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REVIEW ARTICLE

Blood-Based Testing for Colorectal Cancer Screening

Karen A. Heichman

Abstract Colorectal cancer (CRC) is the third most common non-skin cancer diagnosed in men and women in the USA and worldwide. While it has been clearly established that screening for CRC, using a variety of methods, is cost effective and has a significant impact on overall survival, screening rates have proven to be sub-optimal. It has been long conjectured that a simple blood-based test, with a specimen drawn at a routine doctor's office visit, would encourage those individuals who have refused or ignored screening recommendations to undergo screening. This article reviews the currently available blood-based screening tests for CRC, including the ColonSentry™ messenger RNA (mRNA) expression panel and the *SEPT9* methylated DNA test, and explores newer biomarkers that are near clinical implementation. Also discussed are additional applications for blood-based CRC testing, such as assessing prognosis, disease surveillance, and expansion of screening tests to high-risk populations, such as the estimated 1.4 million individuals in the USA with inflammatory bowel disease.

1 Introduction

Colorectal cancer (CRC) is the third most common non-skin cancer diagnosed in men and women in the USA and worldwide, with over 140,000 new cases predicted in the USA for 2013 [1]. American men and women have a 1 in 20 lifetime risk of getting CRC. CRC is the second leading cause of cancer death in the USA if both sexes are taken together as a group, with an estimated 50,000 deaths attributed to the disease expected in 2013 [1]. Death rates have decreased over the past 20 years, due in part to screening efforts, early detection, and improved treatment options. CRC can be prevented by the removal of polyps during colonoscopy. Tumors detected at an early stage can be more effectively and less expensively treated, with 5-year survival rates of 90 %. Unfortunately, many CRCs are diagnosed when the disease has spread beyond the primary site, with greatly reduced survival rates.

2 Screening and Compliance

Although screening can dramatically lower mortality due to CRC, less than two-thirds of Americans aged 50 years and older currently undergo any kind of screening [2], with much lower screening rates reported in other countries [3]. Globally, stool-based testing predominates; however, in the USA, screening methods other than colonoscopy, such as fecal testing, have declined in recent years [4]. Patient compliance is a major barrier to achieving universal screening [5]. Individuals who otherwise adhere to screening recommendations for other cancers, such as those who routinely undergo mammography, pap screening, and prostate-specific antigen (PSA) testing, do not faithfully follow CRC screening recommendations [5]. American physicians recommend colonoscopy to their patients most often [4], yet patient preference appears to strongly determine what screening method is ultimately used [6]. Reasons for not complying with colonoscopy

K. A. Heichman (✉)
Oncology Technology Development and Licensing, ARUP Laboratories Inc., 500 Chipeta Way, Mail stop #209, Salt Lake City, UT 84108, USA
e-mail: karen.heichman@aruplab.com

K. A. Heichman
Department of Pathology, University of Utah School of Medicine, Salt Lake City, USA

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referral include the time-consuming nature of the procedure, discomfort with the bowel preparation, and concern about invasiveness [6]. Immunochemical fecal occult blood test (FOBT) testing (i.e. fecal immunochemical testing [FIT], immunochemical FOBT [iFOBT]) and stool DNA tests have recently contributed to a renewed interest in fecal testing in the USA, and studies have demonstrated that increased screening-compliance rates can be achieved with some of these methods [7, 8].

3 Blood-Based Screening

In 2003, Dr. David Ransohoff [10], in reference to an article by Cui et al. [9] describing the loss of imprinting of the insulin-like growth factor (*IGF*)-*2* gene as a potential biomarker of CRC, described the search for a noninvasive biomarker test for cancer as the "Holy Grail of cancer biomarker research." In fact, it has been a widely held view that a simple blood test for CRC, with a sample collected at a routine physician's office visit, would encourage more patients to undergo screening and could ultimately prevent CRC deaths. Patients have become accustomed to laboratory screening tests such as those used to measure cholesterol, vitamin D, blood sugar, and PSA, and it is hardly a stretch to imagine that a blood-based test for CRC, with an additional tube of blood collected at the same time as other routine tests, would be widely embraced.

