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A systematic review of the performance of the SEPT9 gene methylation assay in colorectal cancer screening, monitoring, diagnosis and prognosis

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Abstract.
BACKGROUND: The applications of the SEPT9 assay are expanding from CRC early diagnosis to screening, therapeutic effect monitoring and prognosis prediction. Its performance in these areas has not been thoroughly examined.
OBJECTIVE: We aim to evaluate the performance of the SEPT9 assay in CRC screening, diagnosis and therapy by reviewing the current data published in these aspects.
METHODS: The Ovid MEDLINE ,EMBASE, CBMdisc (China Biology Medicine disc) and CJFD (Chinese Journal Full – text Database) database were searched for potential reports on the assay performance. Letters, reviews, meta-analysis and guidelines, basic research studies and articles irrelevant to mSEPT9 detection assays were excluded. Finally, data from 19 studies was summarized and systematically reviewed to clarify the assay performance.
RESULTS: 2/3 algorithm provided the best overall performance in diagnosis and screening, while the 1/3 algorithm exhibited the best sensitivity in screening. The combination of SEPT9 assay with FIT and/or CEA enhanced the CRC detection rate in screening. The SEPT9 assay appeared to be effective in monitoring the therapeutic effect and may potentially predict the CRC recurrence and survival.
CONCLUSION: The SEPT9 assay exhibited satisfactory performance in CRC diagnosis and screening, while more evidence is needed for therapeutic effect monitoring and prognosis prediction.

Keywords: SEPT9, Septin 9, colorectal cancer, adenoma, methylation, CEA, fecal DNA

1. Introduction

Colorectal cancer (CRC) has become the 3rd leading cause of new cancer cases in the world. Regular screening has been proved to be an effective method to reduce morbidity and mortality [1]. There are several methods currently available for CRC screening. The U.S. Preventive Services Task Force (USPSTF) recently listed the gFOBT (guaiac-based fecal occult blood test), FIT (fecal immunochemical test), FIT-DNA (multitargeted stool DNA), SEPT9 tests and the direct visualization tests (colonoscopy, CT colonoscopy and sigmoidoscopy) as the current CRC screening strategies [2].

The blood-based SEPT9 assay was designed to detect aberrant hypermethylation at the promoter region of the *SEPT9* gene V2 transcript. The development of the assay is based on the theory that DNA released from necrotic and apoptotic CRC cells into the peripheral blood (i.e. the circulating tumor DNA, or ctDNA)

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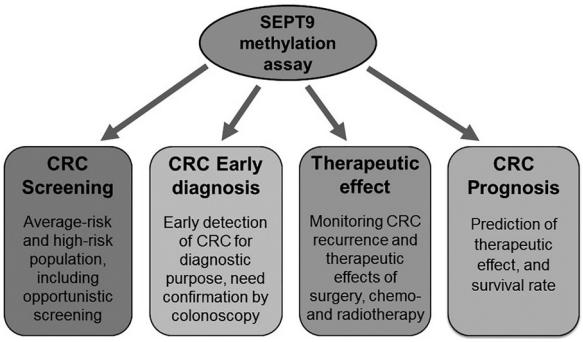

Fig. 1. Four applications of the SEPT9 gene methylation assay. The assay has been used in CRC screening and early diagnosis, while it can potentially be used in monitoring therapeutic effect and predicting CRC prognosis. Description for each application is provided in each individual panel.

can be detected by multiple qRT-PCR method [3–5]. The risk of CRC can be determined by detecting the degree of DNA methylation of the specific region of the *SEPT9* gene from the peripheral blood [6].

The blood-based *SEPT9* assay was developed in 2008, initially aiming at early detection of CRC for patients who have low compliance with other CRC detection methods, such as colonoscopy [3,4]. The assay was later improved and commercialized as a European union approved product for CRC early detection. Large-scale randomized clinical trial aiming at the US FDA approval was later performed to extend its application to CRC screening. The FDA approved Epi proColon (Epigenomics, Inc) as the first blood-based CRC screening test for average-risk population from 50 to 75 years old in April, 2016, based on the clinical trial data involving nearly 8000 subjects [7].

Apart from CRC screening and early detection for diagnostic purpose, the assay has also been used in opportunistic screening (Fig. 1). A recent publication from four Chinese hospitals reported the use of the assay in screening of outpatients and inpatients [8]. Due to the higher risk of CRC occurrence in hospital patients, the positivity rate in this opportunistic screening was higher than that in average-risk population. The assay was proved to be effective to identify the high-risk subjects in hospital population. Moreover, a couple of recent reports suggested that the SEPT9 methylation could be an indicator for post-surgery therapeutic effect, and could be used in monitoring CRC recurrence [9]. It may also be a predictor for the effect of chemo- and radiotherapy and long-term survival rate [10] (Fig. 1). These observations suggest great potentials in application extension for the SEPT9 assay. Since there has been no systematic review on the performance of the SEPT9 assay in CRC diagnosis, screening, opportunistic screening, therapeutic effect monitoring and prognosis prediction, we will provide an overview on the current applications of the SEPT9 assay in the above aspects, with specific focuses on CRC screening and early detection for diagnostic purpose.

