

COLORECTAL CANCER SCREENING

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3.7 Emerging techniques

Several tests for CRC screening are at different stages of development, including new stool-based tests, tests based on visual inspection, and blood-based tests ([Table 3.7.1](#)).

3.7.1 Stool-based tests

CRC develops from normal mucosa through a series of cumulative mutations ([Vogelstein & Kinzler, 1993](#)). Cellular debris from growing neoplastic lesions is released into the stool effluent, and through that mechanism provides an opportunity to screen for CRC. Currently, one such test is commercially available; it is based in part on detection of DNA with mutations in stool.

(a) *Multitarget stool DNA test*

(i) *Technique*

Several stool-based DNA tests have been developed during the past decade. Early efforts focused on panels of genes that are commonly mutated during the progression of cancer (e.g. *KRAS*, *APC*, and *BAT-26*). However, results in screening populations showed poor sensitivity, missing approximately half of the invasive cancers detected at colonoscopy ([Imperiale et al., 2004](#)). Increased recognition of the importance of gene methylation as an early marker of cancer prompted the addition of methylated gene markers to the panel. Also, to improve sensitivity, haemoglobin immunoassay was added to the test, leading to a new designation as the multitarget stool DNA (mt-sDNA) test (see [Table 3.7.1](#)). Currently, the mt-sDNA test detects *KRAS* point mutations and aberrantly methylated *NDRG4* and *BMP3*, as well as haemoglobin. *B-actin* is used to measure DNA quality. Stabilizing buffers have been added to prevent DNA degradation after sample deposit, and improved analytical techniques (e.g. quantitative allele-specific real-time target and signal amplification assay) have

been developed ([Bailey et al., 2016](#); [Sweetser & Ahlquist, 2016](#)).

No adjustment in diet or medication is needed by the participant to perform the test, but testing should be avoided in cases of diarrhoea or of bleeding due to haemorrhoids or menses. Whole stool is collected in a container that is placed in the toilet bowl, and the participant separately probes the stool for FIT. The participant then adds a preservative to the container and sends out both the container and the probe to the laboratory. The laboratory must receive the sample within 72 hours of collection to be able to process the test.

Processing of the samples occurs at the manufacturer's laboratory. The results for each marker are incorporated into a logistic regression equation to determine whether the test result is positive or negative ([Lidgard et al., 2013](#)); a quantitative value of 183 or more indicates a positive test result ([Sweetser & Ahlquist, 2016](#)).

Quality control measures are taken during laboratory processing. Specifically, control samples for the DNA assay and the haemoglobin immunoassay components of the test are run alongside the patient samples to ensure adequate processing.

(ii) *Screening performance*

Test performance of the newest iteration of the mt-sDNA test was examined in a large screening population ($n = 9989$) in the USA ([Imperiale et al., 2014](#); [Table 3.7.2](#)). In this study, all individuals underwent screening with colonoscopy, FIT, and the mt-sDNA test. In that population, 65 individuals had CRC and 757 had at least one advanced colorectal adenoma. The sensitivity of the mt-sDNA test was 92.3% for cancer and 42.4% for advanced neoplasia, significantly higher than that of FIT (73.8% for cancer and 23% for advanced neoplasia). The mt-sDNA test was also significantly more sensitive than FIT in detecting large (≥ 10 mm) serrated lesions (42.4% vs 5.1%); however, the specificity for the

Table 3.7.1 Comparison of the most mature emerging technologies for colorectal cancer screening with respect to key features and patient considerations

Technology	Key advantages	Major disadvantages	Time needed for data acquisition	Time needed for reading	Patient considerations	Suggested follow-up frequency when test result is negative
mt-sDNA	Non-invasive No preparation At-home testing	Expensive Modest sensitivity for cancer precursors	Minutes, by patient, around the time of a bowel movement	Minutes; automated	Requires patient to handle/sample own stool	3 yr
Capsule colonoscopy	Total colon examination No intubation of colon or rectum	Cannot control pace of capsule transit, with potential for non-informative/incomplete examinations Modest sensitivity for cancer precursors	≥ 6 hours	30 minutes or more	Extensive preparation with boosting to propel capsule Long acquisition times	5 yr
Plasma DNA (<i>mSEPT9</i>)	Non-invasive No preparation	Expensive Insensitive for early cancer and cancer precursors	Minutes, for a single blood draw	Hours to complete laboratory assays	A single blood draw	Annual

mSEPT9, methylated *Septin 9*; mt-sDNA, multitarget stool DNA; yr, year or years.

Table 3.7.2 Performance of emerging technologies for detection of colorectal cancer and advanced neoplasia

Technology	No. of prospective studies in screening populations	Cancer		Preneoplastic lesions ≥ 10 mm		References
		Per-lesion sensitivity (%)	Per-patient sensitivity (%)	Per-patient sensitivity (%)	Per-patient specificity (%)	
mt-sDNA	1	NA	92.3	42 ^a	87 ^a	Imperiale et al. (2014) Redwood et al. (2016)
Capsule colonoscopy	2	75	100	85–92 ^b	95–97 ^b	Rex et al. (2015) Spada et al. (2016)
Plasma DNA (<i>mSEPT9</i>)	2 ^c	NA	50–68	11–22 ^d	80–91 ^d	Church et al. (2014) Potter et al. (2014)

mSEPT9, methylated *Septin 9*; mt-sDNA, multitarget stool DNA; NA, not applicable.

