening setting.

Objective: To compare stool DNA and fecal blood testing for detection of screen-relevant neoplasia (curable-stage cancer, high-grade dysplasia, or adenomas >1 cm).

Design: Blinded, multicenter, cross-sectional study.

Setting: Communities surrounding 22 participating academic and regional health care systems in the United States.

Participants: 4482 average-risk adults.

Measurements: Fecal blood and DNA markers. Participants collected 3 stools, smeared fecal blood test cards and used same-day shipment to a central facility. Fecal blood cards (Hemoccult and HemoccultSensa, Beckman Coulter, Fullerton, California) were tested on 3 stools and DNA assays on 1 stool per patient. Stool DNA test 1 (SDT-1) was a precommercial 23-marker assay, and a novel test (SDT-2) targeted 3 broadly informative markers. The criterion standard was colonoscopy.

Results: Sensitivity for screen-relevant neoplasms was 20% by SDT-1, 11% by Hemoccult ($P = 0.020$), 21% by HemoccultSensa ($P = 0.80$); sensitivity for cancer plus high-grade dysplasia did not differ among tests. Specificity was 96% by SDT-1, compared with 98% by Hemoccult ($P < 0.001$) and 97% by HemoccultSensa ($P = 0.20$). Stool DNA test 2 detected 46% of screen-relevant neoplasms, compared with 16% by Hemoccult ($P < 0.001$) and 24% by HemoccultSensa ($P < 0.001$). Stool DNA test 2 detected 46% of adenomas 1 cm or larger, compared with 10% by Hemoccult ($P < 0.001$) and 17% by HemoccultSensa ($P < 0.001$). Among colonoscopically normal patients, the positivity rate was 16% with SDT-2, compared with 4% with Hemoccult ($P = 0.010$) and 5% with HemoccultSensa ($P = 0.030$).

Limitations: Stool DNA test 2 was not performed on all subsets of patients without screen-relevant neoplasms. Stools were collected without preservative, which reduced detection of some DNA markers.

Conclusion: Stool DNA test 1 provides no improvement over HemoccultSensa for detection of screen-relevant neoplasms. Stool DNA test 2 detects significantly more neoplasms than does Hemoccult or HemoccultSensa, but with more positive results in colonoscopically normal patients. Higher sensitivity of SDT-2 was particularly apparent for adenomas.

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Colorectal cancer remains the second most common cause of death among the types of cancer (1). Although screening reduces colorectal cancer mortality (2–6), observed reductions have been modest (6, 7) and more than one half of adults in the United States have not received screening (8). More accurate, user-friendly, and widely distributable tools have the potential to improve screening effectiveness, acceptability, and access.

Several molecular approaches to screening stool for colorectal cancer have been studied and reviewed (9, 10), and stool DNA testing has been jointly endorsed by the American Cancer Society, the U.S. Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology (11). The advantages of stool DNA testing include noninvasiveness, absence of bowel preparation or dietary restrictions, and ease of access via mail courier. However, the reported accuracy of stool DNA tests for the detection of colorectal neoplasia varies. In clinical studies that used different assays and selected groups (12–20), sensitivities ranged from 62% to 100% for colorectal cancer and 27% to 82% for advanced adenomas, with specificities ranging from 82% to 100%. In the only reported multicenter study on asymptomatic average-risk patients (21), a precommercial multitarget DNA assay (SDT-1, a proto-sachusetts) detected 52% of cases of colorectal cancer, compared with 13% by Hemoccult ($P = 0.003$), at specificities of 94.4% and 95.2%, respectively.

The accuracy of stool DNA testing is influenced by both biological and technical factors. A panel of markers must be used to accommodate the molecular heterogeneity of colorectal neoplasia, and marker selection critically affects discrimination (9). Unlike occult bleeding, which is intermittent (22), DNA markers seem to be shed continuously by exfoliation (23). Thus, the multiple stool sampling practiced with fecal occult blood tests may not be necessary with stool DNA tests. However, recovery of the

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Context

Because the colonic mucosa constantly sheds cells, testing stool for cancer-related genes could be better for colorectal cancer screening than testing for occult bleeding, which is intermittent.