In May 2011, the nonprofit Colon Cancer Alliance and Quest Diagnostics (a large US-based clinical laboratory) engaged an independent research company to conduct a survey in the USA to measure awareness of CRC screening in the population aged 50 years and older [11]. The study confirmed widespread usage of colonoscopy; however, bowel preparation was a major deterrent for many patients. Interestingly, the study demonstrated that even previously screened individuals did not follow guidelines for periodic testing. Three-quarters of all respondents, including both screened and unscreened individuals, indicated that they would undergo testing more frequently if a blood test was available. In fact, many respondents indicated they would be willing to give up a modern convenience, such as beer or wine, chocolate, or the use of a cellular phone, for 6 months in exchange for having the option of a blood-based screening method.

Further, additional independent patient preference studies strongly suggest that blood-based screening for CRC would increase screening rates as well as encourage first-time testing of individuals who have otherwise declined CRC testing. Behavioral science researchers from the University of Utah have conducted focus groups and surveys of previously screened and unscreened participants to elicit attitudes and identify predictors of interest in blood-based CRC screening [12]. Perhaps not surprisingly, after presentation of information on four screening methods, colonoscopy, sigmoidoscopy, FOBT, and a new blood test, 64 % of all participants, both previously screened and unscreened, indicated a preference for a blood test over other, well-established options [12].

4 First-Generation Commercially Available Blood-Based Screening Tests for Colorectal Cancer (CRC)

Blood-based screening for CRC first became available in North America and Europe in 2008. In Canada, the company GeneNews introduced ColonSentry™ as a laboratory-developed test (LDT) in the summer of 2008, while Epigenomics AG made the first research use only (RUO) kit based on the Septin 9 (*SEPT9*) biomarker available in Europe later that year. Since then, blood-based testing for CRC has expanded to many countries throughout the rest of the world, including a strong presence in the USA. Improved testing methods for blood-based CRC testing have been developed and implemented in the clinical setting, with in vitro diagnostic (IVD) kits widely available.

One of the first blood-based tests for CRC was brought into clinical use in Canada by the Ontario-based company, GeneNews. The so-called ColonSentry™ test measures messenger RNA (mRNA) from whole blood in a seven-gene expression panel that assesses the following transcripts: *ANXA3*, *CLEC4D*, *LMNB1*, *PRRG4*, *TNFAIP6*, *VNN1*, and *IL2RB* [13, 14]. The ColonSentry™ gene expression signature is measured by a real-time polymerase chain reaction (PCR) method. The results are reported as a 'CURR' score, or the 'CUmulative Relative Risk', which is based on the ratio of a patient's probability of having CRC over the prevalence of the disease. The performance of the test was established in a case–control study of North American subjects, including 202 CRC patients (~60 % stage I or stage II) and 208 matched controls. In this population, the ColonSentry™ test has a reported 72 % sensitivity for detecting CRC, with a specificity of 70 % for distinguishing CRC patients from healthy controls [14]. The test is intended to be used as a decision aid to assess a subject's risk of having CRC at the time of testing, with the recommendation that individuals at increased risk be followed up with colonoscopy. The appropriate ColonSentry™ blood sample may be drawn at a participating laboratory or physician's office. There is no obligation for patient lifestyle modifications prior to specimen collection, such as fasting, withholding medication, or bowel preparation; however, there is a requirement for a specialized collection tube (PAXgene Blood RNA Kit, Qiagen) to be used in order to stabilize the mRNA analytes [15]. The ColonSentry™ gene expression signature has also been

studied in a small case–control study of 210 colonoscopy subjects from Malaysia who donated blood specimens prior to endoscopy [16]. The test is clinically available in Malaysia and China, and more recently has been made available to patients in the New York area of the USA.

The *SEPT9* gene was demonstrated to be differentially methylated in CRC versus non-pathologic tissue [17]. It is well established that tumor DNA from solid cancers can be detected in the bloodstream using sensitive PCR detection methods, and indeed, methylated *SEPT9* DNA has been demonstrated in the blood plasma of CRC patients [17]. The *SEPT9* biomarker has been extensively analyzed in case–control studies of more than 3,000 subjects performed in multiple studies and independent labs in the USA and Europe [17–22]. Table 1 shows that, using different methods and performed in different laboratory settings, the *SEPT9* biomarker exhibited a sensitivity of 71–72 % at a specificity of 86–90 % using first-generation testing protocols [18–20]. The original methods to detect *SEPT9* used a triplicate (three-well) PCR assay in order to maximize the amount of extracted tumor DNA that could be analyzed [18–20]. In the case of the deVos and Solomon assays, the methods were optimized for sensitivity, whereby only a single PCR replicate of the three must contain detectable methylated *SEPT9* DNA in order for the test to reported as positive [19, 20].

