Biomarkers for detecting colorectal cancer non-invasively: DNA, RNA or proteins?

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Abstract

Colorectal cancer (CRC) is a global problem affecting millions of people worldwide. This disease is unique because of its slow progress that makes it preventable and often curable. CRC symptoms usually emerge only at advanced stages of the disease, consequently its early detection can be achieved only through active population screening, which markedly reduces mortality due to this cancer. CRC screening tests that employ non-invasively detectable biomarkers are currently being actively developed and, in most cases, samples of either stool or blood are used. However, alternative biological substances that can be collected non-invasively (colorectal mucus, urine, saliva, exhaled air) have now emerged as new sources of diagnostic biomarkers. The main categories of currently explored CRC biomarkers are: (1) Proteins (comprising widely used haemoglobin); (2) DNA (including mutations and methylation markers); (3) RNA (in particular microRNAs); (4) Low molecular weight metabolites (comprising volatile organic compounds) detectable by metabolomic techniques; and (5) Shifts in gut microbiome composition. Numerous tests for early CRC detection employing such non-invasive biomarkers have been proposed and clinically studied. While some of these studies generated promising early results, very few of the proposed tests have been transformed into clinically validated diagnostic/screening techniques. Such DNA-based tests as Food and Drug Administration-approved multitarget stool test (marketed as Cologuard®) or blood test for methylated septin 9 (marketed as Epi proColon® 2.0 CE) show good diagnostic performance but remain too expensive and technically complex to become effective CRC screening tools. It can be concluded that, despite its deficiencies, the protein (haemoglobin) detection-based faecal immunochemical test (FIT) today presents the most cost-effective option for non-invasive CRC screening. The combination of non-invasive FIT and confirmatory invasive colonoscopy is the current strategy of choice for CRC screening. However, continuing intense research in the area promises the emergence of new superior non-invasive CRC screening tests that will allow the development of improved disease prevention strategies.
INTRODUCTION

Colorectal cancer (CRC) is currently the third most frequently diagnosed cancer worldwide. The global incidence for 2018 is estimated at 180100 new cases, and the number of CRC-related deaths for this period is 861700[1]. Although the highest CRC incidence continues to be observed in economically developed Western countries, it is now rapidly increasing in other parts of the world[2]. Sporadic CRC development can take decades and is in most cases characterised by a slow progression from aberrant crypt formation in the colonic mucosa to benign polyps that may give rise to early cancer, then gradually evolving to invasive and metastasising advanced neoplasms (Figure 1)[2-4]. These pathogenetic features make CRC one of the most preventable and often curable malignancies. However, disease curability entirely depends on its early detection, which is not straightforward as clinical symptoms usually emerge only when CRC is already advanced. The latter factor warrants the necessity of active population screening for CRC, and it has been well proven that screening saves lives[2].

Full colonoscopy is regarded as the gold standard diagnostic technique for colorectal tumour detection[5], and it has become a very popular method for primary CRC screening[6-8] in the United States. One apparent reason for this trend is that diagnostic colonoscopy is usually combined with the simultaneous removal of detected polyps and functions as both a diagnostic and preventive procedure clearly reducing mortality from CRC[9]. Nonetheless, colonoscopy is an expensive and invasive technique that requires unpleasant bowel preparation and occasionally causes serious complications[10]. Moreover, its sensitivity is not perfect, with polyps sometimes missed[11], the latter problem often depending on the operator’s skills[12]. Although colonoscopy as the final (confirmatory) diagnostic step is undisputable, its use in primary CRC screening remains questionable as indiscriminate application of this method inevitably results in frequent negative outcomes and a large health economic burden[13].

In theory, the global introduction of non-invasive tests employing biomarker analysis to select patients that really require endoscopy could dramatically reduce the numbers of unnecessary colonoscopies. Unfortunately, none of the existing non-invasive tests successfully combine high diagnostic sensitivity and specificity with technical simplicity and low cost, the key characteristics of an ideal screening modality. This paper provides a brief overview of the current state of the area encompassing biomarker-based non-invasive tests for CRC detection.

SOURCES OF MATERIAL FOR NON-INVASIVE CRC BIOMARKER TESTING

CRC development is an extraordinarily complex process driven by multiple genetic,
Figure 1 Colorectal cancer pathogenesis and sources of potential diagnostic biomarkers at different stages of colorectal cancer development. CRC: Colorectal cancer.

epigenetic, metabolic and immune alterations at the host level and influenced by numerous environmental factors\(^4,14,15\). Despite intense research, precise mechanisms of CRC development remain largely obscure\(^4,14,15\). Genome-targeting investigations, especially genome-wide association studies, have revealed a highly complex pathogenetic landscape comprising multiple alternative cascades of molecular events that may eventually result in cancer\(^4,16\). This complexity leads some investigators to a hardly satisfactory conclusion that “each patient’s CRC is genetically and epigenetically unique”\(^4\). Nevertheless, colorectal tumours frequently have common molecular patterns that are diagnostically relevant and will be considered below.

The series of morphological events accompanying CRC development is presented in Figure 1. This sequence involves numerous associations with various types of biomolecules that can be characterised as biomarkers. The ideal biomarkers for CRC can be defined as substances that satisfy the following criteria: (1) Are measured easily and inexpensively to identify a patient’s cancer; (2) Identify a patient’s prognosis to improve treatment outcome; and (3) Predict a patient’s response to a specific treatment\(^15\). This paper is focused only on the first category, i.e., diagnostic biomarkers of CRC that can be sampled and tested non-invasively.

Figure 1 outlines the main sources of CRC biomarkers in relation to disease stages. From the morphological point of view, it is obvious that (1) colon tissue; (2) gut lumen; (3) blood/lymph circulation are the main sources of CRC-associated DNA, RNA and protein/polypeptide biomarkers associated with the host; (4) moreover, specific pattern shifts in small metabolite molecules derived from CRC-affected metabolic pathways constitute an additional group of post-metabolic markers that can
be analysed by metabolomics techniques[17,18], and (5) CRC-associated gut microbiome changes[19] deserve to be considered as a separate category of diagnostic markers of non-human origin.

**Normal and neoplastic colon tissue**
Colonic epithelium is the site of neoplastic growth initiation. After that CRC progresses within the colonic wall until advanced stages of the disease, hence pre-malignant and malignant colon tissues are certainly the richest biomarker sources[4]. However, invasive biopsies are required for sampling tissue. Therefore, CRC markers detectable in tissue samples are not discussed here.

**Gut lumen**
Colonic epithelium is the key element of the gastrointestinal barrier between host tissues and microbiota-rich colon contents. Until recently it was presumed that all host cells exfoliated or migrated from the surface of the colonic epithelium were immediately incorporated in the faecal matter. According to this simplistic notion, it seemed to be logical that analysing naturally excreted stool samples constitutes the only perfectly non-invasive approach to investigating CRC biomarkers. It should, however, be stressed that stool is a complex mixture of microbiota-dominated faecal matter and occasional fragments of colorectal mucus secreted by goblet cells of the colonic epithelium. While the prevailing faecal component of stool entirely belongs to the environment, colorectal mucus is host-derived. The two-layered structure and functional significance of the mucus overlaying colonic epithelium have been elucidated only during the last decade[20,21], and it is now clear that colorectal mucus rather than faecal matter is the main receptacle of all cells and biomolecules released from either normal or malignant epithelium[22,23]. Intrarectal collection of colorectal mucus had demonstrated high informativeness of this substance[22,23], which was shown to accept CRC-generated malignant colonocytes exfoliated from tumour surface and transport them distally alongside stool flow without incorporating them into faeces (Figure 1)[20,23]. Biomarker-rich colorectal mucus essentially serves as a border between well oxygenated colonic epithelium and anaerobic gut lumen. Our group has recently developed a simple technique for non-invasive sampling of this mucus[24-26], the analysis of which may constitute a very convenient alternative to stool-based tests.

**Blood/lymph circulation**
Blood-derived biomarker analysis is another area of significant interest in the context of CRC detection since blood collection is regarded as a practically non-invasive procedure. It is evident that a wide range of CRC-associated biomarkers can be detected in the circulating blood and lymph of patients with these malignancies, but lymph collection cannot be performed with minimal invasiveness. For this reason, only biomarkers measurable in blood will be discussed below. In the modern literature the term “liquid biopsy” is often applied to this group of biomarker-based techniques[27]. Nevertheless, despite the easiness of blood sampling and the availability of numerous analytical techniques for biomarker detection in human plasma or serum, the presence of cancer biomarkers in blood may or may not be associated with CRC. Malignancies of other sites should always be excluded if this approach is considered for CRC screening.

**Post-metabolic biomarkers**
The use of metabolomics for revealing CRC-specific changes in patterns of low molecular weight metabolites has recently become another area of active exploration[28]. This new approach can potentially employ a wider range of biological samples comprising blood, stool, colorectal mucus, urine, saliva and exhaled breath, thus bringing about additional diagnostic options.

**Gut microbiome changes associated with CRC**
Recent research has revealed that specific changes in gut microbiome composition may be associated with the development of CRC[19]. In this context stool samples are usually investigated quantitatively for the presence of particular types of bacteria. The limited choice of sample sources for non-invasive testing creates obvious problems. Collecting gut-derived samples looks preferable, but stool samples, albeit containing cells and molecules originating from the colonic mucosa (i.e., colorectal mucus fragments), are usually dominated by the presence of abundant microbiota-rich faecal matter that often interferes with analytical procedures employed for host-related biomarker detection. A recently described analysis of non-invasively collected colorectal mucus presents a very interesting alternative; however, this approach is new and requires further testing. On the other hand, blood collection is very
straightforward and easy to standardise, but molecular changes detectable in blood (or plasma/serum) samples are not necessarily gut-specific. Finally, although the use of easily collectable materials (urine, saliva or exhaled air) is extremely attractive, the presence of CRC-specific biomarkers in such samples remains to be adequately explored. The sources of biological material characterised above may contain several types of diagnostic biomarkers that are discussed in the next section.

**BIOMARKERS ASSOCIATED WITH CRC DEVELOPMENT**

The story of non-invasively detectable CRC markers started due to a 1967 publication by Greegor, describing his observation of the frequent presence of occult blood in stool samples collected from patients with CRC\[29\]. That important discovery resulted in the development and prolonged use of the haemoglobin-recognising faecal occult blood test (FOBT) as the only non-invasive test for CRC detection. The situation had changed considerably in 1992, when a publication by Sidransky et al\[30\] described K-ras gene mutation detection in stool samples obtained from CRC patients and shifted the focus of attention to molecular markers. The area of CRC biomarker research has since exponentially expanded with thousands of papers published, but many initially promising findings failed to transform into clinically relevant diagnostic approaches. The purpose of this paper is to briefly outline the present status of non-invasive biomarkers proposed for detecting asymptomatic CRC. Only the most impressive and clinically relevant observations related to the main groups of these biomarkers (proteins/polypeptides, DNA, RNA, small metabolites, microbiome changes) are highlighted in the text below. However, numerous markers that demonstrated promise in the context of CRC detection are presented in comprehensive Tables 1, 2, 3, 4 and 5. As it was impossible to cover all relevant studies, restrictions had to be applied when the Tables were prepared. Publications describing very small studies or reporting negative results were omitted. Likewise, only papers related to CRC, but not adenoma detection, were included since in most cases diagnostic sensitivity of biomarker tests for adenomatous polyps correlates with that for CRC. In addition, the necessity of non-invasive detection of colorectal polyps is still a debatable question, as the proportion of adenomas likely to progress to malignancy is relatively small, whereas the vast majority of these lesions (especially small polyps) never give rise to CRC\[32,33\].

