MINIREVIEWS

Current noninvasive tests for colorectal cancer screening: An overview of colorectal cancer screening tests

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Abstract

Colorectal cancer (CRC) has become the third most common cancer in the world. Screening has been shown to be an effective way to identify early CRC and precancerous lesions, and to reduce its morbidity and mortality. Several types of noninvasive tests have been developed for CRC screening, including the fecal occult blood test (FOBT), the fecal immunochemical test (FIT), the fecal-based DNA test and the blood-based DNA test (the SEPT9 assay). FIT has replaced FOBT and become the major screening test due to high sensitivity, specificity and low costs. The fecal DNA test exhibited higher sensitivity than FIT but its current cost is high for a screening assay. The SEPT9 assay showed good compliance while its performance in screening needs further improvements. These tests exhibited distinct sensitivity and specificity in screening for CRC and adenoma. This article will focus on the performance of the current noninvasive *in vitro* diagnostic tests that have been used for CRC screening. The merits and drawbacks for these screening methods will also be compared regarding the techniques, usage and costs. We hope this review can provide suggestions for both the public and clinicians in choosing the appropriate method for CRC screening.

Key words: Colorectal cancer; Adenoma; Fecal immunochemical test; Fecal DNA; SEPT9; Septin 9

Core tip: The choice of colorectal cancer (CRC) screening methods is crucial for screening validity and compliance. Currently, the fecal immunochemical test (FIT), fecal DNA and the blood-based SEPT9 assays are the three *in vitro* diagnostic tests for CRC screening. In this article, we reviewed the current application of the three types of assays and compared their performance

in CRC screening. FIT is still the cheapest method with high screening validity, and fecal DNA tests also exhibit high validity but its price is high. In contrast, the SEPT9 assay showed high compliance with acceptable performance. The choice of screening test may depend on the balance of performance, compliance and costs.

INTRODUCTION

Colorectal cancer (CRC) has become the second and the third leading cause of new cancer cases in Europe and in the United States, respectively[1]. There were approximately 142820 new cases with 50830 deaths in the United States in 2013, and approximately 447000 new cases of CRC and 215000 deaths in European countries in 2012[1,2]. The new cases for CRC are approximately 400000 in China in 2012, and it has become the third leading cause of death in the country[3].

Regular screening can achieve early CRC detection and early treatment. However, 60%-70% of patients are found at middle- or late-stage CRC when they are diagnosed[4]. Approximately 60% CRC deaths could be avoided and the average 5-year survival rate could be increased from 46% to 73% if healthy people carry out a regular periodic screening each year[5]. Therefore, an effective early screening method for CRC can reduce CRC morbidity and mortality.

There are four *in vitro* diagnostic (IVD) screening method currently available for CRC screening, including the fecal occult blood test (FOBT), the fecal immuno-chemical test (FIT), the fecal DNA test and the plasma SEPT9 gene methylation test. This review will provide a detailed analysis on the performance of these tests, and compare their merits and drawbacks in CRC screening. It is our aim for this review that the public and the professionals can choose the appropriate methods for CRC screening.

STOOL-BASED TESTS FOR CRC SCREENING

The FIT test

The guaiac FOBT test (gFOBT) has been used for a long time as a screening test for CRC. It exhibited a sensitivity of 12.9%-79.4% with a specificity of 86.7%-97.7% for CRC screening in many studies[6-13]. However, its sensitivity and specificity for CRC detection is lower than the more specific FIT (previous called iFOBT) test. This is because the gFOBT relies on peroxidase-like activity

between heme and guaiac, which can be affected by many factors in daily diet without distinguishment between upper and lower gastrointestinal (GI) tract bleeding, while the FIT test targets the hemoglobin in the lower GI tract, as hemoglobin from upper GI tract will be degraded when it arrives at lower GI tract. This characteristic allows FIT test to specifically detect the bleeding from lower GI tract, and therefore detect the diseases with bleeding, such as adenoma, polyps, inflammatory diseases and CRC, *etc.* As the gFOBT test has many drawbacks in CRC screening, FIT is used more commonly in current CRC screening. We therefore focus on the performance of FIT test in this review.

