COVID Concerns Highlight the Importance of Adequate Fecal Immunochemical Test Sample Collection

“It’s important that ... patients are provided with simple instructions that highlight the importance of adequate fecal sample collection.”
—Erin Symonds, PhD

Recent surveys suggest that many people, concerned about contracting coronavirus disease 2019 (COVID-19) through public exposure, have been forgoing cancer screening tests. (The American Society of Clinical Oncology, for example, found that approximately two-thirds of those who were scheduled to receive cancer screenings during the pandemic reported missing or delaying them.) Although fecal occult blood tests, including FIT, that can be performed at home offer a good solution for most individuals who are concerned about getting a screening colonoscopy at this time, some previous studies have raised concerns that suboptimal stool collection by patients might lead to false-negative results.

Most studies of pre-analytic variables to date have focused on those occurring after collection, such as storage and ambient temperatures and time lags before analysis, resulting in degradation of the fecal Hb, which can lead to false-negative results. However, a new study by Australian investigators investigates whether there needs to be greater emphasis on stricter adherence to the collection of the stool samples themselves, particularly the amount collected, to ensure the diagnostic accuracy of the tests. The research appears in Cancer Epidemiology, Biomarkers & Prevention (doi:10.1158/1055-9965.EPI-20-0984).

“This type of study has never been done before,” says the lead author, Erin Symonds, PhD, a senior research scientist and team leader at Flinders Medical Centre and an associate professor at the College of Medicine and Public Health at Flinders University in Bedford Park, South Australia. “We were aware of several studies that had investigated the effect of the post-collection variables on FIT performance, but no previous studies had assessed the variation in collection technique observed in a clinical setting.”

Dr. Symonds says that her team believed it was imperative to determine whether inconsistencies in sampling quantity affect clinical results for finding advanced neoplasia and other bowel disease because “the use of FIT in the detection of colorectal cancer, whether it is in programmatic or opportunistic screening, surveillance, or symptomatic patients, relies on accurate measurement of Hb to determine which individuals need follow up, usually colonoscopy.”

Study Results
Researchers examined FIT devices (OC-Sensor; Eiken Chemical Co, Tokyo, Japan) returned by mail to their laboratory by patients at elevated risk for colorectal cancer as determined by Australia’s Southern Cooperative Program for the Prevention of Colorectal Cancer, which was developed in 2000 after the release of Australia’s National Health and Medical Research Council Guidelines for colorectal cancer prevention. Participants received FIT kits that included detailed instructions, 2 collection devices, 2 sample collection sheets, and a pouch and envelope with a return address. Sample collections took place between January 2019 and February 2020. Patients were instructed to collect samples at home from the outer surface of the feces of 2 different bowel motions. The collection devices consisted of circumferentially grooved probes for sampling feces. When the collection is performed correctly, the probe is inserted into the device with ideally approximately 10 mg of feces reaching the 2.0 mL of Hb-stabilizing buffer.

Each subject returned both devices. The researchers noted the number of days between the first and second...
sample collections and whether the sample had been collected before or after midday.

Once received at the laboratory, the samples were graded for color from 1 to 5 (from lightest to darkest) by 2 investigators for the first 4 months of the study to develop consistent scoring between them. In addition, approximately 650 collection devices were weighed before they were sent to patients and then were re-weighed upon return to the laboratory after collection to calculate the weight of stool collected. Finally, all samples were assayed to determine fecal Hb levels.

**Study Results**

Approximately 6898 samples were collected from 3449 individuals. More than half were female (53.1%), and the median age of all subjects was 65.3 years. Of the samples, 362 (5.2%) were graded as the lightest in color, 1836 (26.6%) were judged to be color 2, 2569 (37.2%) were judged to be color 3, 1711 (24.8%) were judged to be color 4, and 420 (6.1%) were the darkest in color (color 5).

Of the 3449 sample pairs, 858 (24.9%) had a darker color on the second sample collection, and 872 (25.3%) of the second samples were lighter; the remaining pairs were judged to be the same color. For the 650 collection devices returned that had their mass recorded before and after sample collection, the median mass of feces collected was 33.8 mg.