**Protein markers**

Protein biomarkers considered in CRC early detection and screening are listed in Table 1. Historically, the use of haemoglobin detection in stool for non-invasive CRC detection can be regarded as the most popular approach in terms of population screening. Indeed, the traditional guaiac FOBT was almost exclusively employed for this purpose for several decades, and was attractive due to its simplicity and low cost. Although this test has insufficient sensitivity, it can be credited for saving many human lives\[34,35,137\]. Nevertheless, the outdated FOBT is now being replaced by the faecal immunochemical test (FIT) characterised by a much higher sensitivity. In a recent comprehensive review on FIT, Gies et al\[35\] discussed numerous studies of varying sizes and reported sensitivities between 66% and 74% and specificity levels between 84% and 95% when numbers of analysed CRC cases and controls were over 50. Table 1 also shows that M2-pyruvate kinase (M2-PK) is a relatively well-studied stool marker of CRC\[32,33\]; however, FIT performs better and remains considerably more popular. Other stool tests, including metalloproteinase 9 (MMP9)\[34\] and multimarker protein panels (see Table 1) have been investigated, but these tests have not been clinically accepted so far. It is also intriguing that in a recent small study, our group compared 24 protein biomarkers in non-invasively collected samples of colorectal mucus and concluded that haemoglobin, tissue inhibitor of metalloproteinase 1, M2-PK, peptidyl arginine deiminase 1, C-reactive protein and MMP9 could reliably detect CRC\[32,33\].

Blood (or plasma/serum) testing for CRC-associated proteins has been employed by many research groups (Table 1), but most of those studies produced relatively modest results. Among single protein markers detectable in the serum only CA19-9 marker protein\[36\], cysteine-rich 61 protein of the CCN family (Cyr 61)\[37\], B6-integrin\[38\] and trefoil factor 3 (TFF3)\[39\] can be regarded as promising. A number of protein panels were also examined; however, analysing multiple proteins is usually more technically complex and expensive. Impressive test sensitivity and specificity values (98.7% and 94.8%, respectively) were reported for combined testing for lectins DC-SIGN and DC-SIGNR by Jiang et al\[42\] in 2014, but these results remain to be confirmed in larger studies. Although blood collection is simple and easy to standardise, protein
Table 1  Non-invasive protein (including cytokine) biomarkers used for colorectal cancer detection

| Study setting              | Sample type | Marker type | Biomarker(s) | Sensitivity (or its range) | Specificity (or its range) | Ref. |
|----------------------------|-------------|-------------|--------------|---------------------------|----------------------------|------|
| Screening (reviewed)       | Stool       | Protein     | Haemoglobin (FIT) | 66.0%-74.0% | 84.0%-95.0% | [31] |
| Case-control (reviewed)    | Stool       | Protein     | M2-PK        | 68.0%-93.0% | 70.0%-97.5% | [32,33] |
| Case-control               | Stool       | Protein     | MMP 9        | 89.30%       | 91.20%       | [34] |
| Case-control               | Stool       | Protein panel | Complement C3, Lactotransferrin, Haemoglobin subunit α1 and Haptoglobin | 71.00% | 95.00% | [35] |
| Case-control               | Serum       | Protein     | CA11-19      | 98.00%       | 84.00%       | [36] |
| Case-control               | Serum       | Protein     | MIC-1 (GDF15) | 43.80%       | 96.70%       | [36] |
| Case-control               | Serum       | Protein     | IL-6         | 70.00%       | 91.00%       | [36] |
| Case-control               | Serum       | Protein     | IL-8         | 95.30%       | 93.50%       | [36] |
| Case-control               | Serum       | Protein     | Cyr61        | 83.00%       | 97.00%       | [37] |
| Case-control               | Serum       | Protein     | B6-integrin  | 69.80%       | 100.00%      | [39] |
| Case-control               | Serum       | Protein     | TIMP-1       | 52.0%-85.0% | 60.0%-95.0% | [40] |
| Case-control               | Serum       | Protein     | RBP4         | 74.90%       | 81.70%       | [40] |
| Case-control               | Serum       | Protein     | THBS2        | 64.90%       | 87.10%       | [40] |
| Case-control               | Serum       | Protein     | TFF3         | 74.20%       | 94.80%       | [40] |
| Case-control               | Serum       | Protein     | COL3A1       | 98.80%       | 69.10%       | [40] |
| Case-control               | Serum       | Protein     | COL10A1      | 63.00%       | 85.00%       | [40] |
| Case-control               | Serum       | Protein     | AZGP1        | 55.80%       | 85.00%       | [40] |
| Case-control               | Serum       | Protein     | Angiopoietin-2 | 79.30%   | 82.40%       | [40] |
| Case-control               | Serum       | Protein     | Kininogen    | 63.60%       | 65.90%       | [40] |
| Case-control               | Plasma      | Protein     | Melanotransferrin | 48.20%   | 92.50%       | [40] |
| Case-control               | Serum       | Protein panel | RBP4 and CEA | 80.80%       | 91.20%       | [40] |
| Case-control               | Serum       | Protein panel | TFF3 and CEA | 89.40%       | 87.80%       | [40] |
| Case-control               | Serum       | Protein panel | sDC-SIGN and sDC-SIGNR | 98.70%   | 94.80%       | [40] |
| Case-control               | Serum       | Protein panel | IGFBP-3 and CEA | 75.00%   | 90.00%       | [40] |
| Case-control               | Serum       | Protein panel | AZGP1, CEA and CA19-9 | 67.50%   | 82.50%       | [40] |
| Case-control               | Serum       | Protein panel | IGFBP2, DKK3 and PKM2 | 73.00%   | 95.00%       | [40] |
| Case-control               | Plasma      | Protein panel | BAG4, IL6ST, VWF, EGFR and CD44 | 73.00%   | 90.00%       | [40] |
| Case-control, prospective  | Serum       | Protein panel | CEA, hs-CRP, CYFra21-1 and Ferritin | 60.0%-70.0% | 81.0%-89.0% | [40] |

FIT: Faecal immunochemical test.

Biomarkers of CRC present in stool or colorectal mucus currently look more diagnostically reliable than those detectable in blood.

An additional advantage of using protein biomarkers for CRC detection is defined by the fact that their immunochemical detection can be easily presented as point of care (POC) tests, which are already available for FIT[139].

**DNA and mRNA markers**

This sub-section briefly discusses studies on CRC detection using DNA and mRNA markers that are listed in Table 2.