The performance of FIT test in CRC screening in asymptomatic, average-risk adults has been listed in Table 1. Data from 19 studies showed that the overall sensitivity for CRC was 0.79 (95%CI: 0.69-0.86) and the overall specificity was 0.94 (95%CI: 0.92-0.95)[12,14-31]. This includes a total of 113360 subjects with 437 CRC cases confirmed by colonoscopy or 2-year follow-up. As the overall sensitivity and specificity are satisfactory for a cancer screening test with low costs, FIT is currently the most commonly-used method for CRC screening. The overall CRC positivity rate of 0.39% (437/113360) appeared to be significantly lower than the other two screening reports with asymptomatic, average-risk adults using fecal DNA (0.65%, 65/9989; $\chi^2 = 15.93$, $P < 0.001$)[32] and *SEPT9* gene methylation assay (0.67%, 53/7941; $\chi^2 = 14.66$, $P < 0.001$)[33], respectively, indicating that the use of 2-year follow-up as a confirmatory methods may result in underestimation of CRC cases.

The use of qualitative FIT or quantitative FIT has always been an issue in choosing the FIT test for screening. A strip test (colloidal gold immunochro-matographic method) is currently the main technique for qualitative FIT. It does not need specific instruments and the interpretation of test results relies on human recognition of test bands, although instruments are available to digitize the chrominance of the bands. In contrast, immunoturbidimetry is the main method for quantitative FIT, and the current devices include automated instrument for samples processing and colori-metry. Therefore, the current qualitative FIT appears to be faster, more convenient, less costly while more subjective than the quantitative FIT.

The performance between the qualitative and quantitative FIT showed significant differences. As shown in Table 1, the overall sensitivity of the qualitative FIT was 0.82[12,15-21], which was significantly higher that of the quantitative FIT (0.73) ($\chi^2 = 3.933$, $P = 0.047$)[22-31], while the qualitative FIT exhibited significantly lower specificity than the quantitative FIT (0.93 *vs* 0.95) ($\chi^2 = 81.64$, $P < 0.001$), although the difference was small. This comparison needs to be interpreted with caution, as different studies used different cutoff values and resulted in distinct sensitivity and specificity. Ideally, they should be compared under the same cutoff value so that the sensitivity and specificity can be directly

Table 1 The sensitivity and specificity of qualitative and quantitative fecal immunochemical test

	Qualitative FIT					Quantitative FIT			
Ref.	Total cases	CRC cases	Sensitivity	Specificity	Ref.	Total cases	CRC cases	Sensitivity	Specificity
Allison *et al*[12], 1996	7493	35	0.69	0.94	Sohn *et al*[22], 2005	3794	12	0.25	0.99
Allison *et al*[15], 2007	5356	14	0.86	0.97	Levi *et al*[23], 2011	1204	6	1.00	0.88
Cheng *et al*[16], 2002	7411	16	0.88	0.91	Levi *et al*[24], 2007	80	3	0.67	0.83
Nakama *et al*[17], 1999	4611	18	0.56	0.97	Morikawa *et al*[25], 2005	21805	79	0.66	0.95
Nakama *et al*[18], 1996	3365	12	0.83	0.96	Launoy *et al*[26], 2005	7421	28	0.86	0.94
Parra-Blanco *et al*[19], 2010	1756	14	1.00	0.93	Itoh *et al*[27], 1996	27860	89	0.87	0.95
Chiu *et al*[20], 2013	8822	13	0.85	0.92	Nakazato *et al*[28], 2006	3090	19	0.53	0.87
Chiang *et al*[21], 2011	2796	28	0.96	0.87	Park *et al*[29], 2010	770	13	0.77	0.94
					de Wijkerslooth *et al*[30], 2012	1256	8	0.75	0.95
					Brenner *et al*[31], 2013	2235	15	0.73	0.96
					Brenner *et al*[31], 2013	2235	15	0.60	0.95
Overall (pooled data)	41610	150	0.82	0.93		71750	287	0.73	0.95

CRC: Colorectal cancer; FIT: Fecal immunochemical test.

