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Review Article

Non-Invasive Colorectal Cancer Screening: An Overview

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Keywords
Colorectal cancer screening · Tumour biomarkers · Diagnostic biomarkers · microRNA · Gut microbiota

Abstract
Background: Colorectal cancer (CRC) follows a protracted stepwise progression, from benign adenomas to malignant adenocarcinomas. If detected early, 90% of deaths are preventable. However, CRC is asymptomatic in its early-stage and arises sporadically within the population. Therefore, CRC screening is a public health priority. ***Summary:*** Faecal immunochemical test (FIT) is gradually replacing guaiac faecal occult blood test and is now the most commonly used screening tool for CRC screening program globally. However, FIT is still limited by the haemoglobin degradation and the intermittent bleeding patterns, so that one in four CRC cases are still diagnosed in a late stage, leading to poor prognosis. A multi-target stool DNA test (Cologuard, a combination of *NDRG4* and *BMP3* DNA methylation, *KRAS* mutations, and haemoglobin) and a plasma *SEPT9* DNA methylation test (Epi proColon) are non-invasive tools also approved by the US FDA, but those screening approaches are not cost-effective, and the detection accuracies remain unsatisfactory. In addition to the approved tests, faecal-/blood-based microRNA and CRC-related gut microbiome screening markers are under development, with work ongoing to find the best combination of molecular biomarkers which maximise the screening sensitivity and specificity. ***Key Message:*** Maximising the detection accuracy with a cost-effective approach for non-invasive CRC screening is urgently needed to further reduce the incidence of CRC and associated mortality rates.

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Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death worldwide, with over 1.8 million new cases and causing approximately 900,000 deaths in 2018 [1]. The incidence rate varies among countries, with a rate about 3-times higher in developed versus developing countries, while the mortality rate has less variation [1]. The improvement in cancer treatment and the introduction of CRC screening programs have further reduced mortality arising from CRC in developed countries [2–4]. However, the pathogenesis of CRC follows a stepwise progression from benign adenomas to malignant adenocarcinomas, often over a course of 10 years. It is often asymptomatic in its early stages and remains undiagnosed until late stages, where prognosis becomes unfavourable [2]. If detected early, up to 90% of deaths can be prevented [5]. As a result, a well-planned public health policy with the development of effective and non-invasive biomarkers could overcome the problem.

Colorectal Cancer Screening Program

Implementation of CRC screening programs in communities allows early detection of colonic neoplasm(s) to lower the treatment need, morbidity, and mortality [6]. However, CRC screening programs in different countries differ in their approach [7]. These programs can be broadly divided by structured opportunistic and population-based organised (pilot) screening programs (Table 1) [4]. Population-based organised programs have been introduced into the United Kingdom (UK), Croatia, and Hong Kong, with the governments providing a well-organised systematic process of inviting a specific group of individuals for testing. By contrast, structured opportunistic screening programs are implemented on an ad hoc basis, usually through fee-for-service reimbursement of physicians, such as the United States (US) (Table 2).

The United Kingdom

The UK is a typical example of a population-based organised screening program, where the National Health Service (NHS) has been providing a free-for-charge nationwide Bowel Cancer Screening Program (BCSP) for UK residents since 2006. The BCSP was originally intended for the population between 60 to 69 years of age and recently extended the age range to between age 50 to 74 for their biannual tests. To increase detection accuracy, the screening guidelines have shifted from guaiac faecal occult blood test (gFOBT) to faecal immunochemical test (FIT) since April 2018. Moreover, the NHS also offers a one-off flexible sigmoidoscopy at the age of 55 [8].

Croatia

The early CRC screening program in Croatia was established in 2007 following recommendations by the European Council in 2003. The program provides a non-invasive gFOBT for the population aged 50 to 74 every 2 years. Positive cases of gFOBT may further be referred for a colonoscopy to confirm the finding [9]. However, the participation rate was below 20% for 5 years (2007 to 2011), the lowest rate in the European Union [4].