SEPT9 has also been the subject of the PRESEPT large prospective study of nearly 8,000 subjects in the USA and Germany, which demonstrated the utility of *SEPT9* in a population-based screening setting. Summary results of the study utilizing three PCR replicates were presented at the 2010 Digestive Disease Week (DDW) conference and indicated that the test could detect 67 % of CRCs (including mostly early-stage tumors) at a specificity of 88 % if only one of three PCR replicates was required to have detectable *SEPT9* [23]. These data are consistent with the previous case–control studies and demonstrate the utility of the *SEPT9* biomarker for detecting CRC in the blood. The test was also reported as a two-well PCR protocol, which not surprisingly, yielded lower sensitivity than if the third well was included [24].

The first kits for the blood-based detection of CRC employing the *SEPT9* methylated DNA biomarker became available in 2008 as an RUO kit developed by Epigenomics, AG in Berlin. Shortly thereafter, two CE-marked (Conformité Européenne; conformity marking in the European Economic Area) IVD kits based on *SEPT9* were launched by two vendors, Epi proColon® (Epigenomics) and mS9™ (Abbott Molecular); these kits were based on the first-generation assays described by deVos et al. [19] and Solomon et al. [20], respectively. The following year, the first blood test for CRC based on *SEPT9* was launched in the USA by Quest Diagnostics as an LDT called ColoVantage® [25]; according to the company's website, the test has similar performance characteristics to the first-generation kits and as described in the preceding case–control studies [18–20]. Warnex Medical Laboratories in Canada began offering a *SEPT9* LDT, also, based on the original *SEPT9* detection methods. Since these first results, efforts have been underway by both kit vendors, as well as independent laboratories in the USA and Europe, to improve the sensitivity of the *SEPT9* tests.

5 Improved Blood-Based Screening Tests for CRC

CRC screening tests based on the *SEPT9* methylated DNA biomarker have undergone significant enhancements with regard to sensitivity for detecting CRC as well as laboratory workflow. In July 2010, Utah-based ARUP Laboratories launched an independently developed and validated *SEPT9* LDT with increased sensitivity compared with the first-generation CE-marked Epi proColon® kit. The improved ARUP method developed by Warren et al. [21] examines the exact same region of the *SEPT9* gene and utilizes the same plasma DNA extraction method as described by deVos et al. [19]. The differences in the two methods are limited to the PCR detection protocols, which employ slightly different concentrations of reagents and

Table 1 Performance characteristics of first-generation *SEPT9* assays

Study	PCR format	Overall sensitivity	Overall specificity	Stage I sensitivity (%)	Stage II sensitivity (%)	Stage III sensitivity (%)	Stage IV sensitivity (%)
Grützmann et al. [18]	2 of 3	72.0 % (63–80)	89.7 % (84–94)	50.0	69.4	79.2	90.9
deVos et al. [19]	1 of 3	72.2 % (62–81)	85.8 % (79–91)	52.6	75.0	77.8	100
Solomon et al. [20]	1 of 3	71.1 % (60–80)	98.7 % (93–100)	52.0	76.0	76.0	100
Church et al. [24]	1 of 2	50.9 % (38–64)	91.4 % (90–93)	36.4	57.1	58.3	80.0
Church et al. [23]	1 of 3	66.7 % (54–79)	88.4 % (87–90)	42.9	78.6	81.8	100