2. The application of SEPT9 assay in CRC early diagnosis

The application of an assay in early diagnosis normally means that a test can be used as a supplementary or subsidiary method to confirmatory diagnostic method or the ‘gold standard’ in diagnosis, and can be used to enhance the early diagnostic rate or positive detection rate. The application of an assay in screening means that a test can be used independently as a method for identifying high-risk individuals for certain diseases in population-based screening activities, while confirmatory diagnosis is normally required following the screening. There are currently 19 studies investigating the performance of the SEPT9 assay at various settings, and most studies performed so far are case-control or cohort studies, except the PRESEPT study [7,11] and the RESEPT study [8], which were screening studies in average-risk and high-risk population, respectively (Table 1). The Ovid MEDLINE ,EMBASE, CBMdisc (China Biology Medicine disc) and CJFD (Chinese Journal Full-text Database) database were searched using the key words ‘SEPT9’, or ‘septin 9’, and ‘colorectal cancer’ or ‘colorectal carcinoma’ to identify all relevant studies. A total of 174 studies were identified from the above database. In the initial screening, a total of 104 articles, including letters, reviews, meta-analysis, guidelines, basic research studies, articles irrelevant to mSEPT9 detection assays and articles published earlier than 2008 (the first study on the development of the SEPT9 assay was published in 2008) were excluded. In the second screening, a further 51 articles were excluded, including studies that were not using plasma or serum samples, or those not detecting gene methylation, or those not having statistically significant number cases, and 19 studies were finally included in the quantitative synthesis for this review.

The test sensitivity, specificity, algorithm, and kit used in each study, were compared in Table 1. The studies are categorized and highlighted by algorithm used for data interpretation. The sensitivity and specificity of the cohort or case-control study were affected

Table 1
Sensitivity and specificity of the blood-based SEPT9 gene methylation assay in CRC detection or screening with various algorithm

Publications	Number of cases	Sensitivity	Specificity	Algorithm	Kit used
Potter et al., 2014 [11]	Total 1544 (44 CRC, 1500 advanced adenoma, small polyps or no evidence of diseases	68.2% (30/44)	80.0% (1200/1500)	1/3	Epi proColon 2.0
Johnson et al., 2014 [12]	301 (101 CRC, 200 advanced adenoma, small polyps or no evidence of diseases)	73.3% (74/101)	81.5% (163/200)	1/3	Epi proColon 2.0
Grutzmann et al., 2008 [4]	309 (126 CRC, 183 control)	72% (90/125)	89.6% (164/183)	2/3	Research kit
Toth et al., 2014 [13]	58 (34 CRC, 24 no evidence of diseases)	88.2% (30/34)	91.7% (22/24)	2/3	Epi proColon 2.0
Kang et al., 2014 [14]	132 (80 CRC, 52 NED)	75.0% (60/80)	98.1% (51/52)	2/3	Epi proColon 2.0
He et al., 2014 [15]	298 (79 CRC, 73 Polyps, 146 NED)	71.1% (54/76)	95.6% (196/205)	2/3	Epi proColon 2.0
Jin et al., 2015 [16]	226 (135 CRC, 91 control)	74.8% (101/135)	96.7% (88/91)	2/3	Epi proColon 2.0
Yu et al., 2015 [17]	123 (70 CRC, 53 NED)	81.4% (57/70)	86.8% (46/53)	2/3	Epi proColon 2.0
deVos et al., 2009 [18]	245 (90 CRC, 155 control)	73.8% (138/187)	86.2% (282/327)	1/3	Research kit
		56.1% (105/187)	96.6% (316/327)	2/3	
Tanzer et al., 2010 [19]	161 (33 CRC, 34 control)	82% (27/33)	88% (30/34)	1/3	Epi proColon 1.0
		73% (24/33)	91% (31/34)	2/3	
Warren et al., 2011 [20]	144 (50 CRC, 94 control)	90.0% (45/50)	88.0% (83/94)	1/3	ARUP Lab LDT assay
		76.0% (38/50)	99.1% (93/94)	2/3	
Toth et al., 2012 [6]	184 (92 CRC, 92 control)	95.6% (88/92)	84.8% (78/92)	1/3	Epi proColon 2.0
		79.3% (73/92)	99% (91/92)	2/3	
Lofton-Day et al., 2008 [3]	312 (133 CRC, 179 control)	69% (92/133)	86% (154/179)	1/1	Research kit
He et al., 2010 [21]	252 (182 CRC, 170 NED)	74.7% (136/182)	96.5% (164/170)	1/1	research kit
Wang et al., 2012 [22]	56 (36 CRC, 20 control)	69.4% (25/36)	90.0% (18/20)	1/1	research kit
Wu et al., 2016 [8]	1031(291 CRC, 295 NED	76.6% (223/291)	95.9% (283/295)	1/1	SensiColon
Weiss and Rosch, 2010 [23]	257 (103 CRC, 154 no evidence of diseases)	67.0% (69/103)	87.7% (135/154)	1/2	Epi proColon 1.0
Liu et al., 2013 [24]	57 (37 CRC, 20 control)	54.1% (20/37)	90.0% (18/20)	1/2	research kit
Church et al., 2014 [7]	Total 7941 (53 CRC, 1457 advanced adenomas, non-advanced adenomas or no evidence of diseases)	48.2% (27/53)	91.5% (1333/1457)	1/2	Epi proColon 1.0