^a Advanced adenomas + sessile serrated lesions.

^b Polyps.

^c The second study is a reanalysis of the test using frozen plasma from the original participants with a revised assay.

^d Advanced adenomas.

non-advanced lesions was 86.6% for the mt-sDNA test compared with 94.9% for FIT. In the mt-sDNA test, the performance of the haemoglobin immunoassay component alone was similar to that of FIT.

[Although this large tandem-design cross-sectional study provided insights into the performance of the mt-sDNA test compared with that of FIT, the Working Group noted that the study did not directly provide information about the comparative effectiveness of the two technologies. The performance of FIT was influenced by the established thresholds for haemoglobin levels, which define a positive test result and determine the sensitivity and the specificity (see Section 3.2.1). To the extent that FIT with a lower haemoglobin threshold is used, the results with FIT can approximate those achieved with the mt-sDNA test in this study, with the potential exception of the detection of serrated lesions and polyps, for which FIT has poor sensitivity ([Brenner et al., 2014](#)). Also, the study did not provide a comparison in which the two technologies were applied over time.]

There is evidence that the levels of methylated stool markers increase with age. A single study in 500 individuals who had normal colonoscopy findings and who had a freezer-archived stool sample examined factors associated with the levels of methylated stool markers ([Ahlquist et al., 2012](#)). All four genes examined (*BMP3*, *NDRG4*, *vimentin*, and *TFPI2*) were significantly more methylated with increasing age across the age range studied (44–85 years). No associations were found with the other host factors examined in this analysis (e.g. sex, race, body mass index, alcohol consumption, and smoking status). Therefore, theoretically, the specificity might decrease with age. In the large-scale study in the USA ([Imperiale et al., 2014](#)), the specificity of the mt-sDNA test was higher in those younger than 65 years (94%) than in those 65 years and older (87%). [The Working Group noted that these studies were performed in the USA. How this

test would function in populations outside the USA is not well studied, and there are some data suggesting that DNA marker panels would need to be adjusted in other populations ([Park et al., 2017](#)).]

(iii) Preventive effects

There are no RCTs or observational studies of the mt-sDNA test with outcomes of CRC incidence or CRC mortality. Like for CT colonography, most studies of mt-sDNA effectiveness have been tandem studies of a single screening event in a cohort, comparing the outcome (detection rates of CRC and/or advanced neoplasia) with that of established techniques such as FIT or colonoscopy as the reference standard.

[Imperiale et al. \(2014\)](#) compared the detection rates of the mt-sDNA test with those of FIT and colonoscopy. The study population was asymptomatic adults aged 50–84 years at average risk (who were scheduled for screening colonoscopy); recruitment was weighted towards adults aged 65 years and older, to ensure a higher prevalence of cancer. Among the 12 776 participants who were enrolled in the study, 9989 had results that could be fully evaluated; on colonoscopy, 65 (0.7%) were found to have colorectal adenocarcinomas (of which 60 were screening-relevant cancers) and 757 (7.6%) had advanced precancerous lesions, i.e. advanced adenomas (adenomatous polyps ≥ 10 mm or with $> 25\%$ villous component or with high-grade dysplasia) or sessile serrated lesions 10 mm or larger. By comparison, the mt-sDNA test detected 0.6% with CRC (vs 0.48% with FIT), 0.56% with screening-relevant cancers (vs 0.44% with FIT), and 3.2% with advanced precancerous lesions (vs 2.2% with FIT) ([Imperiale et al., 2014](#)). [Although the mt-sDNA test showed higher detection rates than the FIT used in this study, use of a different FIT may have resulted in different findings.]

A second screening study comparing the mt-sDNA test with colonoscopy was undertaken in asymptomatic Alaska Native adults aged