Figures in parentheses are 95 % confidence intervals

PCR polymerase chain reaction

multiplexed fluorescent labeling strategies. A clinical case–control study utilizing the ARUP test to analyze plasma specimens collected from 50 known CRC patients and 94 colonoscopy-verified healthy controls demonstrated an overall sensitivity of 90 % for detecting CRC at a specificity of 88 % [21]. Importantly, the improved test identified the majority of early-stage cancers (87 %), as well as all of the late-stage cancers (Fig. 1). A limited prospective collection of 300 individuals aged 24–86 years undergoing colonoscopy demonstrated that the improved sensitivity method still retains good specificity, with an overall *SEPT9* false-positivity rate of 3 %, if both cancers and adenomas are considered true-positives [21]. Since its first use in 2008, the Epi proColon® kit has also undergone significant changes that have improved its performance with regard to sensitivity to detect CRC. Using the Epi proColon® 2.0 CE kit, Tóth et al. [22] demonstrated the enhanced performance of the new kit in a case–control study of 92 CRC and 92 healthy controls. In this study, *SEPT9* methylation was detected in 95 % of cancers and 15 % of healthy controls, consistent with the results obtained by Warren et al. [21] using different DNA extraction, bisulfite, and PCR detection methods. Table 2 lists the performance of the two methods for detecting CRCs of each clinical stage, both of which demonstrate an ability to detect a majority of early stage cancers.

In response to feedback by US clinicians during the development of the ARUP *SEPT9* test, the Warren method is always performed clinically in the more sensitive format, whereby only one in three PCR replicates must have detectable methylated *SEPT9* in order for the specimen to be called positive [21]. However, in Europe, the Epi proColon® 2.0 CE test can be performed in a manner whereby specificity is prioritized over sensitivity; in this modification, at least two in three PCR replicates must have detectable methylated *SEPT9*. For illustration purposes, results for the ARUP (Warren) and Epi proColon® 2.0 CE (Tóth) methods using the increased specificity format (two of three) are shown in Table 2; both tests demonstrate a false-positivity rate of only 1 %, while still retaining an overall sensitivity of 76–79 %.

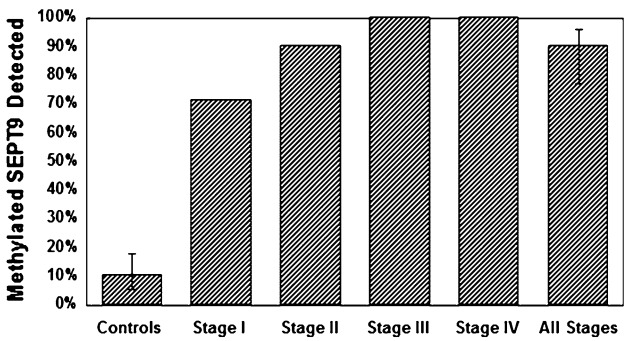

Fig. 1 Sensitivity of the improved *SEPT9* blood-based test in a clinical case–control study. Methylated *SEPT9* DNA was measured in plasma specimens donated by CRC patients and colonoscopy-confirmed control subjects. The fraction of specimens with detectable methylated *SEPT9* DNA is illustrated by the *solid bars*. The test has been maximized for sensitivity by only requiring one out of three of the PCR replicates to have methylated *SEPT9* DNA detected. The specificity of the assay is 88 % under these parameters (modified from an original figure published by BioMed Central in Warren et al. [21]). *CRC* colorectal cancer, *PCR* polymerase chain reaction

6 Comparison of Blood-Based CRC Screening Tests to Other Test Methodologies

There have not been many studies reported in the literature that have described genuine side-by-side comparisons between CRC screening methods. In a 2012 review of blood-based CRC screening tests in *Practical Gastroenterology*, Lofton-Day [26] nicely summarized the characteristics and specifications of commercially available CRC screening tests including the following: sample type, price to payer, sensitivity for CRC, specificity, and availability. Since the focus of her review was blood-based screening methods, she included in her study, for contextual purposes, colonoscopy, FOBT (Hemoccult® SENSA, Beckman Coulter), and FIT (multiple), and discussed the two clinically available blood-based tests, ColonSentry™ RNA expression panel and *SEPT9* methylated DNA biomarker (multiple). Table 3, modified from the Lofton-Day review to include more recent publications, displays her findings and provides a window on how the blood-based tests compare. The performances of the blood-based biomarkers described above were originally established using specimens collected from study subjects clinically characterized by colonoscopy or histopathologic analysis of surgical specimens; however, few studies have directly compared any blood-based testing methodologies versus one another, or versus other laboratory tests for CRC detection.