CRC = colorectal cancer, NED = no evidence of diseases.

by study design, population choice, selection of cases, choice of kits and algorithm, etc. The sensitivity of these studies varied between 54.1% and 95.6%, with specificity at 81.5%–99.1% (Table 1). The effect of different algorithm can be observed in studies with multiple algorithms applied. The positive test results were determined by one positive count out of three PCRs (1/3 algorithm), one positive count out of two PCRs (1/2 algorithm), two positive counts out of three PCRs (2/3 algorithm), or one positive count out of one PCR (1/1 algorithm).

In order to compare the performance of SEPT9 assay at various algorithms, study data from each algorithm were pooled and the sROC curves were plotted

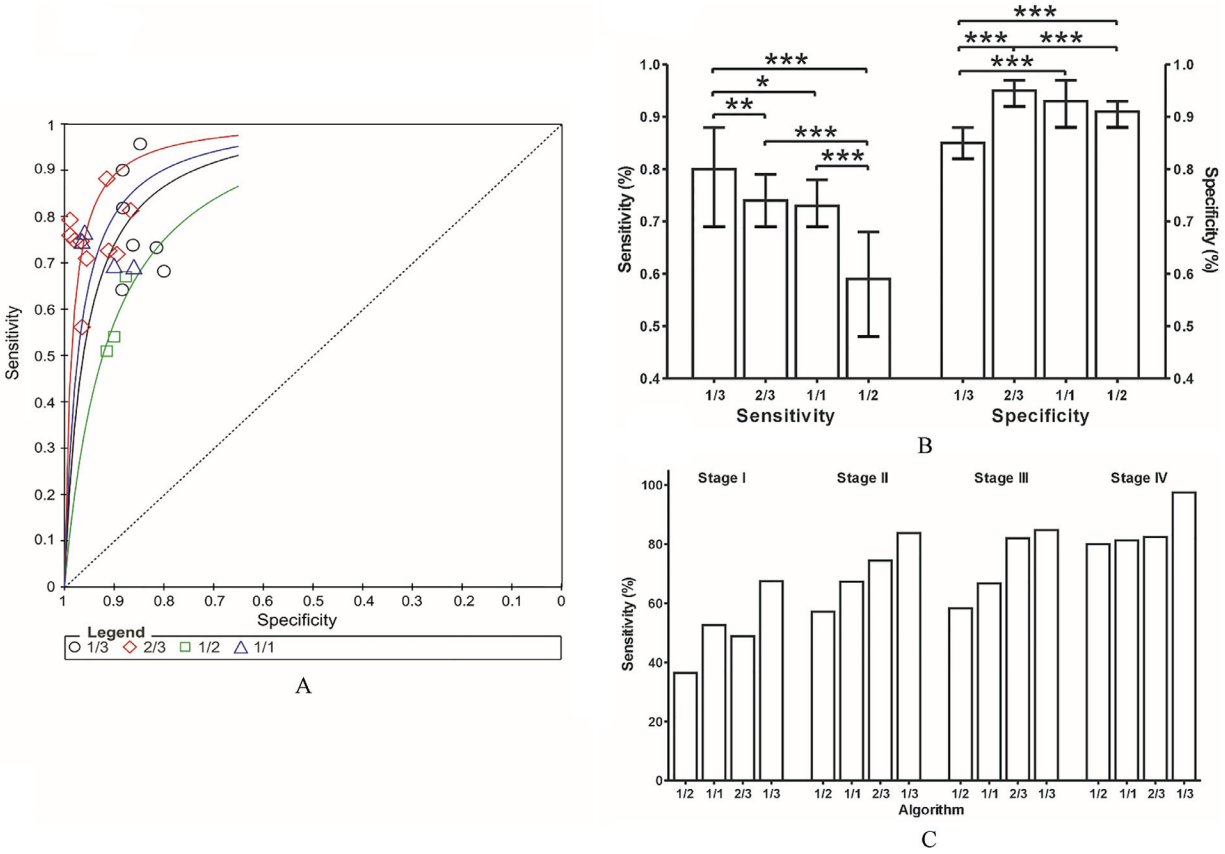

Fig. 2. sROC curves, sensitivity, specificity and the stage-dependent PDR for 1/3, 2/3, 1/2 and 1/1 algorithms in CRC detection. The sROC curves for the four algorithms are shown in panel (A). Circles, diamonds, squares and triangles represent the optimal points for sensitivity against specificity for individual studies for 1/3, 2/3, 1/2 and 1/1 algorithms, respectively, and the sROC curve for each algorithm was plotted. The estimated overall sensitivity and specificity for the four algorithms was compared in panel (B), with the scale bars representing the 95% CI. The sensitivity for each CRC stage at each algorithm was plotted and compared in panel (C). McNemars's test was performed to compare the sensitivity and specificity from various algorithms, and the significant differences were labeled on panel (B). '*','**' and '***' represent significant ($p < 0.05$), highly significant ($p < 0.01$) and very highly significant ($p < 0.001$) differences.

to compare the performance (Fig. 2A). The sensitivity and specificity from each algorithm were also analyzed and compared (Fig. 2B). It can be seen from panel A that 2/3 algorithm exhibited the highest area under the curve (AUC), followed by 1/1 and 1/3 algorithm, while 1/2 had lower AUC than other algorithms (Fig. 2A). However, AUC may not be an appropriate indicator for performance here, as the points in panel A seem to be quite scattered at random, and the number of points were low (only three) for 1/1 and 1/2 algorithms. Therefore, the sensitivity and specificity were compared directly to evaluate the test performance. As shown in panel B, 1/3 algorithm exhibited significantly higher sensitivity (0.80) with significantly lower specificity (0.85) than all the other algorithms, while 2/3 algorithm exhibited the highest specificity (0.95). The sensitivity and specificity of 2/3 and 1/1 algorithm were very similar (sensitivity: 0.74 vs 0.73, specificity: 0.95 vs 0.93). 1/2 algorithm exhibited the lowest sensitivity (0.59) with acceptable specificity (0.91) (Fig. 2B). It can be observed that the 2/3 algorithm provided the best overall performance while the 1/3 algorithm exhibited the best sensitivity.