40–85 years who were scheduled for screening or surveillance colonoscopy ([Redwood et al., 2016](#)). Alaska Native people face several challenges related to CRC screening: (i) they are at high risk of CRC, with earlier onset; (ii) most live in remote areas, where access to endoscopy is limited and regular screening with any test is challenging; and (iii) there is a high prevalence of endemic gastrointestinal bleeding from *Helicobacter pylori* gastritis, and hence the specificity of FOBT is poorer (76%) in this population ([Redwood et al., 2014](#)). Because of these considerations, there was interest in comparing detection rates of FIT with those of the mt-sDNA test in this population. The primary outcome measure was the detection of CRC, advanced adenomas (adenomatous polyps ≥ 10 mm or with $> 25\%$ villous component or with high-grade dysplasia), or sessile serrated lesions 10 mm or larger found on screening or surveillance colonoscopy, described as screening-relevant neoplasia. Advanced adenomas were independently reviewed before unblinding. Among 868 enrolled participants, 661 completed the study, of whom 435 were in the screening group and 226 were in the surveillance group. In the screening group, 50% of the screening-relevant neoplasia were detected by mt-sDNA compared with 31% by FIT ($P = 0.01$), and 45% of the advanced adenomas were detected by mt-sDNA compared with 28% by FIT ($P < 0.05$). Detection rates for sessile serrated lesions ($n = 25$) were not reported separately for the two study groups, but for lesions larger than 10 mm, 67% were detected by mt-sDNA compared with 11% by FIT ($P = 0.07$), and for lesions 10 mm or smaller, 38% were detected by mt-sDNA compared with 6% by FIT ($P = 0.07$). [The Working Group noted that these findings are consistent with those from the study by [Imperiale et al. \(2014\)](#), but that this is a small study in a unique, high-risk population and therefore is not easily generalizable to individuals at average risk of CRC.]

Modelling studies have begun to include the mt-sDNA test in simulations for comparison with other screening tests. [Knudsen et al. \(2016\)](#) examined 17 unique strategies for mt-sDNA screening in their microsimulation model for the USPSTF (for detailed information on the models, see also Section 3.2.6 and Section 3.3.6). The strategies varied the starting and ending ages for screening and used screening intervals of 1 year, 3 years, and 5 years for the test. Annual screening with mt-sDNA at age 50–75 years was efficient (i.e. a strategy that provided the largest incremental increase in LYG per additional screening) or near-efficient (within 98% of the efficient frontier) in all three models; however, mt-sDNA every 3 years, which is recommended by the manufacturer, and every 5 years was not as efficient as the other stool-based tests performed annually. Annual mt-sDNA testing was not a recommended strategy, because its efficiency ratio was lower than the efficiency of the benchmark (i.e. colonoscopy every 10 years at age 50–75 years). However, in terms of deaths averted per 1000 individuals screened, outcomes were similar: 22–24 deaths averted for colonoscopy every 10 years, 22–24 for mt-sDNA testing every year, 19–22 for mt-sDNA testing every 3 years, and 20–23 for FIT every year ([Bibbins-Domingo et al., 2016](#)).

[Barzi et al. \(2017\)](#) included mt-sDNA testing at screening intervals of 1 year and 2 years in their comparison of 10-year outcomes for 13 CRC screening strategies. Screening with mt-sDNA every year compared with every 2 years resulted in a negligible difference in the number of diagnosed CRCs (3870 vs 3860), but with both annual and biennial screening, the CRC detection rate exceeded that with one-time colonoscopy (3462). However, the model predicted a smaller reduction in the risk of CRC for annual and biennial screening with mt-sDNA compared with colonoscopy (14% vs 23%), and a smaller reduction in the risk of death from CRC (21% for annual screening with mt-sDNA and 18% for biennial

screening with mt-sDNA vs 34% for colonoscopy) ([Barzi et al., 2017](#)).

(iv) *Adverse effects*

There are no documented adverse effects from the process of undergoing mt-sDNA screening. As with other stool-based screening tests for CRC, adverse effects are associated with follow-up colonoscopy for positive test results, and with false-positive results (see Section 3.2.4). A positive mt-sDNA test result followed by a negative colonoscopy result may be attributable to failure to detect a visible lesion, to very early neoplastic changes that are not yet visible, to the detection by the molecular panel of aerodigestive or supracolononic neoplasms, or to test specificity.

In two screening studies with mt-sDNA ([Imperiale et al., 2014](#); [Redwood et al., 2016](#)), the percentage of advanced neoplasia or CRC not detected in follow-up colonoscopies after a positive mt-sDNA result was about 10% (range, 7–13%) and did not fluctuate with age. These patients may undergo more aggressive short-term surveillance because of concerns about a greater range of possible explanations for false-positive findings compared with other stool-based tests.

[Cotter et al. \(2017\)](#) measured outcomes (mortality, incidence, and other symptoms) after a false-positive test result among patients with false-positive and true-negative test results with mt-sDNA. Among 1050 eligible patients (patients with a positive mt-sDNA test result and those with a negative colonoscopy result) with a median follow-up of 4 years, the cumulative incidence of aerodigestive malignancies (8 cases, including 1 CRC, 3 pancreatic cancers, 3 lung cancers, and 1 bile duct cancer) did not exceed the expected incidence, and false-positive status was not associated with excess mortality or “alarm symptoms”. Evidence related to patient anxiety in response to false-positive findings has not been reported.

