A variety of noninvasive molecular approaches to colorectal cancer screening are emerging with potential to improve screening effectiveness and user-friendliness. These approaches are based on the sensitive assay of molecular markers in stool, blood, and urine samples. New methods, especially next generation stool-based tests, have been shown to detect both colorectal cancers and precancerous lesions with high accuracy. Validation of these technologies in average-risk populations are needed to establish their role for general colorectal cancer screening. This review addresses the biological rationale, technical advances, recent clinical performance data, and remaining issues with molecular screening for colorectal cancer.

**Target Lesions for CRC Screening**

The critical target lesions for CRC screening include cancers that are at curable stages and precancerous lesions that have the highest risk of becoming malignant tumors. Presymptomatic detection of the earliest stage cancers yields the greatest benefit in mortality reduction. Among the unscreened, average-risk US population, the prevalence of colorectal cancer is 0.5%–1%. Precancerous lesions

**Precancerous Lesions**

Cancer prevention by screening necessitates detection of precancerous lesions. Historically, adenomas have been considered the most important precursor to sporadic CRC. It is estimated that >50% of individuals will develop colorectal adenomas in their lifetime, but only 6% will develop colorectal cancer, so most adenomas do not progress to cancer. The adenomas that should be detected by screening are those that are most likely to progress. Based on collective observations, the so-called advanced adenomas (ie, size ≥1 cm or those that contain high-grade dysplasia or villous elements) confer the highest risk for CRC and are generally considered to be the most relevant subset to detect in screening. Among unscreened Americans between 50 and 80 years of age, the point prevalence of advanced adenomas is 5%–10%

Serrated polyps (ie, serrated adenomas, sessile serrated adenomas, and large hyperplastic polyps) are increasingly recognized as likely precancerous lesions. It is estimated that 20%–30% of CRCs arise from serrated polyps rather than adenomas. Serrated polyps are typically right-sided,

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**Abbreviations used in this paper:** APC, adenomatous polyposis coli; BEAM, beads, emulsion, amplification, and magnetic; CRC, colorectal cancer; mRNA, messenger RNA; PCR, polymerase chain reaction.

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doi:10.1053/j.gastro.2010.01.055
more common among the elderly, and less conspicuous, endoscopically, than adenomas. They appear to grow faster than adenomas and can progress more rapidly to cancer. Serrated polyps are associated with BRAF mutations, which are rarely present in adenomas. Serrated and adenomatous polyps each have aberrant gene methylation patterns. There is no consensus on the features that should be used to identify high-risk serrated polyps; some suggest size ≥1 cm as one of the criteria. Prevalence rates of advanced serrated polyps remain uncertain, and careful population-based observations are needed. Although most screening studies have excluded serrated polyps as relevant targets, these lesions will need to be considered carefully in future studies.

**Biological Context**

The heterogeneity of colorectal neoplasms must be taken into account in selecting markers for molecular detection and determining testing frequency. Detection of these markers depends on a cascade of biological events—markers must be regularly released from tumors or precancerous lesions, dispersed into the medium that is collected for the assay, survive metabolic degradation, and be measurable.

**Molecular Heterogeneity**

A score of well-described molecular alterations drive or accompany oncogenesis; progress has been made toward defining the genome, methylation, transcriptome, and proteome of CRC. DNA changes and the quantitative downstream effects on RNA and protein expression result in many candidate markers that can be detected in different types of media (Table 2). Just as tumor phenotypes vary widely, so do cancer cell genotypes and molecular patterns. There is no single, universal molecular marker of cancer or precancerous lesions, so panels of markers are needed. Only a handful of genes (eg, *P53*, *adenomatous polyposis coli* [APC], and *KRAS*) have been found to be frequently mutated in colorectal neoplasms; genome-wide searches have associated additional genes with CRC, but none that is mutated at comparably high frequency among tumor samples. Aberrant DNA methylation patterns are detected during early stages of tumorigenesis at the same frequency or more frequently than mutations; some specific genes are methylated in the majority of cancers and precancerous lesions and represent attractive marker candidates. However, not all colorectal tumors harbor aberrantly methylated genes. Complementary genetic and epigenetic markers can be selected that cover essentially all forms of CRC tumors and precancerous lesions. Similarly, combinations of RNA expression or protein markers are more informative than single markers alone.