Gene mutations, especially those of K-Ras and APC genes, were the first CRC-associated genetic markers assessed with the purpose of developing new non-invasive modalities for CRC early detection and screening. Regrettably, it soon became clear
| Study setting | Sample type | Marker type | Biomarker(s) | Sensitivity (or its range) | Specificity (or its range) | Ref. |
|---------------|-------------|-------------|--------------|---------------------------|---------------------------|------|
| Screening     | Stool       | DNA mutation panel | 3 K-ras mutations, 10 APC mutations, 8 p53 mutations, microsatellite instability marker BAT-26 and long DNA marker | 51.60% | 94.40% | [49] |
| Case-control  | Stool       | Panel including DNA mutation, DNA methylation, DNA amount and protein testing | K-ras mutation, methylation of Vimentin (VIM), BMP3, NDRG4 and TPPI2 genes, DNA measurement by \( \beta \)-actin assessment and HemoQuant test for haemoglobin | 78.0%-85.0% | 85.0%-90.0% | [47] |
| Screening     | Stool       | Panel including DNA mutation, DNA methylation, DNA amount and protein testing | K-ras mutation, BMP3 and NDRG4 promoter methylation, DNA measurement by \( \beta \)-actin assessment and test for haemoglobin (FIT) | 92.30% | 86.60% | [48] |
| Case-control  | Stool       | Methylated DNA | BMP3 gene | 51.0%-84.0% | 90.0%-100.0% | [49] |
| Case-control  | Stool       | Methylated DNA | CDKN2A gene | 20.0%-40.0% | 84.0%-100.0% | [49] |
| Case-control  | Stool       | Methylated DNA | ECAD gene | 65.20% | 88.00% | [49] |
| Case-control  | Stool       | Methylated DNA | FBN1 gene | 72.00% | 93.30% | [49] |
| Case-control  | Stool       | Methylated DNA | GATA4/5 gene promoter | 42.9%-71.0% | 84.0%-95.0% | [49,50] |
| Case-control  | Stool       | Methylated DNA | HLF gene | 20.0%-37.5% | 90.0%-92.6% | [49] |
| Case-control  | Stool       | Methylated DNA | HIC1 gene | 42.30% | 98.00% | [49] |
| Case-control  | Stool       | Methylated DNA | HPP1 gene | 71.20% | 57.10% | [49] |
| Case-control  | Stool       | Methylated DNA | ING1B gene | 73.70% | 95.00% | [49] |
| Case-control  | Stool       | Methylated DNA | MIT4A4 gene | 40.00% | 96.80% | [49] |
| Case-control  | Stool       | Methylated DNA | MGMT gene | 33.9-55.1% | 52.0%-100.0% | [49] |
| Case-control  | Stool       | Methylated DNA | NDRG4 gene promoter | 53.0%-92.0% | 89.1%-100.0% | [49-51] |
| Case-control  | Stool       | Methylated DNA | P16INK4A gene | 71.70% | 86.00% | [49] |
| Case-control  | Stool       | Methylated DNA | PHACTR3 gene | 55.0%-66.0% | 95.0%-100.0% | [49] |
| Case-control  | Stool       | Methylated DNA | RASSF2 gene | 45.30% | 94.70% | [51] |
| Case-control  | Stool       | Methylated DNA | SEPT1 gene | 81.10% | 93.30% | [51] |
| Case-control  | Stool       | Methylated DNA | SFRP1 gene | 20.0%-84.8% | 80.0%-94.5% | [49] |
| Case-control  | Stool       | Methylated DNA | Vimentin (VIM) gene | 26.4%-89.0% | 86.0%-95.5% | [49] |
| Case-control  | Stool       | Methylated DNA | SFRP2 gene | 32.1%-94.2% | 54.0%-100.0% | [49,51] |
| Case-control  | Stool       | Methylated DNA | SPG20 gene | 80.2%-89.0% | 99.0%-100.0% | [49,51] |
| Case-control  | Stool       | Methylated DNA | SNCA gene | 83.90% | 75.00% | [49] |
| Case-control  | Stool       | Methylated DNA | TPPI2 gene | 63.3%-92.0% | 79.0%-100.0% | [49,51] |
| Case-control  | Stool       | Methylated DNA | TP53 gene | 56.30% | 100.00% | [49] |
| Case-control  | Stool       | Methylated DNA | Vimentin (VIM) gene | 32.6%-86.0% | 82.0%-100.0% | [49,51] |
| Case-control  | Stool       | Methylated DNA | WAF1 gene | 19.3%-60.4% | 96.7%-99.4% | [49] |
| Case-control  | Stool       | Methylated DNA | XAF1 gene | 55.90% | 52.00% | [49] |
| Case-control  | Stool       | Methylated DNA | BMP3 and NDRG4 genes | 98.00% | 90.00% | [49] |
| Case-control  | Stool       | Methylated DNA | MGMT and XAF1 genes | 73.50% | 52.00% | [49] |
| Case-control  | Stool       | Methylated DNA | MGMT-B and SFRP2 genes | 88.30% | 91.20% | [49] |
| Case-control  | Stool       | Methylated DNA | RASSF1A and SFRP2 genes | 75.00% | 89.40% | [49] |
| Study Type | Tissue/medium | Assay | Genes | Sensitivity | Specificity |
|------------|---------------|-------|-------|-------------|-------------|
| Case-control | Stool | Methylated DNA panel | SNCA and FNB1 genes | 84.30% | 93.30% |
| Case-control | Stool | Methylated DNA panel | Vimentin (VIM) and SFRP2 genes | 92.50% | 91.20% |
| Case-control | Stool | Methylated DNA panel | ACTR3, VNT2 and SLIT2 genes | 74.0% - 78.0% | 88.0% - 89.0% |
| Case-control | Stool | Methylated DNA panel | ECAD, MGMT and P16INK4A genes | 72.00% | 88.00% |
| Case-control | Stool | Methylated DNA panel | ITGA4, SFRP2 and P16INK4A genes | 70.00% | 96.80% |
| Case-control | Stool | Methylated DNA panel | MGMT, CDKN2A and hMTH1 genes | 55.00% | 63.00% |
| Case-control | Stool | Methylated DNA panel | MGMT, MLH1 and Vimentin (VIM) genes | 75.00% | 86.50% |
| Case-control | Stool | Methylated DNA panel | SFRP2, HPPI and MGMT genes | 93.70% | 77.10% |
| Case-control | Stool | Methylated DNA panel | WIF-1, ALX-4 and Vimentin (VIM) genes | 25.00% | 98.00% |
| Case-control | Stool | Methylated DNA panel | Vimentin (VIM), OMSR and TFFP2 genes | 86.70% | 87.60% |
| Case-control | Stool | Methylated DNA panel | SFRP2, GATA4/5, NRDG4 and Vimentin (VIM) genes | 96.40% | 65.00% |
| Case-control | Stool | Human DNA content | Total human DNA content | 66.00% | 89.80% |
| Case-control | Bowel Lavage Fluid | Methylated DNA panel | miR-124-3, LOC386758 and SFRP1 genes | 82.00% | 79.00% |
| Case-control | Intraectally collected colorectal mucus | Human DNA content | Total human DNA content | 60.40% | 94.80% |
| Case-control | Serum/plasma | Methylated DNA | ALX4 gene | 23.0% - 90.7% | 72.5% - 100.0% |
| Case-control | Serum/plasma | Methylated DNA | APC gene | 57.0% - 86.5% | 86.0% - 92.1% |
| Case-control | Plasma | Methylated DNA | CDH1 (E-cadherin) gene | 60.00% | 84.00% |
| Case-control | Serum/plasma | Methylated DNA | SDC2 gene | 87.0% - 90.7% | 72.5% - 95.2% |
| Case-control | Serum/plasma | Methylated DNA | SEPT9 gene | 47.1% - 95.6% | 81.0% - 96.7% |
| Case-control | Serum/plasma | Methylated DNA | SFRP2 gene | 54.0% - 69.4% | 40.0% - 98.7% |
| Case-control | Plasma | Methylated DNA | THBD (Thrombomodulin) gene | 70.70% | 80.30% |
| Case-control | Serum/plasma | Methylated DNA | TPEF gene | 65.0% - 81.0% | 69.0% - 90.0% |
| Case-control | Serum/plasma | Methylated DNA | VIM (Vimentin) gene | 59.0% - 90.7% | 72.5% - 93.0% |
| Case-control | Plasma | Hypomethylated DNA | LINE-1 transposable DNA element | 65.80% | 90.00% |
| Case-control | Serum/plasma | Methylated DNA | IKFZ and BCAT1 genes | 62.1% - 95.0% | 92.0% - 95.0% |
| Case-control | Serum | Methylated DNA | SEPT9 and SDC2 genes | 86.50% | 92.10% |
| Case-control | Serum/plasma | Methylated DNA | APC, MGMT, RASSF2A and WIF-1 genes | 86.50% | 92.10% |
| Case-control | Plasma | Methylated DNA | ALX4, BMP3, NPTX2, RARB, SDC2, SEPT9 and VIM genes | 90.70% | 72.50% |
| Case-control | Serum | ALU115 DNA content | Free ALU115 DNA content | 69.20% | 99.10% |
| Case-control | Serum | DNA integrity | ALU247/115 DNA integrity index | 73.10% | 97.30% |
| Case-control | Serum | Free DNA content | ALU-based cell-free DNA | 64.50% | 98.90% |
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| Case-control | Whole blood | mRNA expression | TSPAN8 gene | 83.60% | 58.20% | [36] |
| Case-control | Whole blood | mRNA expression | LGALS5 gene | 82.10% | 61.20% | [36] |
| Case-control | Whole blood | mRNA expression | COL1A2 gene | 73.10% | 59.70% | [36] |
| Case-control | Whole blood | mRNA expression | CEACAM6 gene | 65.70% | 61.20% | [36] |
| Case-control | Whole blood or serum | mRNA expression | SALL4 gene | 85.9%-96.1% | 85.7%-95.0% | [65,66] |
| Case-control | Whole blood | mRNA expression panel | TSPAN8 and LGALS4 genes | 92.50% | 67.20% | [36] |
| Case-control (CRC and high-risk adenomas in the case group) | Whole blood | mRNA expression panel | LGALS4, CEACAM6, TSPAN8 and COL1A2 genes | 75.00% | 87.00% | [67] |
| Case-control | Whole blood | mRNA expression panel | CEA, EpCAM, CK19, MUC1, EGFR and C-Met genes | 87.00% | 85.00% | [36] |
| Case-control | Whole blood | Long non-coding RNA expression | NEAT1 variant 1 | 69.00% | 79% | [36] |
| Case-control | Whole blood | Long non-coding RNA expression | NEAT1 variant 2 | 70.00% | 96.00% | [36] |
| Case-control | Serum | Long non-coding RNA expression | BLACAT1 | 83.30% | 76.70% | [36] |
| Case-control | Plasma | Long non-coding RNA expression panel | ATB and CCAT1 | 82.00% | 75.00% | [36] |
| Case-control | Plasma | Long non-coding RNA expression panel | 91H, PVT-1 and MEG3 | 82.80% | 78.60% | [36] |
| Case-control | Serum | Long non-coding RNA expression panel | LOC285194, RP11-462C24.1 and Nbla12001 | 68.30% | 86.90% | [36] |

FIT: Faecal immunochemical test; CRC: Colorectal cancer.

that using gene mutations alone does not achieve satisfactory levels of diagnostic sensitivity. One demonstrative study evaluating this approach in a representative colonoscopy screening group concluded that the sensitivity of a panel comprising 21 DNA alterations (point mutations in \(K\)-ras, \(APC\) and \(p53\) genes, microsatellite instability marker \(B\)AT-26 deletions and long DNA assay) was only slightly above 50% \([36]\).

The relatively disappointing diagnostic performance of mutation-based assays stimulated the search for CRC-related epigenetic changes, in particular aberrant hypermethylation of CpG islands usually located in gene promoter regions \([68]\). Gene-specific DNA methylation in stool was extensively investigated (Table 2), and several genes, including \(BMP3\), \(NDRG4\), \(septin 9 (SEPT9)\), \(SFRP2\), \(SPG20\), \(TFPI2\), \(vimentin (VIM)\) were shown to have diagnostic sensitivities between 50% and 92% at specificities between 80% and 100% for CRC detection (see recent reviews by Liu et al \([69]\), Lam et al \([70]\) and Rasmussen et al \([71]\)). However, the reproducibility of these results was often problematic, and attempts to combine multiple methylated genes in panels were undertaken to increase assay reliability. It is remarkable that high CRC detection sensitivities and specificity values could be achieved by combining methylation testing for \(BMP3\) and \(NDRG4\) or \(VIM\) and \(SFRP2\) genes, but these results need to be corroborated. The Colosure™ test detecting methylated \(VIM\) in stool was the first methylation-based commercial test for CRC \([72]\). This diagnostic product was marketed in the USA but has recently been replaced by a more efficient multimarker Cologuard™ test considered later in this sub-section.

Table 2 demonstrates that in the context of CRC diagnostics, DNA methylation markers detectable in blood attract at least as much attention as similar markers in stool. Although investigations of different groups often produce conflicting results, it is now apparent that \(SEPT9\) methylation detection is the best studied option amongst these blood tests \([73]\). This test has recently been commercialised and regulated for clinical application as Epi proColon™ 2.0 CE \([74]\), but its use appears to be limited to opportunistic CRC screening \([75]\). Moreover, DNA methylation analysis in biological samples is relatively laborious (especially for multimarker panels) and difficult to present in POC format. These factors limit diagnostic potential of this approach. In addition, Table 2 shows that samples of stool, blood, bowel lavage fluid and colorectal mucus were also tested for total and ALU-based DNA quantification, DNA integrity
assessment, examination of gene expression and long non-coding RNA expression. However, none of these assays could provide sufficiently high values for diagnostic sensitivity and specificity.

It is now becoming clear that tests involving DNA markers tend to perform better only when markers of different types are combined. Long-term research projects led by a United States company, Exact Sciences, allowed the design of a multitarget stool test that demonstrated high levels of sensitivity and specificity for CRC detection. An early version of this test that included K-ras mutation, methylation of VIM, BMP3, NDRG4 and TFF12 genes, DNA measurement by β-actin assessment and the HemoQuant test for haemoglobin achieved diagnostic sensitivity between 78% and 85% at specificity between 85% and 90% in a case-control study[141]. It is remarkable that this test performed significantly better when directly compared with the test for methylated SEPT9 in plasma (similar to Epi proColon)[142]. The multitarget test was then simplified, and its final version includes only determination of K-ras mutation, BMP3 and NDRG4 promoter methylation, DNA measurement by β-actin assessment and FIT. Screening application of this test in a large study produced CRC detection sensitivity of 92.3% at a specificity of 86.6%[143], which makes this assay the best among all available tests involving DNA markers. The test was approved by the United States Food and Drug Administration in 2014 and is now marketed as Cologuard®. However, this test, which can be regarded as an enhanced version of FIT, requires stool collection, remains technically complex, with a multistep analytical procedure required[144], and is very expensive at over $600.

**MicroRNA markers**

MicroRNAs (a sub-class of small non-coding RNA molecules) were discovered and characterised during the last decade of the XX century. Since that time, it was established that microRNAs are important regulators of gene expression intimately involved in the pathogenesis of many diseases including cancer[145]. As many of them are associated with the presence of colorectal tumours, it was suggested that microRNA determination in stool or blood samples may provide a new diagnostic modality for CRC early detection and screening[146]. MicroRNA variants investigated as potential CRC markers are listed in Table 3. Several published studies that used stool sample analysis highlight miR-21 as the best-studied marker of this type, but do not show outstanding sensitivity and specificity values[147]. MiR-451 and miR-223 detectable in stool produced high sensitivity and specificity values in a small study[148]; however, these markers looked less impressive in other studies, when combined with other microRNAs[149,150]. It is impossible to exclude that these discrepancies may be associated with either technical problems or different ethnic composition of the studied patient groups since clinical studies providing material for microRNA analyses were performed mostly in East Asia.