(v) *Cost-effectiveness*

In a comparative microsimulation modelling study, [Lansdorp-Vogelaar et al. \(2010\)](#) used the MISCAN and SimCRC models to evaluate the comparative cost-effectiveness of the mt-sDNA test, FIT, sigmoidoscopy, sigmoidoscopy plus FIT, and colonoscopy. The base case evaluated CRC screening intervals of 3 years and 5 years, performance characteristics of the first- and second-generation mt-sDNA tests for detection of adenoma and cancer, and a reimbursement rate of US\$ 350. With 100% participation in CRC screening, the models obtained similar reductions in the risk of CRC incidence with 3-yearly and 5-yearly screening with mt-sDNA (30–49%) compared with annual FIT screening (32–40%), but the estimated reduction in the risk of CRC incidence was considerably larger (53–72%) with colonoscopy every 10 years. Compared with other screening tests, mt-sDNA at intervals of 3 years and 5 years was the most costly and least effective screening test, but the costs were still within the conventional criteria for cost-effectiveness (< US\$ 15 000 per LYG). The authors concluded that mt-sDNA would be an efficient strategy with the modelled reimbursement rate and screening every 3 years if the participation rate was 50% greater than that of the other screening tests, or if reimbursement costs were less than US\$ 60.

[Ladabaum & Mannalithara \(2016\)](#) used a Markov model to evaluate the comparative cost-effectiveness of CRC screening with mt-sDNA every 3 years, FIT every year, and colonoscopy every 10 years in adults at average risk. Despite the improved performance of mt-sDNA over FOBTs, and better sensitivity compared with FIT (see Section 3.7.1(a)(ii)), annual FIT and colonoscopy every 10 years were more effective and less expensive than mt-sDNA every 3 years, assuming 100% participation and a cost-effectiveness threshold of less than US\$ 100 000 per QALY gained. Sensitivity analyses produced findings similar to those of the previous study; in

an organized screening programme, mt-sDNA every 3 years would need to achieve greater participation rates of regular screeners (68% vs 50%) and of intermittent screeners (32% vs 27%) compared with FIT, or would need to be reimbursed at 60% less than the base case rate, to be preferred to FIT at the highest threshold (< US\$ 100 000 per QALY gained) of cost-effectiveness. With opportunistic screening, based on participation rates of 15% for regular screeners and 30% for intermittent screeners, mt-sDNA would need to achieve nearly twice the participation rate to be under the cost-effectiveness threshold of US\$ 100 000 per QALY gained.

(b) *Other stool-based tests*

Although identification of haemoglobin as a protein marker in stool is a well-recognized screening approach, other stool protein markers have also been evaluated. Generally, evaluation of these other markers has occurred in small observational studies in which detection rates of CRC and advanced neoplasia are estimated. Pyruvate kinase type M2 is a protein that regulates tumour growth and is not specific to CRC. In one meta-analysis that identified 10 individual studies assessing the use of stool pyruvate kinase type M2 in the detection of CRC, the sensitivity of the marker was 79% and the specificity was 81% ([Li et al., 2012](#)). Calprotectin is a calcium-binding protein that is found predominantly in neutrophils. In one large prospective evaluation study in a screening population ($n = 2321$), the sensitivity of this marker for CRC was 63% and the specificity was 76% ([Hoff et al., 2004](#)).

Beyond these single protein markers, there is also interest in developing broader panels of proteins present in stool that might be used for screening ([Ang et al., 2011](#); [Bosch et al., 2017](#)). However, this work remains preliminary. In addition, there is also interest in examining the faecal microbiome as a screening tool, but this development is largely still at the discovery phase ([Yu et al., 2017](#)).

3.7.2 *Capsule colonoscopy*

Building on capsule technology to evaluate the small bowel, a capsule to evaluate the large bowel was first released and evaluated in 2006 ([Eliakim et al., 2006](#)). This technology enables a total colon examination to be performed with no bowel intubation or sedation ([Table 3.7.1](#)).

(a) *Technique*

The technology currently used is a second-generation system with improved performance with respect to imaging of the large bowel. Equipment to perform capsule colonoscopy, which is currently commercially available from a single vendor, requires three components: a capsule, a data recorder, and a workstation to read the acquired images. The ingestible capsule has dimensions of approximately 11 mm × 32 mm. The device has a camera at each end and a battery that enables the capture of images for approximately 10 hours. On the basis of the initial experience, refinements were made to improve the performance of the capsule. The current, second-generation capsule has a viewing angle for each camera of 172°, which enables a near-360° view of the colon. The capsule can capture up to 35 images per second. Another improvement in the technology was the development of an adaptive frame rate; this was made possible in part by real-time communication between the capsule and the data recorder worn by the patient. Finally, data from the recorder are downloaded to a workstation, to assist the clinician with image review ([Spada et al., 2015, 2016](#)).

Preparation for capsule colonoscopy is necessary for several important reasons. As is the case for conventional colonoscopy, the quality of the examination can be compromised by residual stool coating the colonic mucosa. In fact, suboptimal preparation is a greater threat to the test performance for capsule colonoscopy than for traditional colonoscopy, because with capsule colonoscopy there is no opportunity to wash

the bowel or remove residual effluent through suction. In addition, there is a requirement to “push” the capsule to and through the colon to complete the examination during the 10-hour battery life of the device ([Spada et al., 2015](#)). Generally, colon preparation is accomplished with a full dose (4 L) of polyethylene glycol, of which half is taken the day before the procedure and half on the day of the procedure. Boosting of the movement of the capsule is most commonly accomplished with sodium phosphate (NaP). Preparation also includes dietary adjustment (e.g. a clear liquid diet) the day before the procedure, and occasionally the use of suppositories to assist with capsule excretion. Even with these aggressive preparation and boosting regimens, the quality of the examination can be an issue. In a recent meta-analysis of studies of colon capsule colonoscopy, adequate cleansing levels were achieved in 81% of cases and capsule excretion within the usual battery life (10 hours) was achieved in 90% of cases ([Spada et al., 2016](#)).