Hong Kong

The Hong Kong government has a CRC screening program for citizens in the age range of 50 to 74, which is considered an average risk age group. Eligible citizens should receive a FIT every 2 years in this screening program. The guideline from the Department of Health in Hong

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Table 1. Global status of structured and organised colorectal cancer screening by continent in 2018

Continent	Population-based organised	Population-based organised pilot	Structured opportunistic
Europe	1. Belgium 2. Croatia 3. Czech Republic 4. Denmark 5. Estonia 6. France 7. Ireland 8. Italy 9. Lithuania 10. Luxembourg 11. Malta 12. Montenegro 13. The Netherlands 14. Norway 15. Poland 16. Slovenia 17. Spain 18. United Kingdom	1. Austria 2. Cyprus 3. Georgia 4. Hungary 5. Portugal 6. Serbia 7. Sweden 8. Switzerland	1. Austria 2. Germany 3. Greece 4. Latvia
North and Latin America	1. Canada 2. Uruguay	1. Argentina 2. Brazil 3. Chile	1. USA 2. Colombia
Africa	–	–	1. Morocco
Central, West, South Asia	1. Israel 2. UAE	1. Bahrain 2. Kuwait 3. Kazakhstan 4. Lebanon 5. Qatar 6. Saudi Arabia	1. Iran
Far East Asia and Oceania	1. Taiwan 2. Korea 3. Hong Kong 4. Singapore 5. Australia 6. New Zealand	1. PR China 2. Thailand	1. Japan 2. Malaysia

Kong also recommended self-funded invasive screening, such as sigmoidoscopy every 5 years or colonoscopy every 10 years [10]. A similar screening program can also be found in Macau and Taiwan [11, 12].

The United States

In the US, the CRC screening program is largely opportunistic, and the guidelines are relying on both government institutions as well as national independent bodies, such as the US Preventive Services Task Force and the American Cancer Society (ACS). These organisations provide their professional guidelines for the choice of CRC screening tests according to the latest prevention and evidence-based medicine [4]. Currently, CRC screening is indicated for the patients aged 50 to 75, although the 2018 ACS guideline

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Table 2. Colorectal cancer screening programs in the selected countries

	United States	United Kingdom	Hong Kong/ Macau/Taiwan	Croatia
Screening program	Opportunistic	Population-based	Population-based	Population-based
Age	45 to 75 76 to 85: consult	60 to 75	50 to 75	50 to 74
Non-invasive	Annual gFOBT Annual FIT Triennial mt-sDNA	Biennial FIT	Biennial FIT	Biennial gFOBT
Invasive	CT colonography (every 5 years) Flexible sigmoidoscopy (every 5 years) Colonoscopy (every 10 years)	One-off flexible sigmoidoscopy for age 55	Sigmoidoscopy (every 5 years)* Colonoscopy (every 10 years)*	Colonoscopy (when gFOBT positive)

gFOBT, guaiac faecal occult blood test; FIT, faecal immunochemical test; mt-sDNA, multi-target faecal-based DNA screening test.
* Fee-of-charge service.

Table 3. Clinically available non-invasive CRC screening tools

Screening tool	Sample	Detection target	Specificity, % (95% CI) [Ref.]	Sensitivity, % (95% CI) [Ref.]	Advanced adenoma sensitivity, % (95% CI) [Ref.]	Cost, USD [Ref.]
mSEPT9 (Epi proColon)	Serum	*SEPT9* DNA methylation	92 (89–94) [70]	71 (67–75) [70]	11.2 (7.2–15.7) [71]	273–445 [71]
mt-sDNA (Cologuard)	Faeces	*NDRG4* and *BMP3* DNA methylation, *KRAS* mutations and haemoglobin	89.8 (88.9–90.7) [72]	92.3 (83–97.5) [72]	42.4 (38.9–46) [72]	492.72 [73]
FIT	Faeces	Haemoglobin	90* [74]	78* [74]	39* [74]	20–21.65 [73, 75]
gFOBT	Faeces	Haemoglobin	90.0 (84.2–93.8) [76]	62.6 (34.9–83.9) [76]	–	3.31–5 [71, 75]