Although *SEPT9* assay exhibited adequate sensitivity and specificity in detecting CRC, the ability of detecting early-stage CRC is more important for CRC early diagnosis and intervention. The positive detection rate (PDR) at each stage was pooled and analyzed, as shown in Fig. 2C. It is clear that the detection rate increases with the escalation of clinical stage, indicating a correlation between SEPT9 methylation and the degree of malignancy. Furthermore, the algorithm also exhibited a clear effect on the PDR for every stage, and algorithm can be ranked as 1/3 > 2/3 > 1/1 > 1/2 from

highest to lowest. It is notable that the detection rate for stage I and II reached 67.5% and 83.8%, respectively, at 1/3 algorithm, and the SEPT9 assay with 2/3 or 1/1 algorithm detected approximately half of stage I and 70% of stage II CRC, respectively (Fig. 2C), representing a high PDR for early-stage CRC. It is obvious that algorithms with high PDR should be adopted in CRC screening, while false positive detection may increase with higher PDR algorithms. In brief, these data clearly show that the SEPT9 assay is effective for early stage CRC detection.

Since the SEPT9 assay was designed for detecting trace amount of methylated SEPT9 gene copies in strong background of unmethylated SEPT9 DNA, most studies performed multiple PCRs to enhance test sensitivity. Due to the application of different algorithms in data analysis, sensitivity and specificity vary with different methods of interpretation. The choice of algorithms is based on the specific needs in a test. If the priority is to exclude healthy negative population, such as that in an early detection test for diagnostic purpose, the algorithm with highest specificity should be used, however, if the priority is to identify as many real patients as possible to enhance the disease detection rate, such as that in a screening test, the algorithm with highest sensitivity should be used. The problem of high false positive rate in a screening test can be overcome by further diagnostic examinations, while the high missed diagnosis rate in a diagnostic test can be avoided by routine screening programs.

3. CRC screening with the SEPT9 assay

The PRESEPT study is the only screening study so far performed in average-risk asymptomatic population [7]. SEPT9 assay detected 48.2% CRC, with 35% stage I, 63% stage II, 46% stage III and 77.4% stage IV. The sensitivity reported (48.2%) using 1/2 algorithm was apparently lower than those reported in previous cohort or case-control studies. This could be explained from the following aspects. Firstly, duplicate PCR reactions, instead of triplicate PCRs, were applied in this study. Therefore, the chance to detect abnormally methylated SEPT9 DNA was lower for duplicate PCRs than those previous studies performing triplicate PCRs. Potter and colleagues [11] later performed triplicate PCRs using samples from the same study. The sensitivity increased to 68.2% and the specificity decreased to 80.0%. The US FDA approved the commercialized SEPT9 assay based on the data from the PRESEPT study with 1/3 algorithm [7]. Secondly, CRC patients found in the asymptomatic population are more likely to be those with early-stage CRC. As the PDR in early-stage CRC appeared to be lower than the overall PDR from all stages, the sensitivity from the asymptomatic population tends to be lower than those with symptomatic population.