**Marker Release**

Tumors release markers at different stages of progression, by different mechanisms, into different media that can be assayed. Tumor cells and most tumor markers likely enter into stool at earlier stages than into blood or urine—an advantage of stool testing for cancer precursor lesions and early-stage tumors. Dysplastic cells and their constituents are released into stool by exfoliation from the surface of precancerous lesions and early-stage cancers. Exfoliation from colorectal neoplasms appears to be a continuous process that occurs more frequently than exfoliation from normal epithelium. Factors that might contribute to the high rate of exfoliation from tumors include increased proliferation and reduced cell—cell or basement membrane adhesion. In normal colon, epithelial renewal does not necessarily lead to exfoliation, but instead, often involves engulfment of effete colonocytes by subepithelial phagocytes.

Tumor cells gain entry into blood via blood vessel invasion, which occurs in cancers but not precancerous lesions. Histological analyses have shown that blood vessel invasion occurs more frequently from advanced than early-stage tumors and that there is more abundant release of tumor cells into the circulation with

### Table 1. Ideal Features of a Noninvasive Screening Test for Colorectal Neoplasia

<table>
<thead>
<tr>
<th>Features</th>
<th>Beneficial effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly sensitive</td>
<td>Reduces cancer mortality</td>
</tr>
<tr>
<td>For curable-stage cancer</td>
<td>Prevents cancer</td>
</tr>
<tr>
<td>For advanced precancerous lesions</td>
<td>Reduces program cost</td>
</tr>
<tr>
<td>Highly specific</td>
<td>Increases appeal/compliance</td>
</tr>
<tr>
<td>User friendly</td>
<td>Increases acceptance/compliance</td>
</tr>
<tr>
<td>Affordable</td>
<td>Increases availability/compliance</td>
</tr>
<tr>
<td>Widely distributable</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Candidate Stool, Blood, and Urine Tumor Markers for the Noninvasive Detection of Colorectal Neoplasia

<table>
<thead>
<tr>
<th>Stool</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhaged</td>
<td>In circulating tumor cells</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>RNA, DNA, proteins</td>
</tr>
<tr>
<td>Plasma proteins</td>
<td>In circulating phagocytes</td>
</tr>
<tr>
<td>Secreted</td>
<td>RNA, DNA, proteins</td>
</tr>
<tr>
<td>Mucins</td>
<td>In plasma/serum DNA</td>
</tr>
<tr>
<td>Metalloproteinases</td>
<td>Mutated genes</td>
</tr>
<tr>
<td>Exuded</td>
<td>Methylated genes</td>
</tr>
<tr>
<td>Leukocyte proteins</td>
<td>Proteins</td>
</tr>
<tr>
<td>Plasma proteins</td>
<td>Urine DNA</td>
</tr>
<tr>
<td>Exfoliated</td>
<td>Mutated genes</td>
</tr>
<tr>
<td>Fecal whole colonocytes</td>
<td>Mutated genes</td>
</tr>
<tr>
<td>DNA</td>
<td>Nucleosides</td>
</tr>
<tr>
<td>Mutated genes</td>
<td>Proteins</td>
</tr>
<tr>
<td>Methylated genes</td>
<td>Other</td>
</tr>
<tr>
<td>Long (nonapoptotic) DNA</td>
<td></td>
</tr>
<tr>
<td>RNA (gene expression)</td>
<td></td>
</tr>
<tr>
<td>Tumor-derived proteins</td>
<td></td>
</tr>
</tbody>
</table>
advanced cancers. Tumor markers can also enter blood indirectly via inflammatory cells that infiltrate tumors, phagocytose dysplastic cells (part of the immune response), and then re-enter the circulation carrying detectable patterns of tumor-derived nucleic acids or proteins. This alternative route of circulatory marker release via phagocytic leukocytes can occur during all stages of tumorigenesis and could potentially allow for detection of precancerous lesions as well as cancers by a blood test. Tumor markers enter urine via the circulation, where they are metabolized into fragments small enough for glomerular passage. Urine, therefore, represents a surrogate for blood testing.