After the capsule is excreted, the images that were obtained need to be interpreted. Given that the capsule generates thousands of images, reading them can be cumbersome. The reading time can exceed 50 minutes in more than 25% of cases ([Farnbacher et al., 2014](#)). There have been efforts to develop computer-assisted reading algorithms, building on similar technology used for the capsule to evaluate the small bowel. The proprietary software can develop previews of significant findings that are tailored by the reader. For example, images of interest can be set to various percentages to enable the reader to have a quick overview of the study. This software was formally assessed in one recent study ([Farnbacher et al., 2014](#)). By using such settings, the reader could cut the reading time by 90% while still identifying 98% of patients with at least one significant polypoid finding. The use of such software might facilitate same-day examination of individuals, enabling them to be moved quickly from capsule to colonoscopy

because they have at least one lesion that requires polypectomy.

[The Working Group concluded that compared with other structural screening options (e.g. colonoscopy and CT colonography), capsule colonoscopy is more demanding for the patient and the examiner, requiring more intensive procedural preparation, longer patient examination, and extended clinician interpretation times. Prolonged examination and interpretation limit the opportunity for same-day colonoscopy, which avoids two separate colon preparations.]

Some individuals may not be suitable for screening with capsule colonoscopy. For example, individuals with a swallowing disorder are probably not well suited for this test. To reach the colon, the device must traverse the entire proximal gastrointestinal tract, including the small bowel. For individuals with extensive prior surgery and/or known adhesive disease, there is a risk of the capsule becoming stuck in the small bowel. Finally, certain individuals are at higher risk of complications with NaP (e.g. people with hypertension who are taking angiotensin-converting-enzyme inhibitors), and therefore screening by capsule colonoscopy entails more risk for those individuals.

The European Society of Gastrointestinal Endoscopy guideline addressed the use of the colon capsule ([Spada et al., 2012](#)). Key recommendations included the use of split-dose polyethylene glycol for the preparation and the use of sodium phosphate boosters where possible. Also, the guideline recommended standardized reporting practices, including for the quality of the preparation, the completeness of the examination, and the description (i.e. size, morphology, location) of identified polyps. The guideline recommended follow-up colonoscopy for those with a polyp 6 mm or larger or three or more polyps of any size, and a follow-up interval of 5 years in a CRC screening setting after a negative examination result.

(b) Screening performance

A large tandem study assessed the performance of capsule colonoscopy in a screening population ([Rex et al., 2015](#)). In the final cohort ($n = 695$), capsule colonoscopy identified individuals with one or more polyps 6 mm or larger with a sensitivity of 81% (95% CI, 77–84%) and a specificity of 93% (95% CI, 91–95%). Four cancers were identified by colonoscopy, and three were detected during the blinded capsule interpretation (per-lesion sensitivity, 75%); the other cancer (a 10 mm sessile lesion) was visible during unblinded review. [In the individual with that cancer, other lesions were identified that would have prompted total colonoscopy.]

A second tandem study in the Czech Republic ([Suchanek et al., 2015](#)) assessed the performance of capsule colonoscopy in 236 consecutively enrolled adults older than 50 years. The sensitivity of capsule colonoscopy was 77% for polyps 6 mm or larger, 88% for polyps 10 mm or larger, and 100% for adenomas 10 mm or larger; the specificity was 97% for polyps 6 mm or larger and 99% for polyps 10 mm or larger. Two cancers were diagnosed with both methods. The overall sensitivity and specificity for lesions 10 mm or larger were similar in the two studies ([Spada et al., 2016](#)).

Capsule colonoscopy, also referred to as capsule endoscopy, is approved in the USA for examining the proximal colon in patients with incomplete colonoscopies, or for patients who are not candidates for colonoscopy or sedation, but it is not approved for CRC screening in adults at average risk, probably because of the limited data available on performance in a screening population ([Rex et al., 2017](#)).

(c) Preventive effects

There are no RCTs or observational studies of capsule colonoscopy with outcomes of CRC incidence or CRC mortality. Like with other emerging technologies, single-test tandem

studies comparing the detection rates of cancer and of precursor lesions with capsule colonoscopy and with conventional colonoscopy as the reference standard have been conducted [most of these investigations have been in small, heterogeneous study populations]. In one study evaluating the detection rates with capsule colonoscopy in 689 subjects ([Rex et al., 2015](#)), the detection rate for adenomas 6 mm or larger was [14%], and the detection rate for adenomas 10 mm or larger was [5.8%]. The cancer detection rate was [0.4%] (3 of the 4 cancers identified by colonoscopy), and after unblinding the cancer was visible in multiple photos taken by the capsule.