Table 2 Comparison of sensitivity and specificity between Cologuard and fecal immunochemical test

	Pathological categories	Cologuard	FIT
Sensitivity[32]	CRC	92.3%	73.8%
	Advanced precancerous lesions	42.4%	23.8%
	Polyps with high-grade dysplasia	69.2%	46.2%
	Serrated sessile polyps	42.4%	5.1%
Specificity[32]	Nonadvanced or negative findings	86.6%	94.9%
	Negative results on colonoscopy	89.8%	96.4%

CRC: Colorectal cancer; FIT: Fecal immunochemical test.

compared. The pooled data analyzed here provides a reference for comparing the two types of FIT tests. It can be suggested that the quantitative FIT may be a good choice for CRC screening tests that do not need high accuracy or are performed in hospitals where automated instruments are not available.

However, it should be mentioned that the cutoff value for qualitative FIT is preset, while the cutoff value for quantitative FIT can be adjusted to balance the sensitivity with specificity. Therefore, the data format for qualitative FIT is "positive" or "negative" without traceability, while the results from quantitative FIT are digitized with traceability. This is extremely useful when the relationship between the amount of bleeding in a certain disease and the population/personal information (such as diet, age, habit, sex, *etc.*) is investigated. Future model for predicting CRC incidence might partially relies on the data from quantitative FIT.

The fecal DNA test

The detection of abnormal DNA or epigenetic markers from colorectal lesions is based on natural exfoliation of cancerous or precancerous cells into the colorectal tract. The fecal DNA test aims at detecting the DNA mutations, microsatellite instability, impaired DNA mismatch repair and abnormal methylation. There are many studies focusing on the detection of CRC by fecal DNA markers[34,35], and the overall sensitivity for CRC

detection by various fecal DNA marker combinations ranged from 53% to 87%, with specificities beyond 76%[34,35]. Although there are a large number of fecal DNA markers available in these studies, the first commercial fecal DNA test was not available until the approval of Cologuard (Exact Sciences, Madison, WI, United States) by the United States FDA in 2014. Imperiale *et al*[32] published the leading study on Cologuard in 2014. By randomizing subjects to Cologuard or FIT screening, it showed that the sensitivity of Cologuard was superior to that of FIT in CRC, advanced precancerous lesions, polyps with high-grade dysplasia and serrated sessile polyps, while its specificity appeared to be lower than that of FIT (Table 2).

The Cologuard DNA test includes quantitative molecular assays for KRAS mutations, aberrant NDRG4 and BMP3 methylation, and β-actin, plus a hemoglobin immunoassay. As the hemoglobin immunoassay is essentially a FIT test, Cologuard is actually a combination of gene mutation, methylation and occult blood tests. The multitarget stool DNA test provides a new way that combines various detecting technology to detect CRC and early colorectal lesions with high sensitivity and specificity. The high detection of precancerous lesions, HGD and serrated sessile polyps is extremely useful for a screening test, as these lesions may develop into CRC if they are not resected. The only obstacle for broad application of Cologuard is the cost, as the detection of multitargets increased the cost of the test. Its current expense of $599 per test is high for a routine screening assay.