Two side-by side comparisons have been published that illustrate how the newer *SEPT9* tests compare with other existing methodologies. In order for lab-based comparisons to be meaningful, the samples for each of the tests being compared should either be identical, in the case of examining two or more different blood tests, or in the case of disparate specimen types, specimens should be collected from the same study subjects at approximately the same timeframe. Using analytical specimens comprising methylated genomic DNA spiked into human blood plasma at various concentrations near the limit of detection, Warren et al. [21] demonstrated that the ARUP method performed with improved sensitivity for detecting methylated *SEPT9* DNA as compared with the first-generation Epi proColon® kit (Fig. 2). More recently, Tóth et al. [22] conducted a small study in order to compare the performance of the Epi

Table 2 Performance characteristics of improved *SEPT9* assays in case–control studies

Study	PCR format	Overall sensitivity	Overall specificity	Stage I sensitivity (%)	Stage II sensitivity (%)	Stage III sensitivity (%)	Stage IV sensitivity (%)
Warren et al. [21]	1 of 3	90.0 % (77–96)	88.3 % (80–94)	71.4	90.3	100	100
Tóth et al. [22]	1 of 3	95.6 % (89–99)	84.8 % (76–91)	84.0	100	100	100
Warren et al. [21]	2 of 3	76.0 % (62–86)	98.9 % (93–100)	57.1	74.2	85.7	100
Tóth et al. [22]	2 of 3	79.3 % (70–87)	98.9 % (94–100)	60.0	92.8	88.6	77.8

Figures in parentheses are 95 % CIs

PCR polymerase chain reaction

proColon® 2.0 CE *SEPT9* detection kit versus a commonly used guaiac FOBT (Hema Screen, Immunostics, Inc., NJ, USA) utilized for CRC screening. In addition, the same group compared Epi proColon® 2.0 CE kit with the blood-based carcinoembryonic antigen (CEA; Cobas, Roche Diagnostics) test, usually utilized in the clinical setting of CRC for monitoring therapeutic response and disease recurrence. In this study, blood specimens were collected for the *SEPT9* analysis from individuals prior to colonoscopy. For some of these subjects, CEA (blood) and/or FOBT (stool) specimens had previously been collected (at least 2 days prior to endoscopy) and were retrospectively procured for this study. If pair-wise comparisons are made between the same patient subsets, the following sensitivities are measured for each of the tests, *SEPT9*, FOBT, or CEA as shown in Table 4. Also shown is the three-way comparison of the 16 CRC patients for whom all three specimens were available. This pilot study, using matched same-subject clinical specimens, strongly suggests that *SEPT9* has better sensitivity for detecting CRC than either of the other two methods. However, this conclusion could only be definitively demonstrated had all of the specimens been prospectively collected. Comparison studies using specimen sets collected from different patient cohorts should be interpreted with caution, and should always consider the clinical context under which the specimens have been collected, processed, and analyzed.

7 Would Blood-Based Screening for CRC Be Cost Effective?

It has been well established that CRC screening by a variety of methods can provide a benefit of overall survival, and in some cases can prevent the development of CRC by the detection of precancerous lesions [27–40]. Furthermore, it has been demonstrated that CRC screening can be cost effective [41]. Early-stage CRC can usually be treated surgically, with ~90 % overall survival and no need for further treatment, such as targeted therapy, chemotherapy, or radiation [1]. In contrast, CRC that has spread locally or metastasized to distant sites portends a poorer prognosis as well as a much more extensive, and expensive, course of treatment. Thus, detecting CRC early has both medical as well as financial benefits.

In 2009, Lansdorp-Vogelaar and colleagues [42] published their studies utilizing the MISCAN-Colon microsimulation model to predict the cancer treatment savings of several CRC screening methodologies as compared with no screening at all. The investigators included in their study the following screening tests, each exhibiting distinct performance characteristics and costs as illustrated above: colonoscopy, flexible sigmoidoscopy, FOBT, and FIT. The authors ultimately concluded that all of the modalities would be cost effective given the high (and rising) cost of treating later-stage CRC as compared with no CRC screening at all.