* No 95% CI reported from the meta-analysis. 95% CI, 95% confidence interval; gFOBT, guaiac faecal occult blood test; FIT, faecal immunochemical test; mt-sDNA, multi-target stool DNA test; mSEPT9, plasma *SEPT9* DNA methylation test.

recommended that screening should begin at the age of 45 and does not recommend CRC screening for anyone over 85. People in the age range between 76 and 85 should consult their medical providers. The ACS guideline also recommended a regular faecal-based non-invasive examination, such as FIT (every year) and mt-sDNA (every 3 years) [13]. gFOBT is no longer recommended due to the high false-positive rate as well as the dietary and pharmaceutical restrictions [14, 15]. The ACS guideline also proposed visual invasive examinations, such as CT colonography (every 5 years), flexible sigmoidoscopy (every 5 years) or colonoscopy (every 10 years) [16]. Although opportunistic screening is effective in reducing CRC-related mortality in the US [17], access to CRC screening is not equal [4]. Residents who are in poverty, uninsured, or underinsured are less likely to undergo regular CRC screening [18, 19].

Approved Colorectal Cancer Screening Tools

Faecal Occult Blood Test and Faecal Immunochemical Test

Currently, the most common and low-cost non-invasive faecal tests for CRC screening are gFOBT and FIT (Table 3). Both gFOBT and FIT enable detection of a tiny amount of blood by targeting haemoglobin [20]. Individuals with a positive gFOBT or FIT result may receive a gold-standard, invasive colonoscopy to confirm the results and/or removal of polyp(s). A meta-analysis of four randomised controlled trials revealed that annual or biennial gFOBT screening caused roughly a 16% reduction in CRC-related mortality with no significant effect on CRC incidence [21]. However, gFOBT is limited by its relatively poor sensitivity in advanced colonic adenoma and also requires repeat screening and dietary restrictions [22]. Thus, it is gradually being replaced by FIT [23, 24]. FIT has a relatively better detection accuracy and can be quantified, providing a tailored screening approach by optimising the cut-off level [25, 26]. A low cut-off reduces the specificity and requires more follow-up with colonoscopy, but increases the sensitivity to identify more individuals with precancerous polyp(s) [23, 27]. Further optimisation for FIT to improve detection accuracy is still ongoing, including the formulation of a FIT buffer for haemoglobin stabilisation [28, 29], the best haemoglobin detection concentration for automated FIT systems, as well as a single-sample (1-FIT) and two-sample (2-FIT) faecal sample protocol per one specimen [30]. Although improvement in FIT detection is still ongoing, a more accurate non-invasive test is urgently needed. At present, the Food and Drug Administration (FDA) in the US approved two other commercially available CRC tests in clinical use, including the multi-target stool DNA (mt-sDNA) test (Cologuard) and plasma *SEPT9* DNA methylation test (Epi proColon) (Table 3).

Multi-Target Stool DNA Test (Cologuard)

Since colonocytes consistently exfoliate and shed into the lumen of the gastrointestinal tract, molecular alterations in faeces, such as DNA methylations, have been widely investigated [31]. The mt-sDNA screening test (also called Cologuard) is an FDA-approved non-invasive CRC screening tool in 2014, developed by EXACT Sciences Corporation (NASDAQ: EXAS) and Mayo Clinic [32]. The test is Clinical Laboratory Improvement Amendments (CLIA) certified and accredited by the College of American Pathologists [33]. It is designed to detect faeces-based DNA biomarkers with occult haemoglobin. The initial development utilised a pre-commercial 23-marker assay, with subsequent findings that there were 3 broadly informative markers for colorectal neoplasia [34]. Based on the findings, the preliminary version of mt-sDNA utilised *NDRG4*, *BMP3*, *VIM*, and *TFP12* genes as DNA methylation targets, with mutant *KRAS* and faecal haemoglobin. At the threshold of 90% specificity from the 293 healthy controls, the sensitivities for CRC (n = 252) and adenomas ≥1 cm (n = 133) were 85 and 54%, respectively [35]. The size of the tumour correlated to the detection sensitivity, increasing from 54% in 1 cm to 92% in ≥4 cm adenomas [35]. Afterwards, the commercially available, FDA-approved mt-sDNA version 2.0 by the "DeeP-C" study utilised *NDRG4* and *BMP3* DNA as methylation markers with *KRAS* mutations plus FIT. The "DeeP-C" prospective study recruited almost ten thousand participants in an average-risk community asymptomatic of CRC [36]. The sensitivity for CRC and pre-cancerous lesions was 92.3 and 42.4%, respectively, and presented a higher sensitivity compared to only one FIT kit at one cut-off (CRC: 73.8%, p = 0.002; pre-cancerous lesions: 23.8%, p < 0.001) [36].