**Marker Distribution and Metabolism**

Multiple factors influence the fate of tumor markers. Following release, neoplastic colonocytes have a survival advantage over normal colonocytes. Cells shed from normal colon epithelium are either apoptotic at the point of exfoliation or, if not, promptly undergo apoptotic involution when separated from the basement membrane—a process called anoikis. In contrast, acquired genetic or epigenetic changes allow neoplastic colonocytes to escape anoikis, survive within the circulation, and eventually spawn metastases. Colonocytes that are exfoliated into the lumen also avoid anoikis—evidenced by the presence of viable colonocytes in the muko-cellar layer that covers the surface of cancers and by the increased numbers of whole colonocytes that can be recovered from stool samples or colon lavage effluents from patients with CRC, compared to healthy controls.

Within the colorectum, shed tumor markers are variably protected within the envelope of intact cells embedded in the muko-cellar layer. Some evidence supports causal migration of this muko-cellar layer, and molecular analysis of colonocytes recovered from rectal swabs may indicate presence of more proximal colorectal neoplasms. However, with fecal mixing during transit, colonocytes are lysed by exposure to bile acids and other cytotytic compounds, which exposes their DNA and other contents to degradation by the metabolically active microflora. Cultured colonocytes from tumor cell lines quickly lyse when added to stool and are completely disrupted after 8–10 hours. Many candidate protein markers that might be used to identify tumors are rapidly catabolized by fecal proteases. The tumor site affects the distribution of markers in stool; exfoliated markers from left-side tumors are less well-mixed and appear to be concentrated on the stool surface. Because urine contains products excreted from blood over a period of time, it could be less vulnerable to temporal fluxes in marker levels than blood. 

**Field Changes**

Mutations in KRAS and APC and abnormal DNA methylation patterns can be present in aberrant crypt foci that are not detected by endoscopy as well as in histologically normal mucosa. Such field changes occur with increasing frequency with aging; senescent epithelial cells appear to be especially vulnerable to acquired mutations that result from oxidative stress. The relationship between field changes and CRC risk is not clear, but field changes can cause false-positive results in stool tests that use KRAS, APC, and methylation patterns as markers.

**Technical Considerations**

Research into methods for noninvasive molecular detection of colorectal neoplasia is a continuously changing field. The samples that can be analyzed, and compartments within those media, vary—multiple candidate markers within each compartment can be considered (Table 2). Most molecular approaches have been evaluated only for feasibility. First-generation assays to analyze DNA in stool samples are the only approaches that have been rigorously scrutinized in multicenter, screening studies; no commercial test has been approved by the US Food and Drug Administration. Thus, generalizations about the broad and evolving field of CRC molecular markers should be made with caution.

Technological advances that have improved test performance include innovative methods to increase analytical sensitivity, development of buffers that prevent marker degradation during transport and storage, and identification of marker panels that effectively cover the various genotypes of colorectal neoplasms. Development of high through-put platforms should expand the capacity of these assays and lower costs.

**Analytical Sensitivity**

Tests to reliably detect the minute quantities of marker analytes in stool, blood, and urine must have high levels of sensitivity—especially if precancerous lesions and small, early-stage tumors are to be identified. The technical challenges differ depending on the medium and the markers tested. Most research has been directed toward identification of DNA markers.

In stool samples, it is a challenge to detect trace amounts of target DNA among large amounts of background DNA and high analytical sensitivity (ie, reliable detection of low analyte concentrations) is required. Hu-
human DNA concentrations average about 100 ng/g, which is roughly 0.01% of the total stool DNA.44 The other 99.99% of stool DNA is nonhuman, mostly bacterial and some dietary. The mutated or aberrantly methylated copies of the tumor genes to be identified are only a small proportion of the minute fraction of stool DNA that is of human origin.45,46 Accordingly, an enrichment step is often needed to capture target gene sequences for use as a polymerase chain reaction (PCR) template and remove PCR inhibitors before the assay is performed.46,47 When targeting specific genes shown to be mutated in tissue from advanced adenomas, analytically sensitive assay methods reveal that an average of only 0.5% of the copies of those gene are mutated in matched stools (95.5% wild-type).46 First-generation stool DNA tests could detect mutant copies of genes if they comprised 1% or more of the total copies of the gene,6,7 so many neoplasms that were important to identify contained percentages of mutant genes that were below the detection limit. A number of new approaches have substantially improved analytical sensitivity. For example, an emulsion PCR method referred to as beads, emulsion, amplification, and magnetics (BEAMing)45 (Figure 1) and a digital melt curve method46 detect <0.1% of mutant copies, providing the requisite analytical sensitivity for detection of precursor lesions. Using archival stool samples from a multicenter study, application of the analytically more sensitive digital melt curve method has been shown to significantly improve adenoma detection rates, compared with first-generation stool DNA methods and conventional fecal occult blood tests46 (Figure 2).