Apart from the screening in average-risk population, the SEPT9 assay was also used in the opportunistic screening for high-risk population. Opportunistic screening refers to the screening of patients who are in contact with health care providers in the hospital environment. Patients involved in opportunistic screening include outpatients and inpatients. Since patients coming to hospitals may have symptoms that drive them to see a doctor, they normally represent a higher-risk population. Therefore, opportunistic screening may exhibit a higher positivity rate than that of the average-risk population. A recent study reported that the SEPT9 assay exhibited a sensitivity of 76.6% with a specificity of 95.9% at a positivity rate of 25.8% in an opportunistic screening performed in four northern Chinese hospitals (RESEPT study) [8]. This study recruited a total of 1031 subjects, including 291 CRC patients, 445 patients of other gastrointestinal disease or other cancer, and 295 subjects with no evidence of diseases. The SEPT9 assay exhibited an adequate sensitivity for early-stage CRC (PDR of 64.9% for stage I and 72.7% for stage II) and was therefore proved to be effective for CRC screening. The low positive detection rate in non-CRC diseases and normal subjects ensured adequate specificity.

4. Monitoring the CRC therapeutic effects by SEPT9 assay

Apart from the application in early detection and screening of CRC, *SEPT9* assay has exhibited the potential for CRC therapeutic effect evaluation and recurrence follow-up. In one study, the plasma *SEPT9* methylation level of 9 CRC patients who received curative radical surgery was measured before and after surgery. It showed that the *SEPT9* methylation became negative for 8 patients (88.9%) 118 days after surgery on average, while positivity was regained in patients with CRC recurrence [9]. Furthermore, patients with positive methylated *SEPT9* result had a tendency of developing distant metastasis and exhibited lower disease-free survival [9]. More recently, we performed the SEPT9 assay with samples from 8 patients

with negative CEA and CA199 tests, and 5 of them exhibited positive SEPT9 results, and all of them exhibited negative results in SEPT9 assay after surgery (unpublished data). We further found that the level of plasma SEPT9 methylation decreased after two cycles of chemotherapy (unpublished data), which was consistent with the indicators of therapeutic effects evaluated by other examinations, such as CEA or CT. It can be suggested that the *SEPT9* methylation level is closely correlated with the development of CRC, and *SEPT9* methylation could be used as a predictive marker for monitoring therapeutic effect.

5. Prediction of CRC prognosis by SEPT9 assay

The SEPT9 assay has also exhibited the potential for survival prediction. Tham and colleagues performed five-year prospective cohort study including 150 stage I-III CRC patients who received surgery and subsequent chemo- or radiotherapy [10]. It showed that high serum methylation levels of *SEPT9* at 1-year follow-up were independent predictors for tumor recurrence and unfavorable cancer-specific survival. *SEPT9* at 1-year follow-up exhibited earlier detection of potential recurrences compared with concurrent serum CEA, and the level of *SEPT9* methylation in serum can be a predictive marker for CRC development and prognosis [10]. It would be intriguing if a model can be established to predict the recurrence, metastasis and survival. Repeated invasive biopsies and radiative examinations may be avoided.

6. Combination of multiple methods in CRC screening and early diagnosis

Combination of multiple markers or methods has become a trend in cancer detection and screening. There are two studies so far combining the SEPT9 assay with FIT and/or CEA in CRC detection. One study reported by Jin and colleagues [16] showed that SEPT9 assay alone detected 76.8% of CRC, and FIT alone detected 58.0% of CRC, while the combination of SEPT9 assay and FIT detected 94.2% of CRC, with the specificity for the combination at 92.6%. This study suggested that the combination of SEPT9 and FIT assays significantly enhanced the sensitivity of CRC detection compared with any assay alone. In another very recent report [8], *SEPT9* assay alone, FIT test alone, or CEA alone showed a sensitivity of 77.0%, 74.6% and 41.3%, respectively. The combination of *SEPT9* + FIT ($\chi^2 = 10.68$, $P < 0.001$), *SEPT9* + CEA ($\chi^2 = 6.89$, $P < 0.01$) or *SEPT9* + FIT + CEA ($\chi^2 = 14.82$, $P < 0.001$) showed significantly higher sensitivity than *SEPT9* alone. Although *SEPT9* assay alone can achieve an adequate sensitivity for CRC detection (77.0%), the combination of SEPT9 + FIT (94.4%) or *SEPT9* + FIT + CEA exhibited significantly higher sensitivity (97.2%) in CRC detection. Screening combining multiple markers should be a future strategy to enhance the detection rate.