Following the approval, further clinical trials were continued. In the Alaska native cohort (n = 661) [37], the test presented a sensitivity of 49% for advanced colorectal neoplasms (n = 92) versus 28% for FIT (p < 0.001). The specificity of mt-sDNA was 93%, which is 3% lower than FIT (p = 0.034) in the subjects in whom no adenomas were detected [37]. Later,

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the mt-sDNA test was applied in frozen samples (n = 1,047) from the Netherlands prospective COCOS study for further FIT comparison in advanced colorectal neoplasia (n = 102). The mt-sDNA had a sensitivity of 49% and specificity of 89%, showing better accuracy than FIT, which had a sensitivity of 25% and specificity of 96% [38, 39]. An additional clinical trial at the Netherlands cancer institute is still ongoing [40]. It should be taken into account that a positive test result with no findings on colonoscopy may be due to other causes as *NDRG4* and *BMP3* methylation can be found from other gastrointestinal diseases such as gastric and pancreatic cancers, although it is rare [41–43].

Plasma SEPT9 DNA Methylation Test (Epi proColon)

In addition to faeces, DNA methylation can also be determined from blood. The *SEPT9* methylation detection in plasma has been evaluated in multiple studies. Epigenomics AG (ECX: FRA) in Germany first implemented the *SEPT9* methylation biomarker in Europe in 2008 [44]. Two years later, the commercialised Epi proColon qPCR kit version 1.0 was launched in Europe and later upgraded to version 2.0 [45]. The Chinese Food and Drug Administration and the US FDA approved the Epi proColon kit in 2015 and 2016, respectively. In the prospective "PRESEPT" study, the methylated *SEPT9* assay demonstrated a sensitivity of 48% for CRC (from stages I–IV, 35, 63, 46, and 77%, respectively) with a specificity of 92%; however, merely 11% of advanced adenomas were identified [46]. The commercially available kit provides 2 different algorithms, the 2/3 algorithm test has a relatively high true negative rate, while the sensitivity is higher in the 1/3 algorithm [47]. A meta-analysis study published in 2017 including 25 research articles found that the *SEPT9* assay is only superior to the FIT in the symptomatic population [48]. Due to its relatively poor sensitivity, the US Preventive Services Task Force and the ACS currently do not include the Epi proColon test in their CRC screening guidelines [49].

Screening Tools under Development

One of the biggest challenges in early cancer diagnosis and/or prognosis is the lack of reliable biomarkers, leading to several screening tools, such as PreGen-Plus™ (*KRAS*, *APC*, and *p53* mutations) [50], ColoSure™ (*VIM* methylation), and COLVERA™ (*BCAT1* and *IKZF1* methylation) [51], being withdrawn from the market [50, 52]. Therefore, developing a low-invasive biomarker that can be easily performed with a clear clinical outcome is necessary. Apart from DNA methylation tests in both faeces and blood samples, other molecular biomarkers are being developed for CRC screening, such as circulating tumour DNA (ctDNA) [53], tumour-derived circulating cell (CTC) [54], circular RNA (circRNA) [55], PIWI-interacting RNA (piRNA) [56], microRNA (miRNA) [57–60], and gut microbes (Table 4). Studies relating to miRNA and gut bacteria will further be discussed.