In contrast to stool, essentially all DNA in a plasma or serum sample is of host origin; tumor-derived DNA can account for >25% of total circulating DNA levels.48 Although the minimal amount of background DNA in plasma or serum may enhance assay discrimination, the amount of altered DNA is often absent or below detectable limits from patients with precancerous lesions or early-stage tumors.48 As with stool and urine, optimized sample processing and removal of PCR inhibitors are required for high levels of sensitivity.48

**Marker Stabilization**

Markers are often degraded during specimen transport or storage, which can reduce test sensitivity. Adding stabilizing buffers to the biospecimen at the time of collection can eliminate or substantially reduce marker degradation. First-generation stool DNA tests did not incorporate stabilizing buffers, which was responsible, in part, for the lower levels of sensitivity in screening studies that used mailed-in specimens6,7 compared with earlier studies that used freshly collected stools47,49 or subsequent studies that used buffered, mailed-in specimens.46,50 Addition of

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Figure 1. Digital quantification of low abundance DNA methylation in clinical samples by methyl-beads, emulsion, amplification, and magnetics (BEAMing) method. (A) Methyl-BEAMing laboratory flow. (B) Representative results of methyl-BEAMing obtained with flow cytometry. A number of beads representing methylated vimentin, unmethylated vimentin, a mixture of these, and virgin beads are indicated in the top right corner of each box. Virgin beads represent those in aqueous nanocompartments that did not contain a template molecule. From Li M, Chen WD, Papadopoulos N, et al. Sensitive digital quantification of DNA methylation in clinical samples. Nat Biotechnol 2009;27:858–863, reprinted with permission.
buffers containing DNAse inhibitors effectively prevents marker degradation during transport and storage.\textsuperscript{50,51} As assays are developed, it will be important to include specifically designed stabilization approaches, based on the markers and medium tested.

**Marker Panels**

Considering the molecular heterogeneity of colorectal neoplasia, screening tests that target panels of complementary markers rather than single markers should achieve the highest clinical sensitivity. A number of panels of marker have been described that provide complete or nearly complete coverage of colorectal neoplasms.\textsuperscript{7,19} Because of background expression of some markers in patients without cancer, marker metabolism, and variability in marker recovery, observations in tissue cannot be directly extrapolated to assay results on urine, blood, or stool. Marker panels for CRC screening should be evaluated in the intended test media and in the appropriate clinical setting.

Practical considerations of assay complexity and cost factor into marker selection. For example, although \textit{APC} and \textit{P53} are mutated in the majority of colorectal cancers, mutational sites spread across both genes number in the hundreds. With most technologies, detection of each mutation requires a separate assay and no single point mutation can determine a patient’s overall risk for CRC. In contrast, numerous genes, such as \textit{SFRP2},\textsuperscript{52} \textit{septin 9},\textsuperscript{53} \textit{vimentin},\textsuperscript{20,54} \textit{BMP3},\textsuperscript{20} and others are aberrantly methylated in most CRC and precancerous lesions; these can be detected with relatively simple, single-assay techniques. Expression levels of some genes, like \textit{COX-2}\textsuperscript{55} and several others,\textsuperscript{56} can also be used to identify patients with CRC.

Some markers are phenotypic and not unique to or even present in colorectal neoplasm tissue. For example, occult blood (ectopically present in stool) has been the most widely used screening marker for CRC. Similarly, the presence of chemically normal epithelial markers, such as guanylyl cyclase,\textsuperscript{57} in plasma can indicate the presence of a colorectal or other epithelial tumor. Factors of the host response to a tumor or precancerous lesion can also serve as potential screening markers—for example, circulating ligands for tumor-derived galectin-3 identify patients with CRC, compared to healthy controls.\textsuperscript{58}

### High Through-Put Platforms

Micro-fluidics, micro-arrays, nanotechnologies, high-speed sequencing, and other innovative technologies have the potential to yield automated high through-put platforms for efficient performance of molecular screening assays in the future. Such systems could allow assay of multimarker panels at high capacity and low cost.