(d) Adverse effects

Few serious adverse events associated with capsule colonoscopy have been documented ([Spada et al., 2012, 2016](#)). In a tandem study in a screening population, [Rex et al. \(2015\)](#) reported no serious capsule-related events. Among 884 patients enrolled in the study, 142 non-serious events were reported in 101 patients, of which 128 were related to bowel preparation. Three non-serious events associated with the capsule were related to the procedure; these included gagging, vomiting, and abdominal cramping ([Rex et al., 2015](#)). Capsule retention, defined as a capsule that remains in the digestive tract for longer than 2 weeks, occurred in fewer than 2% of patients ([Rondonotti, 2017](#)).

(e) Benefit–harm ratio and cost–effectiveness

No data are available on the benefit–harm ratio of capsule colonoscopy. [Hassan et al. \(2008\)](#) used a simulation model to compare capsule colonoscopy with conventional colonoscopy and observed that capsule colonoscopy was less cost-effective than conventional colonoscopy if the participation rates were equal. If the participation rate for capsule colonoscopy was 30% higher than that for conventional colonoscopy, then capsule colonoscopy was more cost-effective than conventional colonoscopy.

3.7.3 Blood-based tests

(a) Single-gene plasma DNA test (*mSEPT9* DNA test)

Blood-based DNA tests are another example of a new class of CRC screening tests. Blood testing as a screening test for CRC has been referred to as the “holy grail of cancer detection research” ([Ransohoff, 2003](#)), because the barrier to participation is reduced to a single blood draw, which may well be combined with other annual tests (e.g. a cholesterol test) ([Table 3.7.1](#)). Although the relative simplicity of this technique is an advantage of this approach, commitment to follow-up testing with colonoscopy (after a positive screening result) remains an important consideration.

(i) Technique

DNA of tumour origin is present in minute quantities in the plasma of patients with cancer ([Pawa et al., 2011](#)). To date, it has been more promising to search for tumour-associated methylation changes. Through a well-described process ([Payne, 2010](#)), investigators evaluated a host of potentially relevant DNA methylation markers. From that group, the methylated *Septin 9* gene (*mSEPT9*) was selected for further development and evaluation, on the basis of favourable test characteristics in case–control studies for this marker compared with other potential candidate markers ([Lofton-Day et al., 2008](#)). Septins as a family of genes have critical functions in multiple cellular processes, including apoptosis ([Hall & Russell, 2004](#)).

From the perspective of the patient, the *mSEPT9* DNA test requires no preparation, including no changes in diet and no use of medication. The individual undergoes a single phlebotomy, and the sample is sent to the laboratory for processing. The details of the processing vary by the version of the test used ([Epi proColon, 2017](#)), but processing requires two steps. In step one, DNA is extracted from the plasma and is

subsequently incubated in bisulfite solution, to alter unmethylated cytosine residues in the DNA. In step two, the bisulfite-converted DNA is assayed using real-time duplex polymerase chain reaction, and the presence of *mSEPT9* is specifically identified through the use of a fluorescence detection probe. The processing time is estimated to be 8 hours ([Lamb & Dhillon, 2017](#)).

The laboratory-based *mSEPT9* DNA test has well-described external and internal controls. External controls include known positive and negative samples that are run alongside the patient sample. As an internal control, β -actin DNA is used as a marker of DNA quality.

Variations of the test are approved for use in different countries. In the USA, the United States Food and Drug Administration approved the test for use in people who are unwilling to undergo any other screening test; in Europe and China, a slightly different version of the test for the same marker is approved. Although both are second-generation tests, the version used in the USA requires only one of three replicates to be reported as positive, whereas the version used in Europe requires two of three replicates to be reported as positive ([Lamb & Dhillon, 2017](#)).

(ii) Screening performance

The performance of the *mSEPT9* DNA test as a screening tool was assessed directly in a large-scale ($n = 7941$) prospective study ([Church et al., 2014](#)). Blood samples were drawn from asymptomatic adults aged 50 years or older at least 1 day before colon preparation was initiated. Colonoscopy was subsequently completed. Results for 53 individuals with cancer and 1457 without neoplasia were available. When the *mSEPT9* DNA test was analysed on the basis of two replicate samples, the sensitivity of the test for cancer was 48.2% and the specificity was 91.5%. The sensitivity for the detection of more advanced lesions was higher (e.g. sensitivity of 77.4% for stage IV cancer vs 35% for stage I cancer). In post hoc analyses, the test characteristics were

recalculated using a third replicate sample. The sensitivity increased to 63.9% and, as expected, the specificity decreased to 88.4% ([Church et al., 2014](#)). A follow-up analysis of this cohort was also performed using the optimized (i.e. second-generation) test. The sensitivity for detection of CRC increased to 68%, and the specificity decreased to 79% ([Potter et al., 2014](#)).