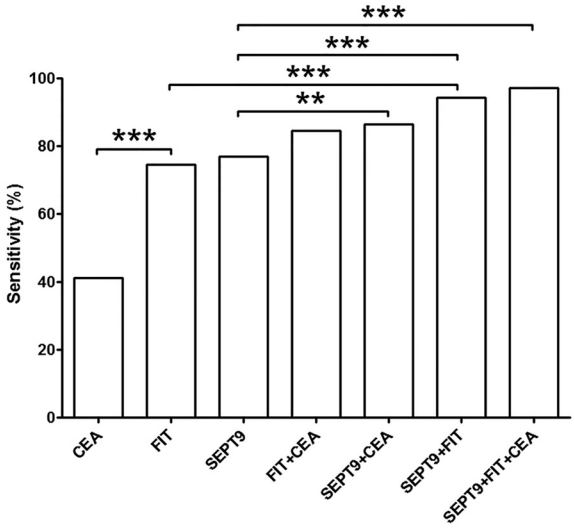

Fig. 3. Comparison of the sensitivity of CEA, FIT, SEPT9 and various combinations of the three markers in CRC detection. Data was pooled from two studies involving the combined detection of CRC using multiple markers. McNemars's test was performed to compare the sensitivity and specificity from various algorithms, and the significant differences were labeled. '*','**' and '***' represent significant ($p < 0.05$), highly significant ($p < 0.01$) and very highly significant ($p < 0.001$) differences.

Here we pooled the data from these reports together and compared the sensitivity of various markers and their combinations in CRC detection (Fig. 3). SEPT9 alone exhibited significantly better sensitivity (77.0%) than FIT (66.4%, $p < 0.05$) or CEA (41.3%, $p < 0.001$) alone in the analysis. For combination of two markers, SEPT9 + FIT (94.3%) exhibited better sensitivity than SEPT + CEA (86.4%, $p < 0.05$) or FIT + CEA (84.5%, $p < 0.05$), while combination of the three markers (SEPT9 + FIT + CEA) showed the best sensitivity (97.2%). It should be noticed that the sensitivity is enhanced with the number of markers increases, but the specificity may be decreased due to accumulation of false positive cases from each marker, therefore, the specificity of these combinations should

be further examined. The best combination may be the one that best balances the sensitivity and specificity.

7. Limitations of the reviewed studies and the SEPT9 gene methylation assay

Since the SEPT9 gene methylation assay detects trace amount of aberrantly methylated DNA of the SEPT9 gene promoter region from peripheral blood, there are some limitations of the assay. First, the detection rate is dependent on two factors, the level of aberrant methylation in colorectal tissues and the amount of DNA that can be released into peripheral blood. Evidence has shown that the targeted region of the SEPT9 gene is nearly 100% methylated at adenoma and CRC stage [25], and this ensures that the methylation level in tissues is applicable for a methylation assay. The detection rate of the assay is then dependent on the amount of circulating tumor DNA (ctDNA), and it is related to the tumor cell turnover rate. When tumor growth, necrosis and apoptosis are active, the cell turnover rate will be high, and there will be more DNA released into blood, and vice versa. Therefore, the level of ctDNA varies individually and distributes widely in a population. Secondly, the detection of single-digit number of aberrantly methylated DNA copies is still challenging for a PCR assay. The Ct values normally falls into the non-linear region of the standard curve at this DNA level, and therefore exhibited huge random variation if the test is repeated. This brings the uncertainty of detection results when the concentration of the targeted DNA is very low, especially for subjects with adenoma or early-stage CRC. Thirdly, the detection rate of the SEPT9 assay is in positive correlation with the degree of malignancy, which means it detects higher ratio of later stage CRC than early stage CRC, and the PDR for adenoma is low. This limits the capability of the assay in detecting precancerous lesions and early stage CRC.

There are also limitations for studies reviewed in the article. First, most studies are cohort or case-control studies that focused on the assay performance in certain groups of subjects, while only two screening studies investigating its performance in population [7,8]. Therefore, more evidence is still needed to evaluate the assay performance in screening setting. Secondly, the sensitivity and specificity in screening appeared to be lower than that of FIT and fecal DNA tests, which did not support the replacement of stool-based tests, however, the blood assay exhibited higher compliance than stool based tests, which supports its use in mass population screening [26]. Thirdly, the use of different algorithms in the reviewed studies leads to difficulties in comparing the test performance, as algorithms greatly affect the detection rate for various diseases. Ideally, all studies should be compared at the same algorithm.

8. Conclusions

The SEPT9 assay has exhibited adequate sensitivity and specificity in CRC screening and early detection for diagnostic purpose, and has great potential in therapeutic effect and recurrence monitoring and prediction of CRC prognosis. Combined test with multiple biomarkers including SEPT9 methylation may be a future option to enhance CRC screening performance and compliance, and to enhance the early diagnosis rate. More studies still need to be performed before the SEPT9 assay becomes a prognostic marker and therapeutic marker.