### Stool Sample Screening

The fecal occult blood test is a noninvasive CRC screening method that has been used for several decades. It is the only screening method shown to reduce colorectal cancer mortality in rigorous, randomized, and controlled trials.\textsuperscript{59–61} Mortality reductions have been modest across studies, and the test has had only modest or no impact on CRC incidence because of the low level of sensitivity for precursor lesions.\textsuperscript{62} Occult bleeding is intermittent,\textsuperscript{63} so testing of multiple stools increases detection rates\textsuperscript{7,63} (Figure 3). The guaiac test is the conventional method for detection of fecal occult blood, but detection rates can be increased using chemically sensitivity guaiac tests or immunochemical tests directed against hemoglobin protein.\textsuperscript{54,65} Many societies, including the American Gastroenterological Association, now recommend these more sensitive approaches be used when fecal occult blood tests are chosen for CRC screening.\textsuperscript{66}

In large screening studies, compared to colonoscopy, sensitive guaiac tests\textsuperscript{7} and immunochemical tests\textsuperscript{67} detected 66% and 63% of colorectal cancers, respectively, but only 20% and 17% of advanced adenomas. Using these stool tests, the detection rates of screen-relevant neoplasms (tumors and advanced adenomas) in the proximal colon were approximately 50% that of neoplasms in distal colorectum. Compared to colonoscopy, sensitive guaiac tests detected 31% of distal screen-relevant neoplasms and 13% of proximal ones (\(P < .01\)), whereas immunochemical tests detected 31% of distal and 16% of proximal lesions (\(P < .001\)). Thus, based on the performance bar set by sensitive guaiac and immunochemical stool blood tests, molecular tests would meaningfully improve the value of stool screening for colorectal neoplasia if they improved sensitivity for early-stage cancers,
DNA Tests

Stool DNA tests have been the most extensively evaluated, noninvasive, molecular approach to CRC screening. The feasibility of testing stool DNA to detect CRC was first demonstrated by assay of a single marker, mutant KRAS, in 1992 and to detect colorectal tumors and advanced adenomas by assay of a panel of markers in 2000. Several configurations of early-generation, multi-marker stool DNA tests have been evaluated in large screening studies. In the first study, a prototype commercial DNA test detected 52% of invasive tumors compared with 13% detected by a conventional guaiac test (P = .003), with comparable levels of specificity. In another study, a 3-marker DNA assay using 1 stool sample from each patient detected 46% of advanced adenomas compared to 10% detected by conventional guaiac testing (P < .001) and 17% by high-sensitivity guaiac testing (P < .001) using 3 stool samples from each patient (Figure 3). However, the DNA test had a lower level of specificity (produced more false-positive results; Table 3). In contrast to the fecal occult blood test, stool DNA test results were not affected by neoplasm site; respective sensitivities for proximal and distal screen-relevant neoplasms were 45% and 48% by the stool DNA test (NS) and 9% and 21% by the conventional stool guaiac test (P = .06). Considering the proportional rise in incidences of CRC and precancerous lesions in the proximal colon, the capacity of stool DNA tests to detect proximal neoplasms is important.

DNA tests have improved detection of screen-relevant neoplasms compared with fecal occult blood tests, despite the fact that the first DNA tests were compromised by use of unbuffered stool samples, suboptimal panels of markers, and analytically insensitive assay techniques. Each of these limitations has been addressed with subsequent technical innovations, as detailed here. Stool DNA testing has been endorsed for CRC screening by the American Cancer Society, the US Multi-Society Task Force, and the American College of Radiology. The 2009 CRC screening guidelines from the American College of Gastroenterology also supported stool DNA tests as an alternative screening approach when used at a frequency of every 3 years. However, the 2008 US Preventive Services Task Force concluded that the evidence from earlier stool DNA tests was “insufficient to assess the benefits and harms,” deferring a final recommendation for the accumulation of additional evidence.