Table 3 Comparison of blood-based CRC assays to other screening methodologies

	Colonoscopy	FOBT	FIT	ColonSentry®	*SEPT9*
Sample	Structural exam	Stool	Stool	Blood	Blood
Sensitivity (for CRC)	95 %	70 % (64–80)	70 % (61–91)	72 %	67–96 %
Specificity	90 %	92.5 % (87–90)	95 % (91–98)	70 %	81–99 %
Availability	Through specialist	IVD FDA cleared	IVD FDA cleared	LDT	LDT (USA) IVD (EU)

Modified from original table: Lofton-Day [26]

Figures in parentheses represent the range of reported values

CRC colorectal cancer, *FDA* US Food and Drug Administration, *FIT* fecal immunochemical testing, *FOBT* fecal occult blood test, *IVD* in vitro diagnostic, *LDT* laboratory-developed test

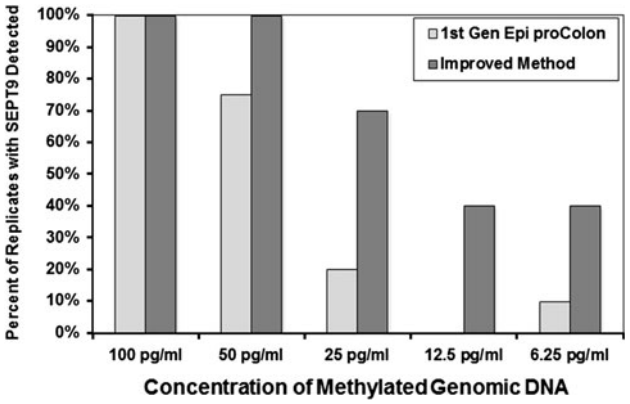

Fig. 2 Comparison of the improved *SEPT9* PCR assay with the first-generation Epi proColon® kit using analytical specimens. Methylated *SEPT9* DNA was measured in pooled normal human plasma spiked with various concentrations of wholly methylated human DNA. Multiple samples of each DNA concentration were prepared and pooled, allowing for the direct comparison of the first-generation Epi proColon® CE-marked kit and improved PCR detection methods using identical DNA substrates (modified from an original figure published by BioMed Central in Warren et al. [21]). *PCR* polymerase chain reaction

More recently, Ladabaum et al. [43] applied their previously published and validated decision analytical model of CRC screening to address outstanding questions pertaining to use of a blood-based biomarker in the screening setting. By way of example, the authors modeled the data generated in the large prospective CRC screening study of the first-generation blood-based *SEPT9* test, and used it to propose an appropriate screening interval, as well as to evaluate effectiveness and cost effectiveness of the early version of the *SEPT9* test as compared with established screening strategies or no screening. Based on the incremental cost of the test and the calculated quality-adjusted life-years (QALYs), the authors concluded that the *SEPT9* test is cost effective as compared with no screening at all, and that the most appropriate screening interval of such a test would be 2 years. Primarily due to the higher cost of the *SEPT9* test compared with other laboratory tests with similar sensitivities for detecting CRC such as FOBT and FIT (both stool-based), *SEPT9* was found to be less cost effective than other methodologies. However, the relative cost effectiveness of the blood-based screening strategy would be greater if the availability of a blood-based option encouraged otherwise unscreened individuals to undergo screening.

Table 4 Comparison of Epi proColon® 2.0 CE *SEPT9* test with FOBT and CEA in matched patient specimens

Test	Number of CRC	Fraction positive	Sensitivity (%)
SEPT9	22	22/22	100
FOBT	22	15/22	68
SEPT9	27	27/27	100
CEA	27	14/27	52
SEPT9	16	16/16	100
FOBT	16	9/16	56
CEA	16	10/16	62

Original data source: Tóth et al. [22]

CEA carcinoembryonic antigen, *CRC* colorectal cancer, *FOBT* fecal occult blood test

8 The Future of Blood-Based Screening for CRC

Although the performance of blood-based CRC screening has improved since clinical testing first became available in 2008, it has not yet been as widely embraced as one might expect even though sensitivities and specificities for detecting CRC of the second-generation tests approach those of colonoscopy, the gold standard. In the USA, blood-based CRC screening has not yet been incorporated into screening guidelines established by the American Cancer Society (ACS) or the National Comprehensive Cancer Network (NCCN). Although it seems obvious that blood-based testing would encourage screening compliance, especially in the population of individuals who have refused physician screening recommendations, additional studies will be required to demonstrate that blood testing for CRC will increase screening uptake even if patient preference studies have clearly demonstrated strong interest and positive attitudes [11, 12].