Content

A total of 3764 healthy adults had screening colonoscopy, fecal occult blood testing with Hemoccult and Hemoccult-Sensa, and both a first- and a second-generation stool DNA test (SDT-1 and SDT-2, respectively) for a battery of cancer genes. The sensitivity of SDT-1 and HemoccultSensa was very similar for screen-relevant neoplasms (20% and 21%, respectively), whereas the sensitivity of SDT-2 was 40%.

Caution

The authors could not measure the specificity of SDT-2.

Implication

A second-generation stool test for cancer genes is substantially more sensitive than fecal occult blood testing.

—The Editors

minute quantities of human DNA and assay of tumor-specific DNA alterations from stool present technical challenges and require exquisite laboratory sensitivity to achieve optimal detection rates.

Our primary aim was to compare the precommercial stool DNA test (SDT-1), which was studied by Imperiale and colleagues (21), with widely used fecal occult blood tests for the detection of screen-relevant neoplasia, defined as curable-stage colorectal cancer (no distant metastases), high-grade dysplasia, or adenomas larger than 1 cm. A secondary aim was to explore neoplasm detection by another stool DNA test 2 (SDT-2), which uses a more broadly informative marker panel.

METHODS

Table 1 lists the genes used in our test panels and defines several key terms.

Design

We conducted this multicenter, prospective, triple-blinded trial, targeting average-risk persons, from 2001 to 2007. A group of national experts on colorectal cancer screening advised on study design, and institutional review boards at each site approved the study. Because we did not know the effect of diet and medications on DNA assays, patients were randomly assigned at entry to group A (restriction of red meat and therapeutic doses of nonsteroidal anti-inflammatory drugs for 3 days before and during stool collections) or group B (no such restrictions). All patients were asked not to ingest vitamin C for the 3 days before and during stool collections. For the companion test, we

nia), the most widely used fecal occult blood test, which was used in the trials that established the benefit of screening for fecal occult blood (2–4). As a second companion test, we chose the next-generation guaiac test Hemoccult-Sensa (Beckman Coulter). We compared fecal blood results from 3 stools per patient with stool DNA on 1 stool. Experienced technicians performed stool DNA and occult blood testing in separate central laboratories without knowledge of clinical findings or the results of other tests. All patients who completed stool collections also had colonoscopy, which served as the criterion standard. We did not have access to data until after they had been analyzed by statisticians and released by a data monitoring board.

Participants

We recruited asymptomatic persons age 50 to 80 years who were at average risk for colorectal cancer from communities surrounding 22 participating academic and regional health care systems through direct mail and multimedia advertisements. The exclusion criteria were structural colorectal evaluation (endoscopic or radiographic) within 10 years; fecal blood testing within 1 year; overt rectal bleeding within 1 month; previous colorectal resection; aerodigestive cancer within 5 years; inability to stop therapeutic doses of nonsteroidal anti-inflammatory drugs or anticoagulants; coagulopathy; contraindications to colonoscopy; chemotherapy within 3 months; high-risk conditions for colorectal cancer, such as familial adenomatous polyposis, the Lynch syndrome, or other cancer syndromes; previous colorectal cancer or adenoma; inflammatory bowel disease; or more than 2 first-degree relatives with colorectal neoplasia. Study assistants at each site registered participants and randomly assigned them by using a Web-based management system; distributed fecal blood test cards, stool collection containers, and colonoscopy preparation materials; and provided instructions.