Within the past 5 years, several groups have reported performance results from stool DNA tests; these studies differed in marker selection, assay methods, and patient populations studied (Table 3). Essentially all of these studies amount to feasibility or early assessments of discrimination by new markers or assay techniques on small sets of referred patients rather than final validation of optimized assay systems in the screen setting. In studies that evaluated assay of just 1 broadly informative methylation marker, detection sensitivity for colorectal cancer ranged from 46% to 94% and for advanced adenomas from 21% to 69% at specificities of 79% to 96%. In studies that evaluated various combinations of markers, sensitivities ranged from 75% to 91% for cancers and 44% to 86% for advanced adenomas, at specificities of 82% to 96%. When individual markers within a panel are quantified, logistic algorithms can be used to optimize discrimination and limit compounding of non-specificity that otherwise occurs with combined markers.

Screening frequency influences programmatic test performance. For example, if a stool DNA test with a point specificity of 90% was given every 3–5 years (as recommended by some because of its capacity to detect precursor lesions), its programmatic specificity would be higher than that of the annually applied fecal blood testing (which has a point specificity of 95%); compounded false-positive results would be fewer with stool DNA testing during the screening years. The program sensitivity of stool DNA tests for advanced adenomas (point sensitivity >50%; Table 3) would be increased by serial testing over multiple years, and the extent of this increase would depend on test frequency and neoplasm dwell time.

Detection of precancerous lesions represents an apparent advantage of stool DNA tests compared to fecal blood tests or blood/plasma tests. Assays with improved...
analytical sensitivity, such as the digital melt curve method (Figure 3), have doubled detection rates of advanced adenomas, compared with first-generation stool DNA tests, and more than tripled or quadrupled the detection rate of guaiac tests.46 Based on data from multimarker stool DNA assay that used newer analytical methods,46 detection rates increase progressively with adenoma size: the assay detected 63% of adenomas \( \leq 1 \) cm, 78% of those \( \geq 2 \) cm, and 90% of those \( \geq 3 \) cm, with a specificity of 90%. This is important considering the well-known association between adenoma size and cancer risk. Preliminary data indicate that serrated polyps can also be detected by stool DNA testing;84 further studies are needed to assess detection methods for this previously disregarded cancer precursor.

Stool DNA testing has potential for expanded clinical application beyond screening for colorectal cancer. Common neoplasms above the colon can be detected by stool DNA tests;76,85,86 therefore, this singular, noninvasive approach might be used for pan-detection of gastrointestinal cancers and precancerous lesions in the future. Other possible roles include dysplasia surveillance in patients with specific premalignant conditions like Barrett esophagus, primary sclerosing cholangitis, and inflammatory bowel disease.

In summary, next-generation stool DNA testing has the potential to reduce cancer incidence by identifying patients with precursor lesions, improve cancer detection rates over other noninvasive approaches, and detect proximal and distal colorectal neoplasms with equal sensitivity. Screen-setting validation studies of optimized, next-generation stool DNA tests are needed.

### Other Tests

Stool tests of non-DNA markers have not been extensively studied. RNA expression levels in stool can be quantified to identify patients with CRC.87 With 100% specificity, analysis of messenger RNA (mRNA) levels of COX-2 in stool detected 87% of patients with referred cancers, analysis of matrix metalloproteinase-7 mRNA levels detected 65%, and analysis of the combination of these 2 mRNA markers detected 90%. Few studies have compared stool RNA tests to other approaches. In 1 study,80 analysis of COX-2 mRNA levels in stool detected 50% of patients with CRC, but only 4% of patients with advanced adenomas, at 93% specificity. Analysis of a panel of methylated DNA markers in stool samples from the same patient population detected 75% of those with cancer and 68% with advanced adenomas, at 90% specificity. Stool tests of blood protein markers, such as calprotectin, lactoferrin, haptoglobin, and albumin have been evaluated;4 these assays have the same, low levels of sensitivity in detecting precancerous lesions as assays for hemoglobin. Additional research and development of tests for tumor-
derived proteins in stool could yield more discriminant assays. An exploratory study of an innovative methodology that used magnetic resonance spectroscopy to analyze fecal extracts identified samples from patients with CRC with a remarkable 92% sensitivity and specificity; this approach warrants further investigation.

Blood Sample Screening

CRC tumor cells circulate in blood and release markers that can be detected in samples of plasma or circulating phagocytes. In general, numbers of circulating tumor cells increase in patients with advanced stages of cancer, but not in those with early-stage tumors or with precancerous lesions. As such, assays for circulating tumor cells might be more relevant to staging, prognosis, and postoperative surveillance than to screening, and will not be considered further in this review.