The second-generation Epi proColon® kit, is currently under review at the US FDA, with anticipated approval by the end of 2014. This improved *SEPT9* kit was recently evaluated in a head-to-head comparison with the standard FIT (OC FIT-Chek®, Polymedco) in a comparative multicenter study, including both screen-identified CRCs as well as prospective subjects prior to screening colonoscopy [44]. In this study, the Epi proColon® test demonstrated an overall sensitivity of 72 % for detecting CRC at a specificity of 81 %. The sensitivity of 72 % compared well with the FIT sensitivity of 68 %, though at a lower specificity (81 and 97 %, respectively). The investigators concluded that the Epi proColon® test was non-inferior to the widely used FIT assay with regard to its ability to detect CRC, perhaps paving the way for FDA approval and eventual inclusion in US national screening guidelines.

There are a number of promising, emerging technologies for the blood-based screening of CRC; however, none of these has yet reached the level of maturity required for clinical use. With regard to DNA methylation biomarkers similar to *SEPT9*, the *SDC2* biomarker was recently identified by a group in South Korea in a genome-wide search for novel genes that are frequently methylated in

early-stage CRC. The authors report that, in a case–control study, the qualitative methylation-specific PCR (MSP) method could detect 87 % of CRCs, with a specificity of 95 %; importantly, >90 % of stage I cancers were detected [45]. Future validation studies of *SDC2* will be eagerly awaited. Another RNA-based assay under development for clinical use for CRC screening, akin to the ColonSentry™ test, is centered on the RNA coding for the *KIAA1199* gene [46]. A recent publication described the discovery of the *KIAA1199* mRNA biomarker in CRC and adenoma tissues, as well as a modest-sized proof-of-concept study in plasma collected from 20 subjects with CRC, 20 subjects with adenomas (size not specified), and 20 healthy controls. The authors report that *KIAA1199* expression levels as measured by quantitative PCR can detect 77.5 % of combined CRCs and adenomas at a specificity of 70 %. However, additional findings document that *KIAA1199* is up-regulated in other cancers such as gastric [47] and breast [48], indicating that the clinical utility of *KIAA1199* for CRC screening may be less straightforward. Another promising CRC screening test under development is the so-called ONC107 mass spectrometry assay, which measures distinct peptides from a small number of serum proteins [49]. Based on the first published description, this multiplexed assay also possesses impressive initial performance specifications, with an overall sensitivity for detecting CRC of 94 % at a specificity of 83 % in a case–control study. Improvements to the ONC107 assay protocol are in development and additional patient cohorts are undergoing testing to augment existing validation data. It will be exciting to learn more about this technology as it becomes confirmed as a clinical test for use in patients.

There have been recent reports of the potential use of the *miR-21* microRNA as a possible biomarker for blood-based screening of CRC [50, 51]. In one study by Toiyama and colleagues [50], the investigators report that in a case–control study, the *miR-21* serum biomarker exhibited an overall sensitivity of 83 % for detecting CRC at a specificity of 91 %. Likewise, in another study by Kanaan et al. [51] published at approximately the same time, the authors demonstrated that plasma *miR-21* achieved a sensitivity and specificity of 90 %. However, it is unclear whether *miR-21* alone will ever be useful as a screening biomarker for CRC since there are also several reports in the literature of elevated *miR-21* in the blood of a number of common solid cancers, such as liver, esophagus, gastric, breast, prostate, and lung (as reviewed in Wang et al. [52]).