A meta-analysis identified 14 studies that evaluated the performance of the *mSEPT9* DNA test ([Zhang et al., 2017](#)). The study design and the application of the test varied across the studies, resulting in significant heterogeneity. For detection of CRC, the pooled sensitivity was 67% (95% CI, 61–73%) and the pooled specificity was 89% (95% CI, 86–92%). In another meta-analysis of 25 studies ([Song et al., 2017](#)), 2613 CRC cases and 6030 controls were included, and the sensitivity ranged from 48.2% to 95.6% and the specificity ranged from 79.1% to 99.1%.

As discussed above (see Section 3.7.1(a)(ii)), there is some evidence that methylation of genes increases with age, which has the potential to decrease test specificity in older individuals. Also, analysis by race suggested some increased false-positivity in African American people compared with White people ([Potter et al., 2014](#)). In fact, the meta-analysis by [Zhang et al. \(2017\)](#) also demonstrated variation in performance across ethnicity.

(iii) Preventive effects

There are no RCTs or observational studies of plasma DNA tests with outcomes of CRC incidence or CRC mortality. As with other emerging technologies, single-test tandem studies comparing the detection rates of cancer and of precursor lesions are the principal methodological strategy for evaluating the efficacy of the test. Only one evaluation of the *mSEPT9* DNA test has been carried out in an asymptomatic screening population, and it is described above ([Church et al., 2014](#)).

[Ladabaum et al. \(2013\)](#) estimated the comparative effectiveness of the *mSEPT9* DNA test, FOBT, FIT, sigmoidoscopy, and colonoscopy using a validated decision analytical model. The performance parameters used for the *mSEPT9* DNA test were those observed by [Church et al. \(2014\)](#) with two and three replicates with biennial screening. All screening strategies assumed 100% participation. In the base case, screening with the *mSEPT9* DNA test decreased CRC incidence by 35–41% and CRC mortality by 53–61%.

In terms of the comparative effectiveness of the *mSEPT9* DNA test, all of the established screening strategies considered in the analysis were more effective than the *mSEPT9* DNA test.

(iv) Adverse effects

There are no reports of adverse effects of CRC screening with the *mSEPT9* DNA test. Potential adverse effects are those related to the process of phlebotomy (blood draw). With the current low specificity, a significant fraction of asymptomatic adults undergoing screening and receiving positive test results would be referred for colonoscopy, and the risks associated with bowel preparation and colonoscopy would apply.

(v) Benefit–harm ratio and cost–effectiveness

No data were available on the benefit–harm ratio associated with CRC screening with the *mSEPT9* DNA test, and only one study on cost–effectiveness was available to the Working Group. [Ladabaum et al. \(2013\)](#) compared the cost–effectiveness of the *mSEPT9* DNA test with that of established screening strategies. In an analysis based on the estimates of specificity and sensitivity from [Church et al. \(2014\)](#), CRC screening with the *mSEPT9* DNA test was cost-effective compared with no screening, but not cost-effective compared with established screening tests, because it was less effective and more costly. Because screening participation is variable and is influenced by test preference, comparisons between strategies may yield different results.

For example, FIT was the preferred strategy with 100% participation, but when the participation rate was reduced to less than 70%, it was no longer more effective than the two-reagent *mSEPT9* DNA test, and when the participation rate was reduced to less than 85%, it was no longer more effective than the three-reagent *mSEPT9* DNA test. [The Working Group noted that where increasing participation in a CRC screening programme depends on the availability of preference-sensitive options, tests that are less effective in head-to-head comparisons than tests that have more favourable characteristics may become more attractive.]

(b) *Multiple-gene plasma DNA test*

A 29-gene marker panel is currently commercially available and has been marketed in some countries as an option for CRC screening. The gene panel is based on the hypothesis that myelomonocytic cells are recruited to neoplasia and that genes in those cells could be used as biomarkers for detection ([Nichita et al., 2014](#)). The test has been assessed within a case-control study that recruited patients in the Republic of Korea and Switzerland. The initial study to develop the panel included 144 individuals, with 46 patients with at least one large polyp, 48 patients with CRC, and 50 controls. The 48 CRCs were equally split ($n = 12$) across the four stages. Through a process of repeated statistical analysis, a final list of 29 genes was developed to compose the DNA panel. To evaluate the clinical relevance of the 29-gene panel, penalized logistic regression was applied to the data set and the models were validated by non-overlapped bootstrap methods. In the validation model, the sensitivity of the test for the detection of CRC was 75% ([Ciarloni et al., 2015](#)).

In a second publication ([Ciarloni et al., 2016](#)), the 29-gene test was again examined in a selected population. Although the second study was larger than the initial study ($n = 594$), only the population in Switzerland was included. Again, a

case-control design was used, with 149 controls, 103 patients with at least one large adenoma, and 97 patients with CRC, with a preponderance of late-stage cancers (44 in stages I and II and 53 in stages III and IV). When both sets of tests were considered, the sensitivity for the detection of CRC was 75%, with a higher sensitivity in those with late-stage cancer (90% for stages III and IV vs 56% for stages I and II). The specificity, determined by a negative colonoscopy result, was reported as 92%.