BLOOD-BASED TESTS FOR CRC SCREENING

The plasma SEPT9 gene methylation assay

An ideal screening test for cancer could be a simple blood test in the foreseeable future. The plasma *SEPT9* gene methylation test Epi proColon (Epigenomics AG, Berlin, Germany) is currently the only commercially

Table 3 The reported positive detection rate for each colorectal cancer stage using 1/3 algorithm

Ref.	Positive detection rate for each colorectal cancer stage			
	I	II	III	IV
deVos *et al*[38], 2009	52.6% (10/19)	75.0% (30/40)	77.8% (21/27)	100.0% (4/4)
Warren *et al*[40], 2011	71.4% (5/7)	90.3% (28/31)	100.0% (7/7)	100% (5/5)
Tóth *et al*[41], 2012	84.0% (21/25)	100.0% (14/14)	100.0% (35/35)	100.0% (18/18)
Lee *et al*[47], 2013	30.8% (8/26)	36.7% (11/30)	25.0% (7/28)	64.7% (11/17)
Johnson *et al*[44], 2014	61.5% (16/26)	80.0% (16/20)	65.2% (15/23)	92.3% (12/13)
Pooled positive detection rate	58.3% (60/103)	73.3% (99/135)	70.8% (85/120)	87.7% (50/57)

Table 4 The reported positive detection rate for each colorectal cancer stage using 2/3 algorithm

Ref.	Positive detection rate for each colorectal cancer stage			
	I	II	III	IV
Grützmann *et al*[37], 2008	50.0% (11/22)	69.4% (25/36)	79% (42/53)	91% (10/11)
deVos *et al*[38], 2009	26.3% (5/19)	60.0% (24/40)	66.7% (18/27)	75.0% (3/4)
Tóth *et al*[41], 2012	60.0% (15/25)	92.8% (13/14)	81.6% (31/35)	77.8% (14/18)
Jin *et al*[46], 2015	66.7% (12/18)	82.6% (19/23)	84.1% (37/44)	100.0% (5/5)
Pooled positive detection rate	51.2% (43/84)	71.7% (81/113)	80.5% (128/159)	84.2% (32/38)

available blood-test for CRC early detection and screening, and was approved recently by the United States FDA as a CRC screening test for average-risk population over 50 years old. Many clinical studies have proved the test to be a method with acceptable sensitivity and specificity for CRC detection[33,36-49]. The test was firstly developed by Lofton-Day *et al*[36] in 2008 as a research kit, and was commercialized by Epigenomics AG as its first generation assay Epi proColon 1.0. At the same time, ARUP lab also developed its SEPT9 assay as a lab-developed test[40]. Abbott developed its real-time mS9 CRC assay, but there was only one report on its performance and the sensitivity of 36.3% was much lower than other SEPT9 tests[47]. The 2nd generation test (Epi proColon 2.0) was launched in 2011-2012 with better performance. Till today, most reports on the SEPT9 assay appeared to be case-control study or cohort study investigating the test perfor-mance in selected population, exhibiting a sensitivity of 36.6%-95.6% with a specificity of 81.5%-99.0% using 1/3, 2/3, 1/2 or 1/1 algorithm[33,36-49]. In contrast, there is only one study (the PRESEPT trial) investigating the application of the assay in CRC screening in average-risk population, exhibiting a sensitivity of 48.2% and 68.2% with a specificity of 91.5% and 80.0% using 1/2 or 1/3 algorithm, respectively[33,43].

Detection of early stage CRC is crucial for early intervention and reduction of mortality. The positive detection rate (PDR) of the SEPT9 assay for stage I, II, III and IV was 26.3%-84.0%, 36.7%-100.0%, 25.0%-100.0% and 64.7%-100.0%, respectively, depending on different algorithm, exhibiting a huge variation for each stage. As 1/3 and 2/3 algorithm are the most commonly used methods for result interpretation, we calculated the PDR for each stage using the two algorithms. The pooled PDR for stage I, II, III and IV was 58.3%, 73.3%, 70.8% and 87.7%, respectively, using 1/3 algorithm (Table 3), and was

51.2%, 71.7%, 80.5% and 84.2%, respectively, using 2/3 algorithm (Table 4)[36-47]. No statistical difference in PDR in any stage between the two algorithms has been found. It can be clearly seen that the PDR for early stage CRC (stage I) was above 50% and fell into 70%-80% for stage II and III, which is acceptable for a blood-based CRC test. However, these PDRs were from case-control or cohort studies, and more studies should be performed at screening settings.