Stool Collection and Processing

Patients collected 3 stools by using plastic buckets mounted to the toilet seat. Promptly after each individual collection, patients smeared stool onto both windows of their Hemoccult and HemoccultSensa cards and then express-shipped smeared cards and the whole stool (sealed in

Table 1. **Definitions**

Gene targets in stool DNA test panels:
- Test 1: point mutations on *K-ras*, *APC*, and *p53*; microsatellite marker BAT-26; long DNA
- Test 2: point mutations on *K-ras*, scanned mutator cluster region of *APC*, vimentin methylation

Screen-relevant neoplasia: colorectal cancer, high-grade dysplasia, adenomas ≥1 cm
Sensitivity: rate of test positivity for those with screen-relevant neoplasia
Specificity: rate of test negativity for those without screen-relevant neoplasia
Test positivity: rate of positive stool test results for individual colonoscopic

a bucket in an insulated container cooled with ice packs) to the Mayo Clinic in Rochester, Minnesota. We froze the first stool from each participant whole at −80 °C on receipt and sent it in batches on dry ice to EXACT Sciences (Marlborough, Massachusetts) for DNA assay; each of the subsequent 2 stools were archived in aliquots at −80 °C. If the first stool weighed less than 30 g or was received more than 48 hours after defecation, it was rejected for DNA analysis and the second or third stool (if it met inclusion criteria) was sent for DNA assay.

Stool Assays

DNA Testing

All assays were polymerase chain reaction–based and were run at EXACT Sciences. Stool DNA test 1 was performed as described in Imperiale and colleagues' study (21). The marker panel for SDT-1 included 21 tumor-specific point mutations (3 on the *K-ras* gene, 10 on the *APC* gene, and 8 on the *p53* gene); the microsatellite-instability marker BAT-26; and long DNA, a marker for delayed apoptosis, which is characteristic of exfoliated neoplastic colonocytes (12). For SDT-2, sequence-specific DNA markers were detected by acrylamide gel electrophoresis, as described by Whitney and colleagues (24); the panel consisted of 3 tumor-specific markers broadly informative for both colorectal cancer and adenomas (25): *K-ras* mutations, scanning of *APC* mutator cluster regions, and methylation of the vimentin gene. We used methods described elsewhere to detect mutant *K-ras* (12), *APC* scanning (25), and vimentin gene methylation (20) assays. We defined any positive component marker result according to the manufacturer's preestablished criteria as a positive test result.

Occult Blood Testing

The manufacturer that developed the Hemoccult and HemoccultSensa cards, without rehydration, trained technicians on-site at the Mayo Clinic. As recommended by the manufacturer, the technicians added the catalyst solution to cards stored at ambient temperature within 48 to 72 hours of collection. We defined a spreading (enlarging) blue color in 60 seconds in any window of the cards as a positive result and any other result as negative.

Colonoscopy

After cathartic preparation, experienced endoscopists performed colonoscopy in all patients. If the examination did not reach the cecum or inspected less than 90% of the mucosa, the patient was disqualified. Endophotographs documented cecal intubation, and the size and location of all lesions were recorded. Costs not covered by third parties were reimbursed by study funding.

Pathologic Examination

Local pathologists examined all endoscopically or surgically sampled lesions. A gastrointestinal pathologist at the

sis. Classification discrepancies of screen-relevant neoplasms were adjudicated by a second expert pathologist. We categorized patients with multiple neoplasms according to the most advanced lesion. For assay of markers in screen-relevant neoplasms, DNA was extracted from microdissected tissue.