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Conflict of interest

The first and the corresponding author of the article, Dr. Lele Song, is currently an employee of BioChain (Beijing) Science and Technology, Inc. BioChain is a collaborator of Epigenomics AG, a Germany-based company who launched the first commercial SEPT9 assay. Other authors of the article declare no conflict of interest.

References

[1] National Cancer Institute. Survival rate for colorectal cancer by stage, PDQ, Treatment, Health Professionals, 1999.

[2] Lin, J.S.; Piper, M.A.; Perdue, L.A.; Rutter, C.M.; Webber, E.M.; O'Conno, E.; Smith, N.; Whitlock, E.P. Screening for Colorectal Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 315 (2016), 2576-94.

[3] Lofton-Day, C.; Model, F.; Devos, T.; Tetzner, R.; Distler, J.; Schuster, M.; Song, X.; Lesche, R.; Liebenberg, V.; Ebert, M.; Molnar, B.; Grützmann, R.; Pilarsky, C.; Sledziewski, A. DNA methylation biomarkers for blood-based colorectal cancer screening. *Clin Chem* 54 (2008), 414-23.

[4] Grützmann, R.; Molnar, B.; Pilarsky, C.; Habermann, J.K.; Schlag, P.M.; Saeger, H.D.; Miehlke, S.; Stolz, T.; Model, F.; Roblick, U.; Bruch, H.P.; Koch, R.; Liebenberg, V.; Devos, T.; Song, X.; Day, R.H.; Sledziewski, A.Z.; Lofton-Day, C. Sensitive detection of colorectal cancer in peripheral blood by septin 9 DNA methylation assay. *PLoS One* 3 (2008), e3759.

[5] Wasserkort, R.; Kalmar, A.; Valcz, G.; Spisak, S.; Krispin, M.; Toth, K.; Tulassay, Z.; Sledziewski, A.Z.; Molnar, B. Aberrant septin 9 DNA methylation in colorectal cancer is restricted to a single CpG island. *BMC Cancer* 13 (2013), 398.

[6] Tóth, K.; Sipos, F.; Kalmár, A.; Patai, A.V.; Wichmann, B.; Stoehr, R.; Golcher, H.; Schellerer, V.; Tulassay, Z.; Molnár, B. Detection of methylated SEPT9 in plasma is a reliable screening method for both left- and right-sided colon cancers. *PLoS One* 7 (2012), e46000.

[7] Church, T.R.; Wandell, M.; Lofton-Day, C.; Mongin, S.J.; Burger, M.; Payne, S.R.; Castanos-Vélez, E.; Blumenstein, B.A.; Rosch, T.; Osborn, N.; Snover, D.; Day, RW.; Ransohoff, D.F.; PRESEPT Clinical Study Steering Committee, Investigators and Study Team. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. *Gut* 63 (2014), 317-25.

[8] Wu, D.; Zhou, G.; Jin, P.; Zhu, J.; Li, S.; Wu, Q.; Wang, G.; Sheng, J.; Wang, J.; Song, L.; Han, X.; Qian, J. Detection of Colorectal Cancer Using a Simplified SEPT9 Gene Methylation Assay Is a Reliable Method for Opportunistic Screening. *J Mol Diagn* 18 (2016), 535-45.

[9] Lee, H.S.; Hwang, S.M.; Kim, T.S.; Kim, D.W.; Park, D.J.; Kang, S.B.; Kim, H.H.; Park, K.U. Circulating methylated septin 9 nucleic Acid in the plasma of patients with gastrointestinal cancer in the stomach and colon. *Transl Oncol* 6 (2013), 290-6.

[10] Tham, C.; Chew, M.; Soong, R.; Lim, J.; Ang, M.; Tang, C.; Zhao, Y.; Ong, S.Y.; Liu, Y. Postoperative serum methylation levels of TAC1 and SEPT9 are independent predictors of recurrence and survival of patients with colorectal cancer. *Cancer* 120 (2014), 3131-41.

[11] Potter, N.T.; Hurban, P.; White, M.N.; Whitlock, K.D.; Lofton-Day, C.E.; Tetzner, R.; Koenig, T.; Quigley, N.B.; Weiss, G. Validation of a real-time PCR-based qualitative assay for the detection of methylated SEPT9 DNA in human plasma. *Clin Chem* 60 (2014), 1183-91.

[12] Johnson, D.A.; Barclay, R.L.; Mergener, K.; Weiss, G.; Konig, T.; Beck, J.; Potter, N.T. Plasma Septin9 versus fecal immunochemical testing for colorectal cancer screening: a prospective multicenter study. *PLoS One* 9 (2014), e98238.