Table 3 also indicates that microRNA markers of CRC were intensely investigated in blood. Hitherto most of these studies produced modest or inconsistent results. Again, miR-21 was assessed by many groups, and conflicting results were published. Although very high diagnostic sensitivity (96.6%) and specificity (97.8%) values were reported by Ng et al[149] for miR-139-3p, which was shown to be downregulated in the serum of CRC patients, this finding remains to be confirmed. Combinations of microRNA markers detectable in plasma or serum were also tested as diagnostic panels. Among these panels (Table 3), combinations of downregulated miR-144-3p, miR-425-5p and miR-1260b[151] and upregulated miR-19a, miR-19b, miR-13b, miR-29a, miR-335 and miR-18a[150] demonstrated sensitivity and specificity levels exceeding 90%.

In addition, it should be noted that a recent small study has revealed that quantification of miR-21 in saliva samples resulted in CRC detection with 97% sensitivity and 91% specificity[152]. However, these highly intriguing results remain to be corroborated.

Although microRNAs constitute a group of promising CRC biomarkers, further research in this relatively new area is needed to establish clinically valid diagnostic techniques using these markers. The relative technical complexity of laboratory procedures used in microRNA analysis (RNA extraction, reverse transcription and qPCR analysis) and the necessity of careful assay optimisation and standardisation[146] should also be taken into account when the diagnostic potential of this interesting approach is considered.

**Volatile organic compounds (VOC) and small metabolite biomarkers**

Metabolomics is a new discipline that focuses on evaluating a wide variety of endogenous metabolites produced by the organism[153,154,155]. These metabolites can serve as late stage biomarkers of either normal physiological or pathophysiological events, and cancer metabolome is defined as the entire suite of low molecular weight (< 1500 Da) cancer-specific metabolites[156]. Interestingly, some of these metabolites are VOC-s
| Study setting | Sample type | Marker type | Biomarker(s) and detection methods | Sensitivity (or its range) | Specificity (or its range) | Ref. |
|---------------|-------------|-------------|-----------------------------------|---------------------------|---------------------------|-----|
| Case-control  | Stool       | MicroRNA    | miR-18a, upregulated              | 61.00%                    | 69.00%                    | [73]|
| Case-control  | Stool       | MicroRNA    | miR-20a, upregulated              | 55.00%                    | 82.00%                    | [73]|
| Case-control  | Stool       | MicroRNA    | miR-21, upregulated               | 56.0%-86.0%               | 73.0%-81.1%               | [73,74]|
| Case-control  | Stool       | MicroRNA    | miR-92a, upregulated              | 72.00%                    | 73.00%                    | [73]|
| Case-control  | Stool       | MicroRNA    | miR-106a, upregulated             | 34.00%                    | 97.00%                    | [73]|
| Case-control  | Stool       | MicroRNA    | miR-135b, upregulated             | 78.00%                    | 68.00%                    | [73]|
| Case-control  | Stool       | MicroRNA    | miR-92a, upregulated              | 72.00%                    | 73.00%                    | [73]|
| Case-control  | Stool       | MicroRNA    | miR-144*, upregulated             | 74.00%                    | 87.00%                    | [73]|
| Case-control  | Stool       | MicroRNA    | miR-223, upregulated              | 62.00%                    | 74.00%                    | [73]|
| Case-control  | Stool       | MicroRNA    | miR-451, upregulated              | 77.00%                    | 96.00%                    | [73]|
| Case-control  | Stool       | MicroRNA panel | miR-223 and miR-92a, both upregulated | 97.00%                    | 75.00%                    | [73]|
| Case-control  | Stool       | MicroRNA panel | miR-17-93 cluster and miR-135b, all upregulated | 74.00%                    | 79.00%                    | [73]|
| Case-control  | Stool       | MicroRNA panel | miR-144-5p, miR-451a and miR-20b-5p, all upregulated | 66.00%                    | 95.00%                    | [74]|
| Case-control  | Plasma      | MicroRNA    | miR-17-3p, upregulated            | 64.00%                    | 70.00%                    | [73,77]|
| Case-control  | Plasma      | MicroRNA    | miR-18a, upregulated              | 73.10%                    | 79.10%                    | [73]|
| Case-control  | Plasma      | MicroRNA    | miR-20a, upregulated              | 46.00%                    | 73.40%                    | [73,77]|
| Case-control  | Serum/plasma| MicroRNA    | miR-21, upregulated               | 65.0%-91.4%               | 74.4%-95.0%               | [73,75-79]|
| Case-control  | Plasma      | MicroRNA    | miR-24, downregulated             | 78.40%                    | 83.80%                    | [73]|
| Case-control  | Plasma      | MicroRNA    | miR-29a, upregulated              | 69.00%                    | 89.10%                    | [73]|
| Case-control  | Serum/plasma| MicroRNA    | miR-29b, downregulated            | 61.4%-77.0%               | 72.5%-75.0%               | [73]|
| Case-control  | Plasma      | MicroRNA    | miR-92, upregulated               | 89.00%                    | 70.00%                    | [73]|
| Case-control  | Serum/plasma| MicroRNA    | miR-92a, upregulated              | 65.5%-84.0%               | 71.2%-82.5%               | [73,77]|
| Case-control  | Plasma      | MicroRNA    | miR-96, upregulated               | 65.40%                    | 73.30%                    | [73,77]|
| Case-control  | Plasma      | MicroRNA    | miR-106a, upregulated             | 74.00%                    | 44.40%                    | [73]|
| Case-control  | Serum       | MicroRNA    | miR-139-3p, downregulated         | 96.60%                    | 97.80%                    | [80]|
| Case-control  | Serum       | MicroRNA    | miR-139a-5p, upregulated          | 76.70%                    | 88.00%                    | [81]|
| Case-control  | Plasma      | MicroRNA    | miR-155, upregulated              | 58.20%                    | 95.00%                    | [81]|
| Case-control  | Plasma      | MicroRNA    | miR-182, upregulated              | 78.00%                    | 91.00%                    | [81]|
| Case-control  | Serum       | MicroRNA    | miR-194, downregulated            | 72.00%                    | 80.00%                    | [81]|
| Case-control  | Serum       | MicroRNA    | miR-196b, upregulated             | 63.00%                    | 87.40%                    | [81]|

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Table 3  Non-invasive microRNA biomarkers used for colorectal cancer detection
| Study Type   | Sample Type | MicroRNA Panel                      | Expression Pattern   | Percentage  |
|-------------|-------------|-------------------------------------|----------------------|-------------|
| Case-control| Plasma       | miR-200c, upregulated               |                      | 64.10% 73.30% |
| Case-control| Serum        | miR-210, upregulated               |                      | 74.6%-88.6% 73.5%-90.1% |
| Case-control| Plasma       | miR-221, upregulated               |                      | 86.00% 41.00% |
| Case-control| Plasma       | miR-320a, upregulated              |                      | 92.80% 73.10% |
| Case-control| Serum        | miR-338-5p, upregulated            |                      | 76.30% 92.50% |
| Case-control| Serum        | miR-372, upregulated               |                      | 81.90% 73.30% |
| Case-control| Plasma       | miR-375, downregulated             |                      | 76.90% 64.60% |
| Case-control| Plasma       | miR-423-5p, downregulated          |                      | 91.90% 70.80% |
| Case-control| Plasma       | miR-506, upregulated               |                      | 76.80% 60.70% |
| Case-control| Plasma       | miR-601, downregulated             |                      | 69.20% 72.40% |
| Case-control| Plasma       | miR-760, downregulated             |                      | 80.00% 72.40% |
| Case-control| Serum        | miR-1290, upregulated              |                      | 70.10% 91.20% |
| Case-control| Plasma       | miR-4316, upregulated              |                      | 78.60% 75.00% |
| Case-control| Plasma       | miR-19a and miR-19b, both          |                      | 78.60% 74.40% |
| Case-control| Serum        | miR-21 and miR-92a, both          |                      | 68.00% 91.20% |
| Case-control| Plasma       | miR-29a and miR-92a, both          |                      | 83.00% 84.70% |
| Case-control| Plasma       | miR-200c and miR-18a, both         |                      | 84.60% 75.60% |
| Case-control| Plasma       | miR-223 and miR-92a, both          |                      | 76.00% 70.80% |
| Case-control| Plasma       | miR-320d, downregulated; miR-1290, upregulated | | 81.20% 90.70% |
| Case-control| Plasma       | miR-431 and miR-139-p3, both upregulated | | 91.00% 57.00% |
| Case-control| Plasma       | miR-601 and miR-760, both downregulated | | 83.30% 69.10% |
| Case-control| Plasma       | miR-19a, miR-19b and miR-15b, all upregulated | | 78.60% 79.20% |
| Case-control| Plasma       | miR-24, miR-320a and miR-423-5p, all downregulated | | 92.80% 70.80% |
| Case-control| Plasma       | miR-144-3p, miR-425-5p and miR-1260b, all downregulated | | 93.80% 91.30% |
| Case-control| Serum        | miR-145, downregulated; miR-106a and miR-17-3p, upregulated | | 78.50% 82.80% |
| Case-control| Plasma       | miR-499-3p, upregulated; miR-7 and miR-93, downregulated | | 82.00% 89.00% |
that are present in the gas phase of various excreted biological materials and can potentially be used for detecting malignancies including CRC\(^9\). The outcomes of metabolomic studies on CRC detection are summarised in Table 4. Remarkably, very impressive results (with CRC detection sensitivity reaching 97% at 99% specificity) were achieved by Sonoda \(\text{et al}\)\(^9\), when dog scent judgment was applied to faeces and exhaled breath samples for discriminating between CRC patients and controls. Unfortunately, it is not realistic to expect that this natural phenomenon could constitute a reliable diagnostic tool. Hence, advanced Electronic Nose technologies are being developed and tested for CRC detection (Table 4) alongside widely used combinations of gas chromatography (GC) and mass spectrometry (MS)\(^18,94,99\). The latter approach, albeit regarded as the technical gold standard, is complex, costly and unsuitable for population screening. This point is especially important because most of the numerous studies applying metabolomic approaches to detecting CRC-related metabolites (non-VOC-s) in biological substances use various versions of MS (Table 4). Although some of the studies listed in Table 4 produced sensitivity and specificity values above 90% for CRC detection\(^102,109,113,116,125\), cost and complexity issues remain major obstacles to the introduction of these assays into routine clinical practice. In this context, the use of electronic noses sensing CRC-associated VOC-s appears to be more promising, especially in view of CRC detection sensitivity and specificity both reaching 95% in a recent study by Zonta \(\text{et al}\)\(^9\).