Although the SEPT9 assay was designed for CRC detection, researchers also studied its detection sensitivity for precancerous adenoma. The pooled PDR for non-advanced adenoma and advanced adenoma (AA) was 10.0% and 18.2%, respectively, from six studies, in which the PDR for AA was significantly higher than the PDR of normal control group (11.8%, χ^2 test, $P <$ 0.001)[30,33,37,40,43,46]. However, as PDR of 18.2% was still too low for an effective test, the SEPT9 assay may not be applicable in adenoma detection.

The SEPT9 assay exhibited high compliance in screening. One recent report showed that 63% of subjects in a CRC screening study refused colonoscopy. 97% of subjects who refused colonoscopy accepted a noninvasive screening test, and 83% chose the SEPT9 test and 15% chose FIT test. The majority of patients who refused colonoscopy chose the SEPT9 assay due to its convenience and less time-consuming procedure[50].

CEA and other serum glycoprotein markers
CEA and carbohydrate antigen 199 (CA199) are the two most common serum-based glycoprotein CRC markers, however, they are not appropriate for CRC screening due to their low sensitivity and the lack of CRC specificity, especially for early-stage CRC[41,51-53]. For example, CEA test exhibited a sensitivity of 40.9%-51.8% and a specificity of 85.2%-95% for CRC detection in three studies[41,51,52]. Therefore, it is more appropriate to be used in monitoring the CRC recurrence or response from

Table 5 Sensitivity and specificity of fecal immunochemical test, fecal DNA and SEPT9 tests in colorectal cancer and advanced adenoma screening

	FIT[12,15-31]	Fecal DNA[32]	SEPT9[43]
Sensitivity (CRC)	79%	92%	68%
Specificity	94%	87%	80%
Sensitivity (AA)	24%	42%	18%

FIT: Fecal immunochemical test; CRC: Colorectal cancer; AA: Advanced adenoma.

Table 6 Positive detection rate of SEPT9, fecal immuno-chemical test and carcino-embryonic antigen tests and various combined tests

SEPT9 alone	FIT alone	CEA alone	SEPT9 + FIT	SEPT9 + CEA	FIT + CEA	SEPT9 + FIT + CEA
77.00%	74.6% (NS)	41.3%[e]	94.4%[c]	86.4%[c]	84.5% (NS)	97.2%[e]
(181/235)	(53/71)	(97/235)	(67/71)	(203/235)	(60/71)	(69/71)

[c]$P < 0.01$; [e]$P < 0.001$ *vs* SEPT9 alone. FIT: Fecal immunochemical test; CEA: Carcino-embryonic antigen; NS: Not significant.

patients to surgical or systemic therapy, rather than screening[53].

The main drawback of serum glycoprotein markers in CRC screening is that the sensitivity and specificity of any single marker is not high enough to make it a reliable indicator. These markers have been found in various cancers other than CRC with low sensitivity for early stage lesions. Combined use of multiple markers may be a way to achieve diagnostic significance in CRC detection. In one report, five glycoprotein markers, including CEA, CA199, CA242, CA72-4, and CA125, are used together as indicators for CRC. It showed that the sensitivity of any single marker was low (18.8%–52.2%) for detecting CRC in stages I and II, while the combination of the five exhibited a sensitivity of 85.3% at the specificity of 95%[54].

COMPARISON OF NONINVASIVE TESTS FOR CRC SCREENING

The sensitivity for CRC and AA, and the specificity in asymptomatic average-risk population for FIT, fecal DNA and SEPT9 tests are shown in Table 5. It can be seen that the fecal DNA test exhibited the best performance in terms of sensitivity for CRC and AA, while its specificity was slightly lower than that of the FIT. It is noteworthy that the fecal DNA test can detect 42% AA, which may reduce the number of subjects progressing to CRC, *i.e.*, reducing the CRC morbidity. The SEPT9 assay is the only blood-base CRC screening assay currently. Although its screening performance was not satisfactory at the moment, it showed very high compliance[50]. The blood-based CRC screening assay may be more popular if the sensitivity and specificity in screening setting could be improved to the level of those in case-control studies (ideally sensitivity > 70% and specificity > 90% for CRC screening).