microRNAs Detection in Blood and Faeces

miRNAs are a class of conserved endogenous, non-coding RNAs with approximately 18–24 nucleotides and play an important role in post-transcriptional regulation of protein-coding gene expression(s) through binding primarily to the 3′-untranslated region of the target mRNA(s), resulting in mRNA degradation and/or translational repression [61]. Thus, aberrant miRNA expression leads to disease progression and thus can be useful as diagnostic and/or prognostic predictors to human diseases. Until now, numerous research articles have reported that both blood- and faecal-based miRNAs can be utilised as biomarkers for CRC screening. Among them, miR-21 and miR-92a are the highly reported miRNAs for CRC screening [62].

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Table 4. Selected developing molecular biomarkers for colorectal cancer screening

Types	Sample	Molecular biomarker(s)	AUC (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Ref.
DNA methylation	Plasma	*APC*, *MGMT*, *RASSF2A*, *WIF1*	0.927	86.5 (81.7–90.8)	92.1 (88.2-95.0)	[81]
	Plasma	*BCAT1*, *IKZF1*	NA	66	94	[82]
	Serum	*SDC2*	NA	87	95.2	[83]
	Plasma cfDNA	*LINE-1*	0.81	65.8	90	[84]
	Faeces	*NDRG4*	T: 0.77	T: 61 (43–79)	T: 93 (90–97)	[85]
			V: NA	V: 53 (39–67)	V: 100 (86–100)	
	Faeces	*TFPI2*	NA	76 (60–88)	93 (77–99)	[86]
Circulating tumour DNA	Serum cfDNA	ALU115	0.8458	69.23	99.09	[87]
		ALU247/115	0.8551	73.08	97.27	
Circular RNA (circRNA)	Plasma	91H, *PVT1*, *MEG3*	0.877	82.76	78.57	[88]
	Serum	LOC285194, RP11-462C24.1, Nbla12061	0.79 (0.71–0.86)	68.33	86.89	[89]
	Whole-blood	NEAT1_v1	0.73 (0.60–0.83)	56.7	83.3	[90]
	Whole-blood	NEAT1_v2	0.85 (0.73–0.93)	86.6	83.3	[90]
PIWI-interacting RNA (piRNA)	Serum	piR-5937	T: 0.8060	71.8	72.5	[56]
			V: 0.7673	73.6	65.3	
		piR-28876	T: 0.8065	75.3	70.0	[56]
			V: 0.7074	66.0	65.3	
microRNA (miRNA)	Plasma	miR-92a	0.885	89	70	[91]
	Serum	miR-210	0.82	74.6	73.5	[92]
	Plasma	miR-24	8.84 (0.79–0.89)	78.4	83.9	[93]
	Faeces	miR-221	0.73 (0.68–0.78)	62 (55–68)	74 (67–80)	[60]
	Faeces	miR-20a	0.73 (0.68–0.78)	55 (47–62)	82 (76–87)	[57]
	Faeces	miR-135b	0.79	78 (69–85)	68 (58–77)	[59]
	Faeces	miR-92a, miR-21, miR-135b, miR-145, miR-133a	0.849	81	80	[94]
	Saliva	miR-21	NA	97	91	[95]
Exosomal microRNA	Plasma	miR-27a	0.87 (0.77–0.96)	81.82	90.91	[96]
	Plasma	miR-130a	0.82(0.73–0.90)	69.32	100	
Tumour-derived circulating cell	Whole-blood	Circulating endothelial cell clusters	0.92 (0.84–1.00)	NA	NA	[54]
Gut microbes	Faeces	*F. nucleatum*, *Parvimonas micra*	0.84	NA	NA	[76]
	Faeces	*F. nucleatum*, *Clostridium hathewayi*, *Lachnoclostridium* sp., *Bacteroides clarus*, and FIT	NA	93.8	81.2	[80]

95% CI, 95% confidence interval; NA, not available; T, training; V, validation; cfDNA, cell-free DNA.