[13] Tóth, K.; Wasserkort, R.; Sipos, F.; Kalmár, A.; Wichmann, B.; Leiszter, K.; Valcz, G.; Juhász, M.; Miheller, P.; Patai, áV.; Tulassay, Z.; Molnár, B. Detection of methylated septin 9 in tissue and plasma of colorectal patients with neoplasia and the relationship to the amount of circulating cell-free DNA. *PLoS One* 9 (2014), e115415.

[14] Kang, Q.; Jin, P.; Yang, L.; Wang, X.; An, H.; Liu, L.; Li, N.; Sheng, J. Significance of Septin9 gene methylation detection of plasma circulation DNA in colorectal cancer screening. *Zhonghua Yi Xue Za Zhi* 94 (2014), 3839-41.

[15] He, N.; Chu, W.H.; Li, Y.Q.; Yang, L.P.; Xie, H.H.; Wang, B.L.; Nie, Y.Z.; Wang, X.; Guo, X.G.; Wu, K.C. Clinical significance of detecting serum methylated Sept9 gene in diagnosis of colorectal cancer. *Chin J Dig* 34 (2014), 726-731.

[16] Jin, P.; Kang, Q.; Wang, X.; Yang, L.; Yu, Y.; Li, N.; He, Y.Q.; Han, X.; Hang, J.; Zhang, J.; Song, L.; Han, Y.; Sheng, J.Q. Performance of a second-generation methylated SEPT9 test in detecting colorectal neoplasm. *J Gastroenterol Hepatol* 30 (2015), 830-3.

[17] Yu, D.; Zhang, X.H.; Lu, X.X. Study on diagnostic value of SEPT9 gene methylation in serum for colorectal cancer. *Chin J Clin Lab Sci* 33 (2015), 687-689.

[18] DeVos, T.; Tetzner, R.; Model, F.; Weiss, G.; Schuster, M.; Distler, J.; Steiger, K.V.; Grützmann, R.; Pilarsky, C.; Habermann, J.K.; Fleshner, P.R.; Oubre, B.M.; Day, R.; Sledziewski, A.Z.; Lofton-Day, C. Circulating methylated SEPT9 DNA in plasma is a biomarker for colorectal cancer. *Clin Chem* 55 (2009), 1337-46.

[19] Tanzer, M.; Balluff, B.; Distler, J.; Hale, K.; Leodolter, A.; Rocken, C.; Molnar, B.; Schmid, R.; Lofton-Day, C.; Schuster, T.; Ebert, M.P. Performance of epigenetic markers SEPT9 and ALX4 in plasma for detection of colorectal precancerous lesions. *PLoS One* 5 (2010), e9061.

[20] Warren, J.D.; Xiong, W.; Bunker, A.M.; Vaughn, C.P.; Furtado, L.V.; Roberts, W.L.; Fang, J.C.; Samowitz, W.S.; Heichman, K.A. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. *BMC Med* 9 (2011), 133.

[21] He, Q.; Chen, H.Y.; Bai, E.Q.; Luo, Y.X.; Fu, R.J.; He, Y.S.; Jiang, J.; Wang, H.Q. Development of a multiplex MethyLight assay for the detection of multigene methylation in human colorectal cancer. *Cancer Genet Cytogenet* 202 (2010), 1-10.

[22] Wang, Z.; Chen, J.C.; He, Q.; Peng, H.Y.; Zhu, Z.Y.; Chen, H.Y.; Li, M. The application of MS-HRM detection of free methylated SEPT9 in early diagnosis of colorectal cancer. *Guangdong Medical Journal* 33 (2012), 1732-1734.

[23] Weiss, G.; Rosch, T. Potential of a new blood test for colorectal cancer screening – the Septin 9 gene biomarker. *Eur. Oncol* 6 (2010), 51-54.

[24] Liu, Y.; Tham, C.K.; Ong, S.Y.; Ho, K.S.; Lim, J.F.; Chew, M.H.; Lim, C.K.; Zhao, Y.; Tang, C.L.; Eu, K.W. Serum methylation levels of TAC1, SEPT9 and EYA4 as diagnostic markers for early colorectal cancers: a pilot study. *Biomarkers* 18 (2013), 399-405.

[25] Semaan, A.; van Ellen, A.; Meller, S.; Bergheim, D.; Branchi, V.; Lingohr, P.; Goltz, D.; Kalff, J.C.; Kristiansen, G.; Matthaei, H.; Pantelis, D.; Dietrich, D. SEPT9 and SHOX2 DNA methylation status and its utility in the diagnosis of colonic adenomas and colorectal adenocarcinomas. *Clin Epigenetics* 8 (2016), 100.

[26] Adler, A.; Geiger, S.; Keil, A.; Bias, H.; Schatz, P.; DeVos, T.; Dhein, J.; Zimmermann, M.; Tauber, R.; Wiedenmann, B. Improving compliance to colorectal cancer screening using blood and stool based tests in patients refusing screening colonoscopy in Germany. *BMC Gastroenterol* 14 (2014), 183.