For some of the blood-based biomarkers of CRC, there have been suggestions that the analytes may provide useful prognostic information even if they may lack adequate specificity for screening. The *miR-21* biomarker has been discussed as a significant blood-based marker of prognosis in a variety of different cancers, with six studies demonstrating that higher levels of *miR-21* expression correlated with poorer overall survival [52]. In a meta-analysis of all 'digestive' cancers, highly elevated *miR-21* concentrations were associated with worse patient survival outcomes, with a calculated hazard ratio of 5.77 (95 % confidence interval 2.65–12.52) [52]. Conversely, *KIAA1199* may also have a role in determining cancer prognosis; mRNA expression levels of *KIAA1199* have been shown to be inversely associated with patient survival rates in gastric [47] and breast cancer [48].

While most of the blood-based CRC screening tests in clinical use or under development have primarily focused on individuals at an average risk of CRC, some of these assays may be extensible to surveillance of populations at increased risk of developing CRC, such as those with a strong family history or genetic predisposition such as Familial Adenomatous Polyposis (FAP), Lynch (hereditary nonpolyposis CRC [HNPCC]), or Peutz–Jeghers syndrome. In addition, these blood-based tests may someday find utilities in monitoring the approximately 1.4 million individuals in the USA with inflammatory bowel disease (IBD; e.g. Crohn's disease or ulcerative colitis), who have an approximately 10 % lifetime risk of developing CRC and often require annual colonoscopies with dozens of biopsies collected throughout the length of the colon [53, 54]. Studies are currently underway to determine whether the *SEPT9* biomarker may be capable of sensitively and specifically detecting early-stage cancer from the blood of IBD patients, as would be expected. Importantly, preliminary studies have shown that methylated *SEPT9* concentrations remain at background levels in IBD patients with no evidence of cancer as determined by colonoscopy [18, 21]. Intriguing is the notion that a sensitive *SEPT9* assay may be capable of detecting high-grade dysplasia in the IBD population due to the high degree of vascularity of the large intestine [55].

At the present time, the only blood-based biomarker used clinically for measuring disease recurrence is carcinoembryonic antigen (CEA); however, one can readily imagine that in the future, some of the newer blood-based biomarkers of CRC may be employed in this capacity. Important considerations for candidate surveillance biomarkers include universal expression in cancers under surveillance and correlation with disease recurrence. In the case of CEA, the tumor marker has been demonstrated to be elevated in the vast majority of colon adenocarcinomas [56, 57]; likewise, elevated *SEPT9* DNA methylation levels have been reported to be present in virtually all CRC tumor tissues [21, 58–60]. It has been well established that increased CEA levels correlate with disease recurrence and progression in CRC; however, CEA has not been demonstrated to be an accurate reflection of tumor burden, nor can it be used quantitatively as an indicator of clinical stage

[56]. Although the *SEPT9* real-time (RT)-PCR assays in clinical use are more qualitative in nature, blood-based *SEPT9* detection rates tend to increase with clinical stage [18–22], suggesting that *SEPT9* levels in the blood trend upward with disease stage. Furthermore, it has been established using a quantitative method that median methylated *SEPT9* DNA concentrations increase with clinical stage [18]. The *SEPT9* biomarker has not been extensively evaluated for applications related to monitoring disease recurrence or therapeutic response in CRC patients; however, a small exploratory study analyzing plasma specimens collected from stage II and III CRC patients before and after surgery suggests that methylated *SEPT9* levels decrease following surgical resection [61]. Given the sensitivity of the improved assays to detect minute quantities of methylated *SEPT9* in spiked analytical specimens (Fig. 2) [21], as well as its superiority compared with CEA in detecting CRC from clinical specimens [22], *SEPT9* appears to be a good tumor marker candidate.

9 Conclusions

None of the blood-based CRC screening tests that are currently commercially available is meant to replace colonoscopy. Rather, it is advised that upon positive blood-test results that patients be referred for follow-up with a physician, which will usually include recommendation for colonoscopy. In the USA, physicians most often emphasize the importance of colonoscopy to their patients; however, it is clear that patient preferences weigh heavily on the decision of whether to undergo screening and what method is utilized [5]. Since one-third of Americans, and even higher fractions of individuals from other countries around the rest of the world, reject or ignore CRC screening guidelines, alternative strategies to encourage screening compliance are needed. Blood testing for CRC, while perhaps not reaching the most optimal sensitivity and specificity profile, is a reasonable and cost-effective method that just might increase screening rates and ultimately save lives.

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