Statistical Analysis

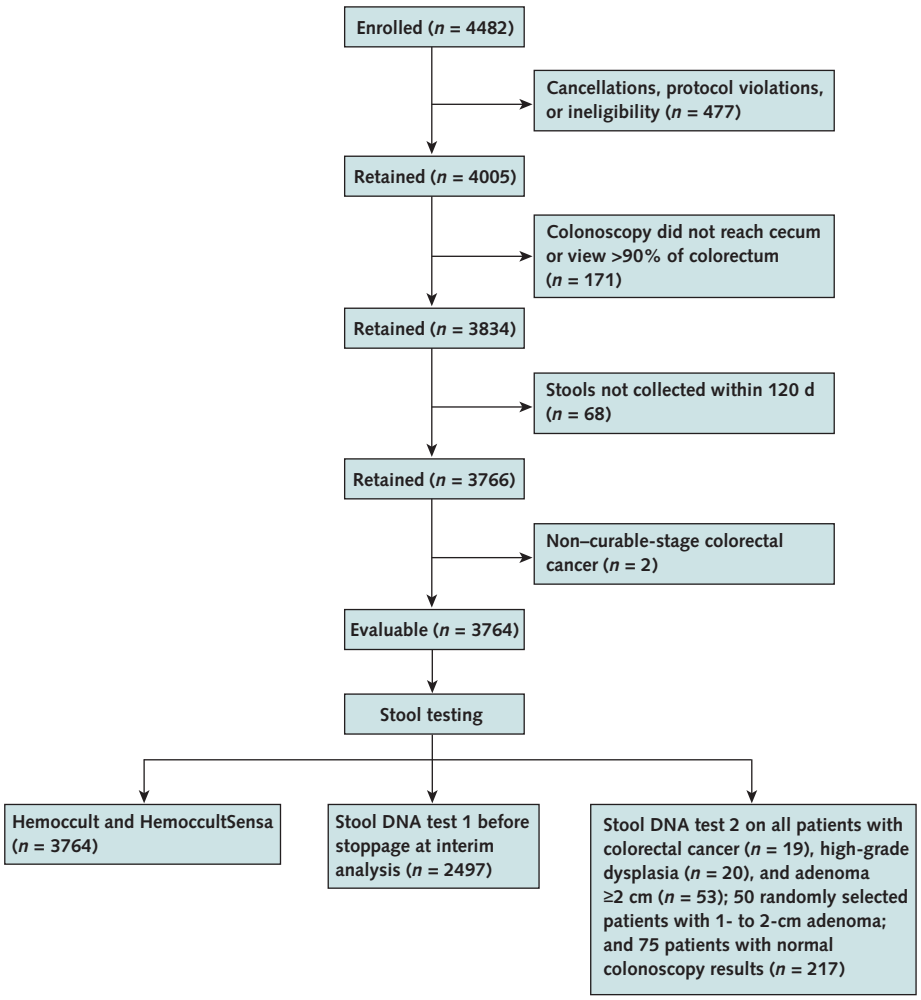
We calculated sample size to ensure adequate power to detect differences in sensitivity comparisons. We powered the study to ensure an adequate number of cases of curable-stage colorectal cancer and high-grade dysplasia and assumed their combined prevalence to be at least 1.5%. A sample size of 2900 would yield an expected 43 curable-stage cancer or high-grade dysplasia cases, which would provide 90% power to detect a 35% improvement in sensitivity of SDT-1 over the Hemoccult test by using a 2-sided McNemar test with $\alpha = 0.05$ (assuming Hemoccult sensitivity of 25%). The protocol specified interim analyses at one half and three quarters of full enrollment to see whether it was necessary to stop the study early if test sensitivities differed significantly or to revise sample size requirements on the basis of observed prevalence of the target lesion. At the first interim analysis, lesion prevalence was lower than expected, and we readjusted the sample size to 4434 patients. However, before we completed enrollment, the manufacturer altered the SDT-1 assay, which prompted an unplanned interim analysis after 2497 patients. On the basis of these interim results, we stopped SDT-1 testing and began doing the SDT-2 test.

To accomplish a secondary aim of this trial (to see whether restricting diet and medication affects the specificity of the SDT test), we randomly assigned persons to pretest restrictions or no restrictions. The sample size calculated for the sensitivity comparison provided 85% power to detect a 4% difference in specificity between randomization groups. Because SDT specificity was the same in both groups, we pooled the results for all analyses.

We included all patients tested with SDT-1. We compared stool test sensitivities and specificities by using the McNemar test. We used a chi-square test or the Fisher exact test to compare baseline characteristics between cohorts and assay performance in subsets of patients. All *P* values are 2-sided.