**Markers of CRC-associated changes in gut microbiome**

The structure of the gastrointestinal tract engenders permanent interactions between its epithelial tissue and luminal microbiota, thus significant microbial impact in colorectal carcinogenesis appears to be likelier than in any other neoplasia. Steadily accumulating evidence indicates a pivotal role for the gut microbiome in influencing the development of CRC\(^19\). It is now believed that bacterial effects predisposing to CRC include impacts in gut surface barrier disruption, induction of colonic inflammation, direct genotoxic action against epithelial cells and dysbiosis leading to CRC-promoting shifts in gut microflora composition and the colonic microenvironment\(^19,147\). These advances prompted interest in evaluating gut microbiome shifts as possible diagnostic markers for CRC\(^148\). The results of several recent studies (presented in Table 5) show that alterations in gut microbiome composition can potentially serve as non-invasive diagnostic markers for this disease.
| Study setting | Sample type | Marker type | Biomarker(s) and detection methods | Sensitivity (or its range) | Specificity (or its range) | Ref |
|---------------|-------------|-------------|-----------------------------------|--------------------------|--------------------------|-----|
| Case-control  | Stool       | VOCs        | Hydrogen sulphide, Dimethylsulphide, Dimethyldisulphide, mlz 90 - detected by selected ion flow tube (SIFT) mass spectrometry (MS) | 72.00%                   | 78.00%                   | [94] |
| Case-control  | Stool       | VOCs        | Propan-2-ol, 3-methylbutanoic acid - detected by gas chromatography (GC) and MS | 87.90%                   | 84.60%                   | [95] |
| Case-control  | Stool       | VOCs        | Methyl mercaptan (increased) and hydrogen (decreased) – detected by GC | 90.00%                   | 57.70%                   | [96] |
| Case-control  | Stool       | VOCs        | Pattern recognition technique - canine scent judgment | 97.00%                   | 99.00%                   | [97] |
| Case-control  | Stool       | VOCs        | Pattern recognition technique (eNose Cyranose® 320) | 85.00%                   | 87.00%                   | [94] |
| Case-control  | Stool       | VOCs        | Pattern recognition technique (SCENT A1) | 95.00%                   | 95.00%                   | [98] |
| Case-control  | Urine       | VOCs        | Ion mobility spectroscopy technology (FAIMS) | 88.00%                   | 60.00%                   | [99] |
| Case-control  | Urine       | VOCs        | Ion mobility spectroscopy technology (FAIMS) | 63.00%                   | 63.00%                   | [100] |
| Case-control  | Urine       | VOCs        | Pattern recognition technique (eNose applied) | 78.00%                   | 79.00%                   | [101] |
| Case-control  | Breath      | VOCs        | Pattern recognition technique - canine scent judgment | 91.00%                   | 99.00%                   | [102] |
| Case-control  | Breath      | VOCs        | Acetone (increased), ethyl acetate (increased), ethanol (decreased) and 4-methyl octane (decreased) detected by GC-MS | 85.00%                   | 94.00%                   | [103] |
| Case-control  | Breath      | VOCs        | Nonanal, decanal, 4-methyl-pentanone, 2-methylbutane, 4-methyloctane, 4-methylundecane, 2-methylpentane, methylcyclopentane, cyclohexane, methylocyclohexane, trimethyldecane-1,2-pentadiene, 1,3-dimethylbenzene, 1,4-dimethylbenzene - detected by GC-MS | 86.00%                   | 83.00%                   | [104] |
| Case-control  | Stool       | Magnetic resonance spectra | Magnetic resonance spectra patterns | 85.20%                   | 86.90%                   | [105] |
| Case-control  | Stool       | Small metabolites | Acetate - detected by proton magnetic resonance spectroscopy (PMRS) | 94.70%                   | 92.30%                   | [106] |
| Group          | Sample | Metabolite Class                  | Detected by Method                        | Sensitivity (%) | Specificity (%) |
|---------------|--------|-----------------------------------|-------------------------------------------|-----------------|-----------------|
| Case-control  | Stool  | Small metabolites                 | Succinate - detected by PMRS              | 91.20%          | 93.50%          | [102] |
| Case-control  | Serum  | Aromatic carboxylic acids         | Benzoic acid - detected by CE-time of flight (TOF) MS | 89.00%          | 82.00%          | [103] |
| Case-control  | Serum  | Fatty acids                        | GTA-446 - detected by flow injection analysis MS | 83.30%          | 84.80%          | [104] |
| Case-control  | Plasma | Amino acid metabolites             | L-kyurenine - detected by high-performance liquid chromatography (HPLC) | 85.20%          | 100.00%         | [105] |
| Case-control  | Plasma | Fatty acids                        | Decanoic acid - detected by CE-TOFMS      | 87.80%          | 80.00%          | [106] |
| Case-control  | Serum  | Multiple metabolites               | 38 metabolites detected by GC-MS          | 85.00%          | 86.00%          | [107] |
| Case-control  | Serum  | Phospholipids (sphingomyelins and phosphatidylcholines) | SM (34:1), PC (34:1), PC (34:2), PC (36:4), PC (36:2), PC (36:3) - detected by MS | 77.3%; 80.8%; 592.4%; 85.9% | [108] |
| Case-control  | Serum  | Unsaturated free fatty acids (panel) | C16:1, C18:3, C20:4, C22:6, all downregulated - detected by MS | 93.80%          | 92.20%          | [109] |
| Case-control  | Serum  | Amino acids (panel)               | 8 amino acids - detected by LC-MS/MS      | 65.00%          | 95.00%          | [110] |
| Case-control  | Serum  | Amino acids, fatty acids, carbohydrates | 13 metabolites - detected by LC-MS/MS     | 96.00%          | 80.00%          | [111] |
| Case-control  | Serum  | Metabolite panel                   | 2-hydroxy-butyrate, aspartic acid, kyurenine, cystamine - detected by GC-MS | 83.10%          | 81.00%          | [112] |
| Case-control  | Serum  | Lipid metabolites (panel)          | Palmitic amide, oleamide, hexadecanenoic acid, octadecanoic acid, eicosatrienoic acid, LPC(18:2), LPC(20:4), LPC(22:6), myristic acid, LPC(16:0) - detected by ion cyclotron resonance MS | 98.10%          | 100.00%         | [113] |
| Case-control  | Serum  | Panel of hydroxylated polyunsaturated ultra long-chain fatty acids | C28H46O4, C28H48O4 and C28H50O4, all downregulated - detected by LC-MS/MS and nuclear MR | 75.00%          | 90.00%          | [114] |
| Case-control  | Serum  | Multiple metabolites (panel)       | 11,14-eicosadienoic acid, 12a-hydroxy-3-oxocholadienic acid, 12-ketodeoxycholic acid, 12-keto-tetrahydro-leukotriene b4, 13cis-retinoic acid, 1b-hydrocholic acid, 1-methylhistamine, 1-monopalmitin, 2,3-dihydroxybutanoic acid, 24-hydroxycalcitriol - detected by GC-TOFMS and UPLC-QTOFMS | 83.70%          | 91.70%          | [115] |
| Study Type  | Sample Type | Metabolites Detected | Detection Method | Sensitivity | Specificity |
|------------|-------------|----------------------|-----------------|-------------|-------------|
| Case-control | Plasma | Amino acids, fatty acids, carbohydrates | 8 metabolites – detected by CT-TQMS | 99.30% | 93.80% |
| Case-control | Plasma | Choline-containing phospholipids (panel) | Total saturated lysophosphatidylcholines (LPCs), 18:2 LPC and sphingosylphosphorylcholine – detected by LC-MS/MS | 88.30% | 80.00% |
| Case-control | Plasma | Choline-containing phospholipids (panel) | Total saturated lysophosphatidylcholines (LPCs) and the difference between 18:2 LPC and 18:1 LPC – detected by LC-MS | 82.00% | 93.00% |
| Case-control | Dried blood | Amino acids and acylcarnitines (panel) | CI6, Arg, C4/C8, CS/C3, Val, Fhe/Tyr, Ala, C4/C3 – detected by direct infusion MS | 81.20% | 83.90% |
| Case-control | Urine | Polyamines | N1, N12-diacetylspermine – detected by ELISA | 75.80% | 96.00% |
| Case-control | Urine | Polyamines and amino acid metabolites | N1, N12-diacetylspermine and kynurenine – detected by LC-MS | 80.00% | 80.00% |
| Case-control | Urine | Amino acids and acetoacetate (panel) | Alanine, glutamine, aspartic acid and acetoacetate – detected by PMRS | 87.50% | 91.30% |
| Case-control | Urine | Nucleosides (panel) | 5-hydroxymethyluracil and 8-oxo-7,8-dihydroguanine – detected by UPLC-MS/MS | 78.60% | 75.00% |
| Case-control | Urine | Nucleosides (panel) | Cytidine, 3-methylcitidine, 1-methyladenosine, 2-deoxyguanosine, adenosine, inosine – detected by HPLC-MS/MS | 69.00% | 98.00% |
| Case-control | Urine | Metabolite panel | Citrate, Hippurate, p-cresol, 2-amino butyrate, myristate, putrescine and kynurenate – detected by UPLC-QTOFMS | 97.50% | 100% |
| Case-control | Urine | Nucleosides (panel) | Adenosine, N4-acetylcytidine, cytidine, guanosine, inosine, 1-methyladenosine, 1-methylguanosine, 1-methylinosine, 2-methylguanosine, 2,2-methylguanosine, N6-methyladenosine, uridine, 3-methyluridine+5-methyluridine, pseudouridine – detected by reverse phase HPLC | 76.90% | 90.40% |
One remarkable common feature of all the studies listed in Table 5 is the obligatory presence of *Fusobacterium nucleatum* (*F. nucleatum*) as one of the components of all tested panels. Indeed, *F. nucleatum*, an anaerobic oral commensal, is now identified as a pathogenetic factor contributing to multiple disorders comprising among others inflammatory bowel disease and CRC[19,148,149]. This interesting diagnostic approach is being actively investigated; however, further studies are necessary to firmly establish the value of the gut microbiome in non-invasive CRC detection.

**NON-INVASIVE BIOMARKER TESTING USE IN CRC SCREENING TODAY AND FUTURE CHALLENGES**

The existing plethora of potential non-invasive approaches to CRC detection briefly reviewed in this paper looks impressive in terms of numbers, but often disappointing in terms of outcome. Most of the published results clearly fail to transform into diagnostic or screening tests that would be highly sensitive and specific, simple to perform and not associated with excessive cost. As a matter of fact, the choice of available biomarker-based tests practically used for CRC screening remains strictly limited. Today FIT is by far the most popular option[2,9,31] owing to its relative simplicity and affordability. The recently introduced and widely advertised multitarget Cologuard® stool test or Epi proColon test targeting *SEPT9* methylation in plasma, albeit approved for clinical use, are technically complex and prohibitively expensive. Comparative studies addressing the health economics of CRC screening have demonstrated that the multitarget stool test, being more cost-effective that no screening, is significantly less cost-effective when compared to the FIT or invasive endoscopic testing[150-152]. Likewise, methylated *SEPT9* detection in plasma samples[153] is clearly less cost-effective than the FIT. Considering a unit cost of $8 for the FIT (sampling kit and analysis only), Lansdorp-Vogelaar *et al*[154] concluded that a biomarker-based test that detects CRC with higher levels of sensitivity and specificity (up to 100%) should never be more expensive than $57 to be cost-effective. These estimates seem to indicate that in practical terms the FIT is currently the most cost-effective test for non-invasive CRC screening. Other authors argue that a highly specific non-invasive biomarker with an improved sensitivity for advanced adenomas (that progress to CRC) would probably be cost-effective at higher threshold costs[155], but the $600 price tag currently attached to Cologuard® is obviously excessive.