In a meta-analysis of the blood-based miRNA study, the overall sensitivity and specificity of blood-based miRNAs for CRC is 76% (95% CI, 72–80%) and 76% (95% CI, 72–80%), respectively [63]. The most predictive miRNA was miR-92a, ranging from a sensitivity of 65.5% to 89%, and from a specificity of 70% to 82.5%, with the area under the receiver-operating characteristics (AUC) between 0.786 and 0.890 [63]. However, the major shortcoming of using blood-based miRNA for CRC screening is the detection specificity. This is because miRNAs might arise from other cancer(s) [64, 65], depression [66], and virus infection(s) [67, 68]. Therefore, faecal-based miRNA detection may be an alternative option [57, 59, 60, 69]. It has been demonstrated that miRNAs are highly stable short sequences which remain detectable within samples throughout a 72-hour incubation period due to protection from ribonuclease degradation by exosomes [70, 71]. A meta-analysis showed that miR-21 is the most reliable miRNA [72]. However, as faeces contain abundant amounts of proteins and DNA from gut microbes, the purity of RNA samples from faeces may determine the result outcomes. As a result, faecal-miRNA detection in combination with FIT is a reliable approach to enhance the detection accuracy. Previous studies indicated that the combination of miR-21 and miR-92a with FIT had a specificity of 96.8% and sensitivity of 78.4%, while FIT alone only had a specificity of 98.4% and sensitivity of 66.7% [73].

Faecal-Based Microbe Detection

The gut flora habitat has a vast amount of microbes and plays an important role in maintaining our health. Changes in microbiome composition have been linked to multiple diseases including cancer. The study of biomarkers from the gut microbiome has a particular focus on CRC, where clinical use is already on the horizon [74]. It is known that dysbacteriosis alters metabolic activities and induces inflammatory stimuli to the gastrointestinal tract, and eventually induces mutations to colonic cells, thus contributing to the development of the CRC. Among multiple microbial taxonomic markers, intensive research showed that *Fusobacterium nucleatum* is enriched in tumour neoplasms as well as in faeces from CRC patients [75, 76]. *F. nucleatum* belongs to the class of asaccharolytic bacteria. Enrichment of *F. nucleatum* in the gut does not only recruit tumour-infiltrating immune cells and induces a pro-inflammatory microenvironment, but also contributes to CRC tumorigenesis through its strong adhesive abilities and invasive effects on epithelial cells [77]. Furthermore, *F. nucleatum* survives and divides in the hypoxic tumour microenvironment, contributing to the cell proliferation and angiogenesis. A meta-analysis indicated that the use of *F. nucleatum* as a biomarker for CRC screening has a sensitivity of 71% (95% CI, 61–79%) and a specificity of 76% (95% CI, 66–84%), with the AUC of 0.80 (95% CI, 0.76–0.83); the sensitivity and specificity for advanced colorectal neoplasia is 36% (95% CI, 27–46%) and 73% (95% CI, 65–79%), respectively, with the AUC of 0.60 (95% CI, 0.56–0.65) [78]. The use of *F. nucleatum* together with FIT may improve the detection accuracy for advanced colorectal neoplasia [79]. A most recent report indicated that the combination of *F. nucleatum*, *Clostridium hathewayi*, *Lachnoclostridium* sp., *Bacteroides clarus*, and FIT presented a high detection accuracy, with a specificity of 81.2% and sensitivity of 93.8% [80].

Conclusion

CRC is the third most aggressive cancer worldwide with a high mortality rate due to the lack of robust biomarkers. Current CRC screening programs are mostly only available to those above age 50 or 55, despite the latest guidelines recommending that screening should begin from the age of 45. Although colonoscopy is the gold standard for CRC, it being labour-intensive and invasive means that it cannot be applied for everyone. Thus, there is a great

need for a cost-effective and non-invasive CRC screening test to improve the screening accuracy and acceptability. The use of circulating and/or faecal-based miRNAs, as well as gut bacteria, could be the next generation CRC screening biomarkers.

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The authors have no conflicts of interest to declare.

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Author Contributions

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