Per agreement with EXACT Sciences, we did the SDT-2 test on all patients with cancer, high-grade dysplasia, and adenomas larger than 2 cm from the full enrollment period as well as on a random sample of 50 patients with 1- to 2-cm adenomas and 75 with normal colonoscopy results. To estimate the population-level sensitivity for the SDT-2 test, we used all case patients tested with SDT-2 and reweighted the calculation to be proportional to the observed prevalence of each screen-relevant neoplasia category in the entire population with screen-relevant

Figure 1. **Study flow diagram.**



subsets without screen-relevant neoplasia, we could not calculate specificity for screen-relevant neoplasia. To compare test positivity rates in patient subsets, we used the McNemar test.

Role of the Funding Source

The National Cancer Institute funded this study and monitored conduct. EXACT Sciences performed DNA assays at no cost, and Beckman Coulter provided Hemoccult and HemoccultSensa cards at no cost. EXACT Sciences limited SDT-2 coverage to screen-relevant neoplasms and a subset of normal control participants. Neither company influenced study oversight, data analysis, or reporting.

RESULTS

Patients

Of the 4482 persons enrolled, 3764 (84%) were evaluable. We excluded 545 patients because of cancellations, protocol violations, or ineligibility; 171 because of incom-

(**Figure 1**). **Table 2** shows demographic and colorectal lesion characteristics. We found screen-relevant neoplasms in 290 (7.7%) patients; 39 had nonmetastatic cancer or high-grade dysplasia and 251 had adenomas that were 1 cm or larger. Major complications from colonoscopy occurred in 4 patients; no procedure-related deaths were reported.

Occult Blood Testing: Hemoccult versus HemoccultSensa

Detection sensitivities for the 290 screen-relevant neoplasms found among all 3764 evaluable participants were 10% (95% CI, 7% to 13%) with Hemoccult and 18% (CI, 13% to 22%) with HemoccultSensa ($P < 0.001$). Based on all 3474 participants without screen-relevant neoplasia, the Hemoccult specificity of 98% (CI, 98% to 99%) was slightly higher than that of HemoccultSensa (97% [CI, 96% to 97%]) ($P < 0.001$).

Hemoccult and HemoccultSensa positivity rates for the 39 patients with colorectal cancer or high-grade dysplasia were 33% (CI, 19% to 48%) and 44% (CI, 28% to

Table 2. **Baseline Characteristics and Colorectal Findings**

Characteristic	All Patients (*n* = 4482)	Evaluable Patients* (*n* = 3764)	Patients Tested with SDT-1† (*n* = 2497)	Patients Tested with SDT-2‡ (*n* = 217)
Age, *y*				
Mean (SD)	63.8 (8.29)	63.7 (8.25)	60.4 (7.86)	66.4 (7.17)
Median (range)	65 (50–81)	65 (50–80)	59 (50–80)	67 (51–80)
Women, *n (%)*	2341 (52.2)	1964 (52.2)	1348 (54.0)	108 (49.8)
White, *n (%)*	4184 (93.4)	3522 (93.6)	2314 (92.7)	201 (92.6)
Colorectal findings, *n (%)*				
Screen-relevant neoplasia	–	290 (7.7)	157 (6.3)	142 (65.4)
Cancer				
Stage I	–	11 (0.3)	6 (0.2)	11 (5.1)
Stages II and III	–	8 (0.2)	6 (0.2)	8 (3.7)
Cancer + high-grade dysplasia	–	39 (1.0)	22 (0.9)	39 (18.0)
Adenoma ≥1 cm	–	251 (6.7)	135 (5.4)	103 (47.5)
Adenoma >2 cm	–	53 (1.4)	21 (0.8)	53 (24.4)
Adenoma <1 cm	–	785 (20.9)	469 (18.8)	Not tested
Hyperplastic polyps	–	492 (13.1)	341 (13.7)	Not tested
Other	–	86 (2.3)	57 (2.3)	Not tested
Normal	–	2111 (56.1)	1473 (59.0)	75 (34.6)