In any case, it is apparent that the FIT is not a perfect screening test. Its specificity reaching 95% is sufficiently high to be deemed satisfactory, but the sensitivity of this test remains relatively modest[31]. There is, however, an opinion that repeated FIT testing with one-year intervals may compensate for the lack of sensitivity[12]. Moreover, accurate identification of individuals with different levels of CRC risk could lead to creating objective approaches to risk stratification and personalised screening[12,155,156].

The effectiveness of a screening strategy is defined not only by screening test performance characteristics, but also by screening participant adherence[12]. One additional practical problem in CRC screening programmes employing faecal tests is insufficient screening uptake[159,160] that often results from participants’ reluctance to collect stool samples[159,160]. The use of non-invasively collected colorectal mucus samples[124,130] in FIT-like tests can help solve this problem, but this new approach remains to be thoroughly evaluated, and this will require large comparative randomised trials that usually take several years to complete[154]. The existing
### Table 5 Non-invasive faecal bacterial biomarkers used for colorectal cancer detection

| Study setting | Sample type | Marker type | Biomarker(s) | Sensitivity (or its range) | Specificity (or its range) | Ref. |
|---------------|-------------|-------------|--------------|---------------------------|---------------------------|------|
| Case-control  | Stool       | Bacterial   | *Fusobacterium nucleatum* | 54.0%-92.8% | 79.8%-91.0% | [128-131] |
| Case-control  | Stool       | Bacterial   | clbA-positive bacteria | 56.4% | 81.5% | [131] |
| Case-control  | Stool       | Bacterial panel | *Fusobacterium nucleatum*, *Bacteroides clarus*, *Roseburia intestinalis* and *Clostridium hathewayi* | 92.8% | 79.8% | [130] |
| Case-control  | Stool       | Bacterial panel | clbA-positive bacteria and *Fusobacterium nucleatum* | 84.6% | 63.1% | [131] |
| Case-control  | Stool       | Bacterial panel | Ratio of *Fusobacterium nucleatum* to *Bifidobacterium* | 84.6% | 92.3% | [132] |
| Case-control  | Stool       | Bacterial panel | Combination of ratios of *Fusobacterium nucleatum* to *Bifidobacterium* and *Fusobacterium nucleatum* to *Faecalibacterium prausnitzii* | 90.0% | 90.2% | [132] |
| Case-control (CRC and adenomatous polyps in the case group) | Stool | Bacterial panel | *Fusobacterium nucleatum*, *Enterococcus faecalis*, *Streptococcus bovis*, *Enterotoxigenic Bacteroides fragilis*, and *Porphyromonas spp* | 91.4% | 93.5% | [133] |

CRC: Colorectal cancer.

Combination of the FIT and confirmatory colonoscopy is the strategy of choice today, and its further optimisation is currently regarded as the main factor in improving CRC screening effectiveness.

The present strong position of the FIT as the test of choice for non-invasive CRC screening will certainly be temporary as this test has one intrinsic deficiency that is impossible to eliminate. The FIT detects blood, which is shed but not produced by tumours, and bleeding may not occur in some CRC patients. For this reason, FIT sensitivity will never approach 100%, and it is likely that this target will become achievable only when a screening test employing CRC-specific biomarker(s) is developed. As no single biomarker detectable in all colorectal tumours has been identified so far, multitarget strategies combining either multiple markers of the same type or different assays (such as Cologuard®) emerge as CRC screening options advocated by some experts. However, these complex assays usually require sophisticated laboratory equipment and are laborious and expensive. Although future technological advances can help in eliminating these deficiencies, the search for more reliable and easily detectable single CRC biomarkers should continue.

It can be expected that rapid progress in cancer biomarker research accompanied by accelerated development of new non-invasive tests promises forthcoming breakthroughs in CRC screening and prevention of this disease.

### REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019; 144: 1941-1953 [PMID: 30350310 DOI: 10.1002/ijc.31937]

2. Brenner H, Chen C. The colorectal cancer epidemic: challenges and opportunities for primary, secondary
Loktionov A. CRC biomarkers

2 and tertiary prevention. Br J Cancer 2018; 119: 785-792 [PMID: 30287914 DOI: 10.1038/s41416-018-0264-x]

3 Grady WM, Markowitz SD. The molecular pathogenesis of colorectal cancer and its potential application to colorectal cancer screening. Dig Dis Sci 2015; 60: 762-772 [PMID: 2549249 DOI: 10.1007/s10620-014-4444-4]

4 Carethers JM, Jung BH. Genetics and Genetic Biomarkers in Sporadic Colorectal Cancer. Gastroenterology 2015; 149: 1177-1190.e3 [PMID: 26216840 DOI: 10.1053/j.gastro.2015.06.047]

5 Hazeijkens Y, Dekker E. Colonoscopy: basic principles and novel techniques. Nat Rev Gastroenterol Hepatol 2011; 8: 554-564 [PMID: 2189402 DOI: 10.1038/nrgastro.2011.11]

6 Hoff G, Dominitz JA. Contrasting US and European approaches to colorectal cancer screening: which is best? Gut 2010; 59: 407-414 [PMID: 20207645 DOI: 10.1136/gut.2009.192948]

7 Lieberman DA, Williams JL, Holub JL, Morris CD, Logan JR, Eisen GM, Carney P. Colonoscopy utilization and outcomes 2000 to 2011. Gastrointest Endosc 2014; 80: 133-143 [PMID: 24565067 DOI: 10.1016/j.gie.2014.01.014]

8 Young GP, Rabencek L, Winawer SJ. The Global Paradigm Shift in Screening for Colorectal Cancer. Gastroenterology 2019; 156: 843-851.e2 [PMID: 30776340 DOI: 10.1053/j.gastro.2019.02.006]

9 Zauber AG, Winawer SJ, O'Brien MJ, Lansdorpp-Vogelaar I, van Ballegoijen M, Hankey BF, Shi W, Bond JH, Schapiro M, Panish JF, Stewart ET, Waye JD. Colonic polypectomy and long-term prevention of colorectal-cancer deaths. N Engl J Med 2012; 366: 687-696 [PMID: 22356322 DOI: 10.1056/NEJMoa1105370]

10 Lieberman D. Colon cancer screening and surveillance controversies. Curr Opin Gastroenterol 2009; 25: 422-427 [PMID: 19463649 DOI: 10.1097/01.mog.0000332832.1628a]

11 Zhao S, Wang S, Pan P, Xia T, Chang X, Yang X, Guo L, Meng Q, Yang F, Qian W, Xu Z, Wang Y, Wang Z, Gu L, Wang Y, R, Pan F, Yao J, Li Z, Bai Y. Magnitude, Risk Factors, and Factors Associated With Adenoma Miss Rate of Tandem Colonoscopy: A Systematic Review and Meta-analysis. Gastroenterology 2019; 156: 1661-1674.e11 [PMID: 30738046 DOI: 10.1053/j.gastro.2019.01.260]

12 Ladabaum U, Dominitz JA, Kahi C, Schoen RE. Strategies for Colorectal Cancer Screening. Gastroenterology 2019; [PMID: 31394803 DOI: 10.1053/j.gastro.2019.04.013]

13 Corte CJ, Leong RW. Improving the utility of colonoscopy: Recent advances in practice. J Gastroenterol Hepatol 2016; 31: 32-44 [PMID: 26211821 DOI: 10.1111/jgh.13056]

14 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674 [PMID: 2176239 DOI: 10.1016/j.cell.2011.02.013]

15 Ogawa Y, Grady WM, Goel A. Epigenetic Alterations in Colorectal Cancer: Emerging Biomarkers. Gastroenterology 2015; 149: 1204-1225.e12 [PMID: 26216839 DOI: 10.1053/j.gastro.2015.07.011]

16 Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. Science 2013; 339: 1546-1558 [PMID: 23535954 DOI: 10.1126/science.1235122]

17 Aboud OA, Weiss RH. New opportunities from the cancer metabolome. Clio Chem 2013; 59: 138-146 [PMID: 23150057 DOI: 10.1373/clinchem.2012.184598]

18 Liu X, Locasale JW. Metabolomics: A Primer. Clin Chem 2017; 63: 274-284 [PMID: 28196646 DOI: 10.1016/j.clinchim.2017.01.004]

19 Chen J, Pitmon E, Wang K. Microbiome, inflammation and colorectal cancer. Semin Immunol 2017; 32: 43-53 [PMID: 28982615 DOI: 10.1016/j.smim.2017.09.006]

20 Johansson ME, Larsson JM, Hansson GC. Composition and functional role of the mucus layers in the intestine. Cell Mol Life Sci 2011; 68: 3635-3641 [PMID: 21947475 DOI: 10.1007/s00018-011-0822-3]

21 Loktionov A. Cell exfoliation in the human colon: myth, reality and implications for colorectal cancer screening. Int J Cancer 2007; 120: 2281-2289 [PMID: 17351899 DOI: 10.1002/ijc.22647]

22 Loktionov A, Bandalekova T, L, L., Lecchini MH, Tu, Rothenberg P, H., Beckstrom H, B. cell exfoliation in the human colon: myth, reality and implications for colorectal cancer screening. Gastroenterology 2017; 32: 992-1002 [PMID: 27787913 DOI: 10.1111/j.1532-5415.2017.13627]

23 Yamada T, Matsuda A, Kozumi M, Shinji S, Takahashi G, Iwai T, Takeda K, Ueda K, Yokoyama Y, Hara K, Hotta M, Matsumoto S, Yohara H. Liquid biopsy for the management of Patients with Colorectal Cancer. Digestion 2019; 99: 39-45 [PMID: 30554222 DOI: 10.1159/000494411]

24 Erben V, Bhardwaj M, Schrotz-King P, Brenner H. Metabolomics Biomarkers for Detection of Colorectal Neoplasms: A Systematic Review. Cancers (Basel) 2018; 10: 246 [PMID: 30064069 DOI: 10.3390/cancers10020246]

25 Greger DH. Diagnosis of large-bowel cancer in the asymptomatic patient. JAMA 1967; 201: 943-945 [PMID: 6072632 DOI: 10.1001/jama.1967.0310120051012]

26 Sidransky D, Tokino T, Hamilton SR, Kinzler KW, Levin B, Frost P, Vogelstein B. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. Science 1992; 256: 102-105 [PMID: 1566048 DOI: 10.1126/science.1566048]

27 Gies A, Bhardwaj M, Smith S, Schrotz-King P, Brenner H. Quantitative fecal immunochromatographic test for colorectal cancer screening. Int J Cancer 2018; 143: 234-244 [PMID: 29277897 DOI: 10.1002/ijc.31233]

28 Uppara M, Adaba F, Askari A, Clark S, Hanna G, Aithanasiou T, Faiz O. A systematic review and meta-analysis of the diagnostic accuracy of pyruvate kinase M2 isoenzymatic assay in diagnosing colorectal cancer. World J Surg Oncol 2015; 13: 48 [PMID: 25888768 DOI: 10.1186/s12957-015-0446-8]

29 Sithambaram S, Hilly I, Goh KL. The Diagnostic Accuracy of the M2 Pyruvate Kinase Quick Stool
DNA isolated from directly collected exfoliated colonocytes.