Markers in Circulating Plasma

DNA markers in plasma have been frequently studied (Table 4). The analytically sensitive BEAMing method detected APC mutations, which were present in primary tumor tissue, in matched plasma samples from 73% of patients with CRC, but in only 9% of patients with advanced adenoma; the APC mutations were more frequently detected in plasma samples from patients with more advanced-stage tumors (Figure 4). In multiple studies, analysis of methylation of septin 9 in plasma samples detected 58%–69% of patients with CRC and 18% with advanced adenomas, with specificities of 86%–90%. Because this assay does not reliably detect CRC precursor lesions, it would need to be performed annually or biennially to be effective—similar to fecal occult blood testing.

Assays for CRC based on protein markers in plasma samples are less mature and their levels of performance

<table>
<thead>
<tr>
<th>Table 4. Molecular Blood Tests for Detection of Colorectal Neoplasia: Selected Reports From the Past 5 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity, % (n)</strong></td>
</tr>
<tr>
<td><strong>Specificity, % (n)</strong></td>
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<tr>
<td><strong>Studies</strong></td>
</tr>
<tr>
<td>DNA in plasma</td>
</tr>
<tr>
<td>Diehl* 2006</td>
</tr>
<tr>
<td>Li* 2008</td>
</tr>
<tr>
<td>deVos* 2009</td>
</tr>
<tr>
<td>Lee* 2009</td>
</tr>
<tr>
<td>Proteins in plasma or serum</td>
</tr>
<tr>
<td>Liu* 2006</td>
</tr>
<tr>
<td>Habermann* 2008</td>
</tr>
<tr>
<td>Hurst* 2007</td>
</tr>
<tr>
<td>Leman* 2007</td>
</tr>
<tr>
<td>Walgenbach-Brunagel* 2008</td>
</tr>
<tr>
<td>Kim* 2009</td>
</tr>
<tr>
<td>NOTE. All studies are based on referred patients.</td>
</tr>
<tr>
<td>*Refers to adenomas ≥1 cm.</td>
</tr>
<tr>
<td>aPlasma assays performed on specific adenomatous polyposis coli mutation present in matched tumor tissue.</td>
</tr>
<tr>
<td>bNot all adenomas in this series were advanced.</td>
</tr>
</tbody>
</table>

Figure 4. Fraction of mutant APC fragments in the plasma of patients with colorectal neoplasms [advanced adenomas (Ad) and Dukes’ stage A, B, and D carcinomas]. For each mutation analyzed, DNA from normal lymphoid cells or plasma DNA from healthy donors was used as a control (Normal). The red lines represent the mean, minimum, and maximum values of the normal controls. From Diehl F, Li M, Dressman D, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci USA 2005;102:16368–16373, reprinted with permission.
An extensive exploratory proteomic study that used surface-enhanced laser desorption/ionization mass spectrometry and sophisticated bioinformatics to analyze plasma samples identified protein patterns that could separate patients with CRC from healthy controls with 95% sensitivity and 95% specificity. It is unclear how this impressive level of discrimination might be translated to a simplified assay for the clinic or if patients with precancerous lesions could be identified by this method. Another assay detected colon cancer-specific antigens-3 and -4 in plasma samples from a small group of patients with extraordinarily high accuracy: it identified patients with CRC with 100% sensitivity and those with advanced adenomas with 78%; specificity was 96%. A subsequent study of an assay that detected colon cancer-specific antigens-2 in plasma identified patients with cancer with 89% sensitivity and those with advanced adenomas with 20% sensitivity; specificity was 84%. Other small series studies of assays for plasma proteins have had lower levels of specificity. Further studies are needed to corroborate, extend, and validate assays of plasma protein markers for CRC screening.

**Markers in Circulating Phagocytes**

Circulating macrophages are an intriguing compartment of blood to consider for early detection of colorectal neoplasia. A subset of circulating lymphocytes appears to contain debris scavenged from dysplastic lesions (cancers and precancerous lesions). Only pilot studies of assay of tumor markers in circulating macrophages have been published. Preliminary data on an assay to detect CD24 in circulating leukocytes indicates that this assay approach might efficiently detect colorectal cancers and adenomas. Given the potential for precursor lesion detection, this intriguing approach merits careful further evaluation.