SDT = stool DNA test.
* Patients who met all inclusion criteria. Both Hemoccult and HemoccultSensa (Beckman Coulter, Fullerton, California) were performed on all evaluable participants.
† On the basis of results from an interim analysis, SDT-1 was terminated.
‡ All participants with cancer, high-grade dysplasia, and adenomas ≥2 cm from the full enrollment period are included, as are random samples from 50 patients with 1- or 2-cm adenomas and 75 with normal colonoscopy results.

adenomas 1 cm or larger, the positivity rates were 6% (CI, 3% to 9%) versus 14% (CI, 9% to 18%) ($P = 0.001$).

Stool DNA versus Occult Blood Testing

SDT-1 versus Occult Blood Testing

Based on the first 2497 evaluable participants (**Table 3**), the sensitivity of SDT-1 for screen-relevant neoplasia was higher than that of Hemoccult (20% [CI, 14% to 26%] vs. 11% [CI, 6% to 16%]; $P = 0.020$) but not that of HemoccultSensa (21% [CI, 15% to 27%]; $P = 0.80$). For all target lesion groupings, specificities were slightly but significantly lower for SDT-1 than for Hemoccult but not HemoccultSensa, and the positive likelihood ratios for SDT-1 were lower than for either Hemoccult or HemoccultSensa for the more advanced groupings of screen-relevant neoplasms (**Table 3**).

Based on test positivity data in subsets of screen-relevant neoplasms (**Table 4**), SDT-1 had higher detection rates than Hemoccult for 1- to 2-cm adenomas but not for any other subset. Stool DNA test 1 had a lower positivity rate for detecting invasive cancer than did HemoccultSensa (25% [CI, 5% to 57%] vs. 75% [51% to 100%]; $P = 0.010$).

SDT-2 versus Occult Blood Testing

Table 3 shows the sensitivity of SDT-2, Hemoccult, and HemoccultSensa for screen-related neoplasia. The

Table 3. **Summary of Test Performance**

Index Test	Screen-Relevant Neoplasia, *n**	Positive Test Result, *n*	Sensitivity (95% CI)	No Screen-Relevant Neoplasia, *n*	Negative Test Result, *n*	Specificity (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
Hemoccult (*n* = 2497)	157	17	11 (6–16)†	2340	2297	98 (98–99)‡	5.9 (3–10)	0.9 (0.9–1.0)
HemoccultSensa (*n* = 2497)	157	33	21 (15–27)§	2340	2258	97 (96–97)‖	6.0 (4–9)	0.8 (0.8–0.9)
SDT-1 (*n* = 2497)	157	31	20 (14–26)	2340	2246	96 (95–97)	4.9 (3–7)	0.8 (0.8–0.9)
SDT-2 (*n* = 217)	142	66	40 (32–49)¶	75	NA**	NA	NA	NA

NA = not available; SDT = stool DNA test.
* Includes curable-stage cancer, high-grade dysplasia, and adenomas ≥1 cm.
† $P = 0.02$ for STD-1 vs. Hemoccult.
‡ $P < 0.001$ for STD-1 vs. Hemoccult.
§ $P = 0.80$ for STD-1 vs. HemoccultSensa.
‖ $P = 0.40$ for STD-1 vs. HemoccultSensa.
¶ We calculated the weighted sensitvity for SDT-2 with the following equation: reweighted sensitivity = (% [colorectal cancer + high-grade dysplasia] × PR) + (% adenomas ≥2 cm × PR) + (% adenomas 1–2 cm × PR) = (0.13 × 0.49) + (0.18 × 0.57) + (0.68 × 0.34). PR = proportion of participants for that category of screen-relevant neoplasia in the entire population with screen-relevant neoplasia. See "Comparison of Stool DNA Tests" for statistical comparisons of SDT-1 and SDT-2 in

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