[The Working Group noted that the validation strategies used suggested extreme overfitting of the model and that the test had not been evaluated for test characteristics in a large prospective asymptomatic screening population using colonoscopy as a reference standard. The spectrum of cases was weighted to later-stage disease, and there was evidence that the test characteristics were better in those with more advanced disease.]

3.7.4 *Tests based on other markers*

(a) *Protein markers in serum*

There are a host of potential pathways to abnormal protein production during carcinogenesis that could be detected in serum. The protein could be the direct product of an abnormally modified gene. Alternatively, protein products released from inflamed or bleeding tissue associated with cancer might also be detected. Finally, approaches to identify antibodies made in response to neoplastic tissue have also been studied. Tumour-derived markers such as carcinoembryonic antigen and carbohydrate antigens (e.g. CA 19-9) have been extensively studied in serum ([Hundt et al., 2007](#)). [The studies on test performance are generally small and have a case-control design.] The results varied according to a variety of factors, including the spectrum of patients with cancer studied (early- vs late-stage disease) and the thresholds used to define a positive test result. Generally, results with such markers have been disappointing, with

sensitivities of about 50% and skewed towards those with more advanced disease. Given the relative lack of sensitivity for individual serum markers, efforts have been made to more broadly study the proteome. Several groups have used mass spectrometric analysis to examine protein patterns in individuals with cancer and those without. Although the studies that have compared the test characteristics of protein patterns with those of tumour markers alone show more promising results ([Pawa et al., 2011](#)), those studies are small and cross-sectional in nature.

(b) RNA markers in stool or serum

During the past decade there has been increasing interest in the role of the transcriptome in colorectal carcinogenesis. The initial work focused more directly on protein-coding RNA. Free messenger RNA (mRNA) is not stable in blood. However, mRNA isolated from circulating leukocytes can be analysed. Analyses have been performed of mRNA coding for a host of potentially relevant biological markers (e.g. carcinoembryonic antigen, cytokeratins, and mucins) ([Hundt et al., 2007](#)). [In these small studies comparing selected cancer cases with controls, the test characteristics were not appropriate for screening.]

There is growing interest in non-coding RNA as a biomarker for cancer, including CRC ([Esteller, 2011](#); [Kita et al., 2017](#)). Perhaps the best-studied species are the microRNAs (miRNAs). These are short (18–22 bp) segments of non-coding RNA. Compared with mRNA, these short segments are more stable and less prone to degradation even at extremes of temperature or pH, and therefore they are better suited as biomarkers ([Yiu & Yiu, 2016](#)). miRNAs serve as modulators of the translation of mRNA and can thus influence the growth and development of tumours through a variety of mechanisms. Importantly, they are detectable in both serum and stool. In one representative study examining miRNA in stool, investigators compared miRNA expression in 29 individuals

undergoing colonoscopy (10 with CRC). Levels of miR-21 and miR-106a in stool were significantly increased in individuals with CRC compared with those without CRC ([Link et al., 2010](#)). [The Working Group noted that although this area of research is developing quickly, the studies are preliminary, focusing on panels of markers in CRC cases and controls. Large-scale studies in screening populations are not available at this time.]

(c) Volatile organic compounds in breath-based tests

There has been some interest in examining volatile organic compounds exhaled through breath as a potential biomarker. Tumour growth has been associated with cell membrane peroxidation and the subsequent emission of volatile organic compounds ([Peng et al., 2010](#)). Volatile organic compounds can be identified in several different ways ([de Boer et al., 2014](#)). Individual volatile gases can be separated out from a breath sample using gas chromatography–mass spectrometry (GC-MS). Alternatively, a sensor array, which has been called an “electronic nose”, can more broadly evaluate smell prints, driven by the underlying profile of volatile organic compounds.

Studies evaluating the role of volatile organic compounds as a biomarker for CRC are relatively limited ([Markar et al., 2015](#)). A proof-of-principle study used trained canine olfaction to distinguish between individuals with cancer and those without. A Labrador retriever, with 33 breath samples, appropriately detected CRC confirmed with colonoscopy, with a sensitivity of 91% and a specificity of 99% ([Sonoda et al., 2011](#)). From a clinical standpoint, it would be difficult to implement canine detection into standard laboratory practice, but the study has prompted further work using more traditional approaches to detect volatile organic compounds. For example, separate investigators ([Altomare et al., 2013](#); [Amal et al., 2016](#)) have used GC-MS in CRC cases and controls to identify potentially discriminating

volatile organic compounds. Using this type of approach in small numbers of individuals results in sensitivities of approximately 85% and specificities in the range 83–94%. [The Working Group noted that the studies are small and preliminary and require validation. Large-scale prospective evaluations of volatile organic compounds in screening populations have not been performed.]

(d) Urinary markers

Urinary markers are under development but are not reviewed here.

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