Int J Cancer 2016; 144: 519-528

Xie H, Guo JH, An WM, Tian ST, Yu HP, Yang XL, Wang HM, Guo Z. Diagnostic value evaluation of trefoil factors family 3 for the early detection of colorectal cancer. World J Gastroenterol 2017; 23: 2159-2167 [PMID: 28405145 DOI: 10.3748/wjg.v23.i12.2159]

Jiang Y, Zhang C, Chen K, Chen Z, Sun Z, Zhang Z, Ding D, Ren S, Zuo Y. The clinical significance of DC-SIGN and DC-SIGNR, which are novel markers expressed in human colon cancer. PLoS One 2014; 9: e114748 [PMID: 25504222 DOI: 10.1371/journal.pone.0114748]

Hou YL, Liu P, Ji GY, Chen H. Clinical significance of serum lFGFP-3 in colorectal cancer. J Clin Lab Anal 2019; 33: e22912 [PMID: 31218761 DOI: 10.1002/jla.22912]

Rho JH, Ladd JJ, Li CI, Potter JD, Zhang Y, Shelley D, Shibata D, Coppola D, Yamada H, Toyoeda H, Tada T, Kumada T, Bremner DE, Hanash SM, Lampe PD. Protein and glycomic plasma markers for early detection of adenoma and colon cancer. Gut 2018; 67: 473-484 [PMID: 27821464 DOI: 10.1136/gutjnl-2016-312794]

Wilhelmsen M, Christensen J, Rasmussen L, Jorgensen LN, Madsen MR, Vilandt J, Hiltig K, Klaerke M, Nielsen KT, Laurberg S, Bruinier N, Gweli S, Yang X, Davis G, Heijboer A, Martens F, Nielsen HJ. Detection of colorectal neoplasia: Combination of eight blood-based, cancer-associated protein biomarkers. Int J Cancer 2017; 140: 1436-1446 [PMID: 27955033 DOI: 10.1002/ijc.31058]

Imperiale TF, Ransohoff DF, Izkowitz SH, Turnbull BA, Ross ME; Colorectal Cancer Study Group. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an age-risk population. N Engl J Med 2004; 351: 2704-2714 [PMID: 15616205 DOI: 10.1056/NEJMoa033403]

Ahquist DA, Zou H, Domanico M, Mahoney DW, Yab TC, Taylor WR, Butz ML, Thibodeau SN, Rabeneck L, Paszai LF, Kinzler KW, Vogelstein B, Bjerregaard NC, Laurberg S, Sorensen HT, Berger BM, Lidgard GP. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. Gastroenterology 2012; 142: 248-56; quiz e25-6 [PMID: 22966375 DOI: 10.1053/j.gastro.2011.09.031]

Imperiale TF, Ransohoff DF, Izkowitz SH, Levin TR, Lavin P, Lidgard GP, Ahlquist DA, Berger BM. Multitarget stool DNA testing for colorectal-cancer screening. N Engl J Med 2014; 370: 1287-1297 [PMID: 24645800 DOI: 10.1056/NEJMa1311194]

Liu R, Su X, Long Y, Zhou D, Zhang X, Ye Z, Ma J, Tang T, Wang F, He C. A systematic review and quantitative assessment of methylation biomarkers in fecal DNA and colorectal cancer and its precursor, colorectal adenoma. Mutat Res 2019; 779: 45-57 [PMID: 31097151 DOI: 10.1016/j.mrrev.2019.01.003]

Lam K, Pan K, Linnekamp JF, Medema JP, Kandimalla R. DNA methylation based biomarkers in colorectal cancer: A systematic review. Biochim Biophys Acta 2016; 1866: 106-120 [PMID: 27385266 DOI: 10.1016/j.bbadis.2016.07.001]

Rasmussen SL, Krarup HB, Sunesen KG, Pedersen IS, Madsen PH, Thorlacius-Ussing O. Integrating protein and glycomic plasma markers for the early detection of colorectal cancer. Clin Chem 2014; 60: 154-163 [PMID: 24518356 DOI: 10.1373/clinchem.2014.019941]

Teixeira Y, Lima JM, Souza ML, Aguia P, Silva TD, Forones NM. QUANTIFICATION IN THE STOOLS OF PATIENTS WITH COLORECTAL CANCER. Acta Gastroenterol Latinoam 2015; 52: 293-298 [PMID: 26440470 DOI: 10.1016/j.cga.2014.01.041]

Harada T, Yamamoto E, Yamano HO, Nogima M, Maruyama R, Kamekawa A, Ashida M, Yoshikawa K, Kimura T, Harada E, Takagi R, Tanaka Y, Aoki H, Nishizono M, Nakaoka M, Tsuyada A, Nishimura T, Kii M, Shimoda K, Shinomura Y, Sugita T, Imai K, Suzuki H. Analysis of DNA methylation in bowel lumen fluid for detection of colorectal cancer. Cancer Prev Res (Phila) 2014; 7: 1002-1010 [PMID: 25139296 DOI: 10.1158/1940-6207.CAPR-14-0162]

Loktionov A, Ferrer CG, Gibson JJS, Bandaleotova T, Djon C, Llewelyn AH, Lywood HGG, Lywood RGC, George BD, Mortensen NJ. A case-control study of colorectal cancer detection by quantification of DNA isolated from directly collected exfoliated colonicocytes. Int J Cancer 2010; 126: 1910-1919 [PMID: 20725971]
Worm Orntoft MB. Review of Blood-Based Colorectal Cancer Screening: How Far Are Circulating Cell-Free DNA Methylation Markers From Clinical Implementation? Clin Colorectal Cancer 2018; 17: e145-e143 [PMID: 29678513 DOI: 10.1016/j.ccc.2018.02.012]

Zhang M, Ye Y, Zhang X, Zhang M, Kong L. A pooled analysis of the diagnostic efficacy of plasmatic methylated seprin-9 as a novel biomarker for colorectal cancer. Biomed Rep 2017; 7: 353-360 [PMID: 29085631 DOI: 10.3892/br.2017.970]

Fu B, Yan P, Zhang S, Lu Y, Pan L, Tang W, Chen S, Chen S, Zhang A, Liu W. Cell-Free Circulating Methylated SEPT9 for Noninvasive Diagnosis and Monitoring of Colorectal Cancer. Dis Markers 2018; 2018: 6437104 [PMID: 29849824 DOI: 10.1155/2018/6437104]

Xie L, Jiang X, Li Q, Sun Z, Qian W, Duan Y, Li D, Chen T. Diagnostic Value of Methylated Septin9 for Colorectal Cancer Detection. Front Oncol 2018; 8: 247 [PMID: 30013949 DOI: 10.3389/fonc.2018.00247]

Li H, Wang Z, Zhao G, Ma Y, Chen Y, Xue Q, Zhang M, Fei S. Performance of a MethyLight assay for methylated SFRP2 DNA detection in colorectal cancer tissue and serum. Int J Biol Markers 2019; 34: 54-59 [PMID: 30852954 DOI: 10.1177/1724600818820536]

Sun J, Fei F, Zhang M, Li Y, Zhang X, Zhu S, Zhang S. The role of **SEPT9 in screening, diagnosis, and recurrence monitoring of colorectal cancer. BMC Cancer 2019; 19: 450 [PMID: 31088406 DOI: 10.1186/s12885-019-5663-8]

Rasmussen SL, Kraup HB, Sunesen KG, Johansen MB, Stender MT, Pedersen IS, Madsen PH, Thorlacius-Using O. Hypermethylated DNA, a circulating biomarker for colorectal cancer detection. PLoS One 2017; 12: e0180609 [PMID: 28700744 DOI: 10.1371/journal.pone.0180609]

Chen Y, Wang Z, Zhao G, Sun C, Ma Y, Zhang L, Zheng M, Li H. Performance of a Novel Blood-Based Early Colorectal Cancer Screening Assay in Remaining Serum after the Blood Biochemical Test. Dis Markers 2019; 2019: 5232780 [PMID: 31093894 DOI: 10.1155/2019/5232780]

Ardalan Khales S, Abbasszadegan MR, Abdollahi A, Raeissossadati R, Toussi MF, Forghanifard MM. SALL4 as a new biomarker for early colorectal cancers. J Cancer Res Clin Oncol 2015; 141: 229-235 [PMID: 25158018 DOI: 10.1007/s00432-014-1808-y]

Wu HK, Liu C, Fan XX, Wang H, Zhou L. Splot-like transcription factor 4 as a potential diagnostic and prognostic marker of colorectal cancer. Cancer Biomark 2017; 20: 191-198 [PMID: 28869451 DOI: 10.3233/CBM-170204]

Rodia MT, Solmi R, Pasioli F, Nardi E, Matti G, Ugolini G, Ricciardiello L, Strippoli P, Miglio R, Lauriola M. LGALS6, CEACAM6, TSPAN8, and COL1A2: Blood Markers for Colorectal Cancer. Validation in a Cohort of Subjects With Positive Fecal Immunochemical Test Result. Clin Colorectal Cancer 2018; 17: e217-e228 [PMID: 29326242 DOI: 10.1016/j.clco.2017.12.002]

Shou X, Li Y, Hu W, Ye T, Wang G, Xu F, Su M, Xu Y. Six-gen assay as a new biomarker in the blood of patients with colorectal cancer: establishment and clinical validation. Mol Oncol 2019; 13: 781-791 [PMID: 30556647 DOI: 10.1007/1878-0261.12427]

Dai M, Chen X, Mo S, Li J, Huang Z, Huang S, Xu J, He B, Zou Y, Chen J, Dai S. Meta-signature LncRNAs serve as novel biomarkers for colorectal cancer: integrated bioinformatics analysis, experimental validation and diagnostic evaluation. Sci Rep 2017; 7: 46572 [PMID: 28406230 DOI: 10.1038/srep46572]

Abedin P, Fattahi A, Aghaj S, Talebi A, Beyghi AH, Amini SM, Mirzaei A, Akbari A. Expression analysis of circulating plasma long noncoding RNAs in colorectal cancer: The relevance of lncRNAs ATB and CCAT1 as potential clinical hallmarks. J Cell Physiol 2019; 234: 2208-2220 [PMID: 31093977 DOI: 10.1002/jcp.28765]

Liu H, Ye D, Chen A, Tan D, Zhang W, Jiang W, Wang M, Zhang X. A pilot study of new promising non-coding RNA diagnostic biomarkers for early-stage colorectal cancers. Clin Chem Lab Med 2019; 57: 1073-1083 [PMID: 30978169 DOI: 10.1515/ccml-2019-0052]

Wang G, Yang X, Bai H, Sun X, Li Y, Hu W, Wang W, Wang H, Zou Y, Zhu S, Zhang W, Chen X. Evaluation of 15 circulating lung cancer-related microRNAs for early-stage colorectal cancer diagnosis. J Cancer Res Clin Oncol 2016; 142: 2693-2702 [PMID: 28055667 DOI: 10.1007/1878-0261.12427]

Shabry N. Non-coding RNAs as Biomarkers for Colorectal Cancer Screening and Early Detection. Adv Exp Med Biol 2016; 937: 153-170 [PMID: 27573899 DOI: 10.1007/978-3-319-42057-8_8]

Bast examinejad S, Taherikalani M, Ghanbari R, Akbari A, Shahab N, Saidijam M. Investigation of MicroRNA-21 Expression Levels in Serum and Stool as a Potential Invasive Biomarker for Diagnosis of Colorectal Cancer. Iran Biomed J 2017; 21: 106-113 [PMID: 27432735 DOI: 10.18869/acapub.jb.21.2.106]