The current costs for FIT, fecal DNA and the SEPT9 test are $10-50, $599 and approximately $170, respectively. As the recommended screening frequency for FIT, fecal DNA and the SEPT9 is once per year, once per three years and once per year, respectively, FIT might be the cheapest test considering the balance between performance and costs. However, the quality adjusted life year of the three tests should be compared under the same setting to evaluate the cost-effectiveness of them, although some studies have been performed for each

individual method in different settings, such as different health systems.

CRC SCREENING WITH COMBINED TESTS

The combination of fecal DNA (mutation and methylation) with a hemoglobin immunoassay in Cologuard has provided a good example for CRC screening when multiple markers are analyzed together to enhance the detection sensitivity. There are merits and drawbacks for this strategy. First, combination of multiple markers enhances sensitivity at the price of reducing specificity. The number of false positive cases will increase with the increased number of markers. Therefore, to identify the markers with high sensitivity and specificity and to find the best combination of markers remain a challenge for combined screening test development. Ideally, the number of markers should be kept to minimum, while the sensitivity and specificity can be balanced to provide the best performance. Secondly, the detection of multiple markers with distinct methods increases the technical difficulties in an assay. For example, the detection of mutation in Cologuard may use sequencing or PCR method, while the detection of methylation needs to use the methylation specific PCR method containing bisulfite conversion. In contrast, immunoassay is used in the detection of hemoglobin. Furthermore, the sample preparation procedure may also be different for detecting different abnormalities. Therefore, a good combined test needs not only optimization of each individual test, but also an accurate algorithm to maximize the performance of each test. The optimization and interpretation of the combined test must come from clinical trials with large number of cases. Thirdly, a screening test should be accurate, fast, convenient, simple and cheap. These features allow large-scale screening in a certain period of time, and allow easy test in areas where test instruments are not available. In addition, low costs ensure screening tests for average-risk population, in which the CRC incidence could be lower than 1% in people over 50 years old. All the above considerations need to be addressed in future development of combined screening test.

As FIT, SEPT9 and CEA tests are all CRC detection tests with high specificity, the combination of them may provide higher sensitivity with no significant compromise

in specificity. We recently tested this assumption in an opportunistic screening setting, in which blood and stool samples were collected from outpatients and inpatients coming to the GI departments of three Chinese hospitals[55]. Table 6 shows the test results from the screening. SEPT9, FIT or CEA alone detected 77.0%, 74.6% and 41.3% of CRC cases, respectively, while the combination of the three increased the sensitivity to 97.2%, and SEPT9 plus FIT exhibited a sensitivity of 94.4%. Since CEA is more sensitive to late-stage CRC than early-stage CRC, and no significant difference was found between SEPT9 + FIT+ CEA and SEPT9 + FIT, we recommend SEPT9 + FIT as a routine method for CRC screening.

CONCLUSION

The FIT, fecal DNA and the SEPT9 tests are IVD tests currently used for CRC screening. FIT tests exhibited satisfactory sensitivity and specificity with low costs and therefore become the major screening test for CRC at the moment. The sensitivity of the fecal DNA test appeared to be very high due to combination of multiple methods while its high cost is an obstacle preventing the test from broad use. Both sensitivity and specificity for the SEPT9 test in CRC screening were lower than those of the FIT and fecal DNA test, but it showed high compliance with promising future if its accuracy can be improved. Combined tests with multiple markers should be a future direction in CRC screening, however, some hurdles, such as technical integration, test/interpretation optimization, and high costs, etc, need to be overcome before they can be used in large-scale CRC screening aiming at asymptomatic average-risk population.

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