The researchers found that color was significantly related to sample mass: colors 2, 3, 4, and 5 had a significantly greater mass than those graded as color 1 ($P = .002$). They found that the samples with the lightest color had a significantly lower fecal Hb level in comparison with all darker classes ($P < .001$). Low sample mass was not associated with patient age, sex, or other demographic variables.

On the basis of a subset of study participants (467 patients) who underwent colonoscopy regardless of FIT results, they also determined that undersampling of stool (the lightest colored samples) was not associated with false-negative results for advanced neoplasia (colorectal cancer and advanced adenomas, with the latter category comprising adenomas with a size and histology indicating a greater risk of evolution to colorectal cancer) but was for colorectal neoplasia overall and inflammatory bowel disease. The false-negative percentages for the detection of advanced neoplasia were 23.3% and 22.8% for specimens with the lightest color and those in all other color categories, respectively ($P = .947$).

According to Dr. Symonds, the most important findings from the study were their observation that “... those devices with a light color had the lowest measured fecal Hb concentration and lowest FIT positivity rate, and in addition, that the light-colored devices conferred an increased risk for a false-negative result in the detection of any colorectal neoplasia and inflammatory bowel disease together.” However, the study results provide reassurance that suboptimal stool collection is unlikely to reduce sensitivity for identifying patients with the most clinically relevant lesions—colorectal cancers and adenomas—so that they can be referred for diagnostic colonoscopy.

**Pandemic Screening Update From the American Cancer Society**

In addition to fears that some patients may have about entering a hospital setting during the pandemic, Durado Brooks, MD, MPH, former vice president of cancer control interventions at the American Cancer Society in Atlanta, notes that virtually all health organizations discouraged in-house, clinical visits last winter and spring, and this caused the number of colorectal screenings to drop significantly.

This, Dr. Brooks says, is where tests such as FIT that patients can perform at home can fill a need—if they are performed correctly. To this end, the National Colorectal Cancer Roundtable has created a resource entitled “Reigniting Colorectal Cancer Screening as Communities Face and Respond to the COVID-19 Pandemic: A Playbook” (https://nccrt.org/resource/a-playbook-for-reigniting-colorectal-cancer-screening-as-communities-respond-to-the-covid-19-pandemic). According to the coalition, this document includes the most recent “data, research, and clinical guidelines available related to colorectal cancer screening and COVID-19” and provides an action-oriented guide for how members, partners, and colorectal cancer screening advocates can “work together to reignite our screening efforts appropriately, safely, and equally for all communities.”

Regarding self-screening kits, the playbook states that for people at average risk, tests such as FIT or a stool DNA test (eg, Cologuard) can be performed safely at home. The American Cancer Society does not see these tests as a substitute for diagnostic colonoscopies, however.
“If the stool test result is positive, you will need a colonoscopy, and it will be important to talk with your doctor about the safest way to proceed with this. Colonoscopy as a screening test is still an option, but it may be harder to get an appointment now compared to before the COVID-19 pandemic,” says Dr. Brooks.

For those who can opt for screening at home, Dr. Symonds stresses the importance of the study’s findings regarding the quantity and color of the samples. “With more and more people and clinicians encouraging FIT tests, labs should recognize that it’s the devices with the lightest color, a relatively small proportion of all tests, that should raise the suspicion that the amount of feces collected has been suboptimal. Inadequate collection of the fecal sample into the FIT collection device will result in a lower measured Hb concentration and potentially a false-negative test result—and consequently a missed opportunity to detect some adenomas.”

Even though this study did not find any association of false-negative results among patients with advanced adenomas or invasive colorectal cancer, it is possible that this problem might have been detected if a much larger number of patients had been studied. For this reason, “It’s important that participants and patients are provided with simple instructions that highlight the importance of adequate fecal sample collection,” Dr. Symonds adds. “If resources permit it, the collection of two samples would assist in minimizing the risks.”

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