**Urine Sample Screening**

Markers of cancer in the urine come from the circulation and probably require some degree of metabolism to clear the glomerular filter and be excreted, as reviewed. Levels of nucleosides (small-fragment metabolic products of DNA) in urine can accurately discriminate patients with CRC from controls. A single study on a small number of referred patients with colorectal neoplasms suggested that urine testing might be better than plasma testing; there was 83% concurrence in detection of mutant KRAS between urine and matched tumor tissue, but only a 56% concurrence between plasma and matched tumor tissue. Given these findings and the ease with which urine can be collected from patients and tested, this approach warrants more research.

**Assessing Value**

Molecular tests could add value to the collective colorectal cancer screening effort in several ways. They could increase the effectiveness of target lesion detection, reduce cost, and increase safety. Each of these factors will be addressed briefly.

Detection effectiveness at the population level does not equal test sensitivity. Rather, effective detection results from a combination of test sensitivity, patient compliance rates, and access. High levels of sensitivity for early-stage cancer and relevant precancerous lesions on both sides of the colon are necessary but not sufficient for test efficacy. Patient compliance and test accessibility together determine test penetration within a population; without a high level of penetration, even with a perfectly sensitive test, cannot effectively detect CRCs. Real and perceived barriers to screening tests have been evaluated and reviewed. Although noninvasive molecular
screening approaches have user appeal and could be widely distributed by mail, factors of compliance and access will need to be evaluated carefully as variously configured commercial tests emerge.

Value can be viewed in terms of benefits per cost. A conventional approach is to estimate cost effectiveness. In the cost-effectiveness ratio, cost (numerator) is compared to different measures of effectiveness (denominator), such as per neoplasm detected, per cancer prevented, per life saved, or per quality years added. Estimations of screening costs can be complicated because they are leveraged by price of repeated testing over many years, test frequency, the potentially substantial expenses of unnecessary diagnostic procedures that result from false-negative results,104 lost work time, test complications, and other factors.

Cost-effectiveness modeling can be used to establish performance targets for tests that are being developed, as has been done for stool DNA testing.105,106 For example, based on previous Markov modeling,105 for stool DNA tests to be more cost effective than screening colonoscopy, they would have to have CRC detection rates >65% and advanced adenomas detection rates >40%; patients would have to tested at least every 2 years and the unit price of the test would have to be ≤$195. As results from new molecular screening tests are reported and as planned or actual commercial pricing becomes available, updated cost-effectiveness analyses will be instructive, especially if assumptions on compliance and access can be incorporated.107

Value can also be assessed in terms of benefits per risk. The safety of a screening test is very important. Because 94% of persons in a general population will not develop CRC in their lifetimes and will not benefit from screening, the tolerance threshold for any harm imposed by screening should be very low. The likelihood of benefit must outweigh the risk of harm to derive value. On a population basis, benefit and risk can be assessed quantitatively, such as by comparing all-cause morbidity and mortality rates in screened versus nonscreened groups. However, translating such data to decision making can be difficult and is affected by many subjective factors. On an a priori basis, complications of morbidity and mortality from noninvasive tests are lower than from invasive tests. More comparative data are needed from the screening studies to assess relative benefits of molecular tests compared with conventional screening tests.

In the future, the value of noninvasive molecular testing could by expanded beyond control of CRC by additionally detecting supracolic neoplasms.85 The degree to which the benefits of such pan-cancer detection would be offset by costs and risks introduced by downstream diagnostic evaluation remains to be determined.

### Summary

New methods to test molecular markers in stool, blood, and urine offer promising noninvasive approaches to CRC screening. Judicious use of such tests will require further research and development of each candidate approach. Some methods, like stool DNA testing, have been extensively studied whereas others, like urine testing, are nascent. Central biological, technical, and clinical questions need to be fully addressed to best assess the value of and to direct the application of each of these methods (Table 5). Adoption will ultimately depend on assessment of test sensitivities and specificities from screening studies, as well as user appeal, availability, and affordability.

### References

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Conflicts of interest
The author discloses the following: Mayo Clinic is a minor equity investor in and has licensed intellectual property to Exact Sciences. Dr Ahlquist holds patents related to stool DNA testing and, consistent with Mayo Clinic policy, could share in potential future royalties.