Phua LC, Chue XP, Koh PK, Cheah PY, Chan EC, Ho HK. Global fecal microRNA profiling in the identification of biomarkers for colorectal cancer screening among Asians. Oncol Rep 2014; 32: 97-104 [PMID: 24841830 DOI: 10.3892/oo.2014.3193]

Wu CW, Cao X, Berger CK, Foote PH, Mahoney DW, Simonson JA, Anderson BW, Taylor WR, Boardman LA, Kisiel JB, Ahlquist DA. Novel Approach to Fecal Occult Blood Testing by Assay of Erythrocyte-Specific microRNA Markers. Dig Dis Sci 2017; 62: 1859-1994 [PMID: 28604849 DOI: 10.1007/s10620-017-4627-6]

Parker JW, Lifeng T, David C, William H, John L, John M, Paul D, John K, David T. trying analysis and differential expression. Br J Cancer 2017; 116: 762-774 [PMID: 28152545 DOI: 10.1038/bjc.2017.12]

Chen B, Xue D, Deng YN, Yang Y, Zhang P, Zou H, Xu N, Liang S. Emerging microRNA biomarkers for colorectal cancer diagnosis and prognosis. Open Biol 2019; 9: 180212 [PMID: 30958116 DOI: 10.1098/rsob.190212]
Liu X, Xu T, Hu X, Chen X, Zeng K, Sun L, Wang S. Elevated circulating miR-182 acts as a diagnostic biomarker for early colorectal cancer. *Cancer Manag Res* 2018; 10: 857-865 [PMID: 29731665 DOI: 10.2147/CMAR.S158016]

Xu C, Gu L. The diagnostic effect of serum miR-196b as biomarker in colorectal cancer. *Biomed Rep* 2017; 6: 39-45 [PMID: 28123705 DOI: 10.3892/br.2016.815]

Bilegsaikhan E, Liu HN, Shen XZ, Liu TT. Circulating miR-338-5p is a potential diagnostic biomarker in colorectal cancer. *J Dig Dis* 2018; 19: 404-410 [PMID: 29952077 DOI: 10.1111/1751-2981.12643]

Krawczyk P, Powrózek T, Oleksiński T, Dmirtuk A, Dzirwoza J, Kowalski D, Milawanski J. Evaluation of miR-586 and miR-4316 expression in early and non-invasive diagnosis of colorectal cancer. *Int J Colorectal Dis* 2017; 32: 1057-1060 [PMID: 28405738 DOI: 10.1007/s00384-017-2814-8]

Imaoka H, Toiyama Y, Fujikawa H, Hiro J, Saigusa S, Tanaka K, Inoue Y, Mohri Y, Mori T, Kato T, Toden S, Goel A, Kusunoki M. Circulating microRNA-1290 as a novel diagnostic and prognostic biomarker in human colorectal cancer. *Ann Oncol* 2016; 27: 1879-1886 [PMID: 27502702 DOI: 10.1093/annonc/mdw279]

Lundström B, Lindqvist B, Söderberg H, Wentzel T, Hallmans G. Nephropathography in Wegener's granulomatosis. A comparison with panarteritis nodosa. *Acta Radiol Diag (Stockh)* 1975; 16: 641-653 [PMID: 3092 DOI: 10.1080/139463520.1975.11862868]

Wikberg ML, Myte R, Palmqvist R, van Guelpen B, Ljuslinder I. Plasma miRNA can detect colorectal cancer, but how early? *Cancer Med* 2018; 7: 1697-1705 [PMID: 29573205 DOI: 10.1002/cam4.1338]

Herreuez-Villegasova M, Duran-Sanchez S, Martin AG, Perez-Palacios R, Vila-Navarro E, Marcuello M, Diaz-Centeno M, Cubiella J, Diez MS, Bujanda L, Lanas A, Jover R, Hernández V, Quintero E, José Lozano J, García-Cougil M, Martinez-Arranz I, Castells A, Girónola M, Arroyo R. Plasma MicroRNA Signature Validation for Early Detection of Colorectal Cancer. *Clin Transl Gastroenterol* 2019; 10: e00003 [PMID: 30702491 DOI: 10.14398/cTG.0000000000000037]

Zhang H, Zhu M, Shan X, Zhou M, Wang T, Zhang J, Tao J, Cheng W, Chen G, Li J, Liu P, Wang Q, Zhu W. A panel of seven-miRNA signature in plasma as potential biomarker for colorectal cancer diagnosis. *Gene* 2019; 687: 246-254 [PMID: 30058288 DOI: 10.1016/j.gene.2018.11.055]

Liu X, Pan B, Sun L, Chen X, Zeng K, Xu M, Pan Y, Sun H, Xu T, Hu X, Wang S. Circulating Serum Exosomal miR-27a and miR-130a Act as Novel Diagnostic and Prognostic Biomarkers of Colorectal Cancer. *Cancer Epidemiol Biomarkers Prev* 2018; 27: 746-754 [PMID: 29677236 DOI: 10.1158/1055-9965.EPI-17-0067]

Sazanov AA, Kisel'yova EV, Zakharenko AA, Romanov MN, Zaraysky MJ. Plasma and saliva miR-21 expression in colorectal cancer patients. *J Appl Genet* 2017; 58: 231-237 [PMID: 27910062 DOI: 10.1007/s13355-016-0379-0]

Bosch S, Berkthout DJ, Ben Larbi I, de Mej T, de Boer NK. Fecal volatile organic compounds for early detection of colorectal cancer: where are we now? *J Cancer Res Clin Oncol* 2019; 145: 223-234 [PMID: 30554400 DOI: 10.1007/s00432-018-2821-3]

Bond A, Greenwood R, Lewis S, Corble S, Barkar S, O'Toole P, Rooney P, Burkitt M, Hold G, Probert C. Volatile organic compounds emitted from faeces as a biomarker for colorectal cancer. *Aliment Pharmacol Ther* 2019; 49: 1005-1012 [PMID: 30828253 DOI: 10.1111/ajp.15140]

Ishibe A, Ota M, Takeshita A, Tsuibo H, Kizuka S, Oka H, Suzuki S, Nakagawa K, Suzuki M, Moriyama M, Watanabe J, Taguri M, Kunisaki C, Endo I. Detection of gas components as a novel diagnostic method for colorectal cancer. *Ann Gastroenterol Surg* 2018; 2: 147-153 [PMID: 29863156 DOI: 10.1002/ags3.12056]

Sonoda H, Kohnoe S, Yamazato T, Satoh Y, Morizono G, Shikata K, Morita M, Watanabe A, Morita M, Uchiyama K, Ota M, Takeshita A, Tsuboi H, Kizuka S, Oka H, Suwa Y, Suzuki S, Nakagawa K, Suzuki M, Moriyama M, Watanabe J, Taguri M, Kunisaki C, Endo I. Detection of gas components as a novel diagnostic method for colorectal cancer. *Ann Gastroenterol Surg* 2018; 2: 147-153 [PMID: 29863156 DOI: 10.1002/ags3.12056]

Zonta G, Anania G, Feo C, Gaiardo A, Gherardi S, Giberti A, Guidi V, Landini N, Palmonari C, Ricci L, de Togni A, Malagù C. Use of gas sensors and FOBT for the early detection of colorectal cancer. *Actuators B* 2018; 262: 884-891 [DOI: 10.1016/j.snb.2018.01.225]

Bezabeh T, de Meij TG, de Boer NK. Fecal volatile organic compounds for early detection of colorectal cancer: a review. *Acta Radiol Diagn (Stockh)* 1975; 16: 641-653 [PMID: 3092 DOI: 10.1080/139463520.1975.11862868]
Phospholipids as Potential Biomarkers for Differentiating Different Pathophysiological states of lung, stomach, intestine, and pancreas. J Cancer 2017; 8: 2191-2204 [PMID: 28819420 DOI: 10.1715/jca.19120]

Zhang Y, He C, Qiu L, Wang Y, Qin X, Liu Y, Li Z. Serum Unsaturated Free Fatty Acids: A Potential Biomarker Panel for Early-Stage Detection of Colorectal Cancer. J Cancer 2016; 7: 477-483 [PMID: 26918062 DOI: 10.7150/jca.13870]

Gu H, Du J, Carnevale Noto F, Carroll PA, Turner SJ, Chiorean EG, Eisenman RN, Raferty D. Metabolomics method to comprehensively analyze amino acids in different domains. Analyst 2015; 140: 2726-2734 [PMID: 25699245 DOI: 10.1039/c5ana02386h]

Zhu J, Djakovic D, Deng L, Gu H, Himmati F, Chiorean EG, Raferty D. Colorectal cancer detection using targeted serum metabolic profiling. J Proteome Res 2014; 13: 4120-4130 [PMID: 25126899 DOI: 10.1021/pr400494r]

Nishiumi S, Kobayashi T, Ikeda A, Yoshiie T, Kibi M, Izami Y, Okano T, Hayashi N, Kawano S, Takenawa T, Azuma T, Yoshida M. A novel serum metabolites-based diagnostic approach for colorectal cancer. PLoS One 2012; 7: e40459 [PMID: 22792336 DOI: 10.1371/journal.pone.0040459]

Li F, Qin X, Chen H, Qiu L, Guo Y, Liu H, Chen G, Song G, Wang X, Li F, Guo S, Wang B, Li Z. Lipid profiling for early diagnosis and progression of colorectal cancer using direct-infusion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun Mass Spectrom 2013; 27: 24-34 [PMID: 23239314 DOI: 10.1002/rcm.6420]

Ritchie SA, Ahiahonwu PW, Jayasinghe D, Heath D, Liu J, Lu Y, Jin W, Kavianiour A, Yamazaki Y, Khan AM, Hosssain M, Su-Myat Wood KL, Krenitsky T, Takekawa I, Miyake M, Sekimoto M, Mondon M, Matusbara H, Nomura F, Goodenow DB. Reduced levels of hydroxylated, polyunsaturated ultralong-chain fatty acids in the serum of colorectal cancer patients: implications for early screening and detection. BMC Med 2010; 8: 13 [PMID: 2056336 DOI: 10.1186/1741-7015-8-13]

Tan B, Qiu Y, Zou X, Chen T, Xie G, Cheng Y, Dong T, Zhao L, Feng B, Hu X, Xu LZ, Xiao A, Zhang M, Cai G, Cai S, Zhou Z, Zheng M, Zhang Y, Jia W. Metabonomics identifies serum metabolite markers of colorectal cancer. J Proteome Res 2013; 12: 3000-3009 [PMID: 23675754 DOI: 10.1021/pr300337b]

Nishiumi S, Kobayashi T, Kawana S, Uno Y, Sakai K, Okamoto K, Yamada S, Sudo K, Yamaji T, Saito Y, Kanemitsu Y, Okita NT, Saito H, Tsugane S, Azuma T, Qijin M, Yoshida M. Investigations in the possibility of early detection of colorectal cancer by gas chromatography-triple-quadrupole mass spectrometry. Oncotarget 2017; 8: 17115-17126 [PMID: 28179577 DOI: 10.18632/oncotarget.15081]

Li S, Guo B, Song J, Deng X, Cong Y, Li P, Zhao K, Liu L, Xiao G, Xu F, Ye Y, Zhao Z, Yu M, X. Yang J, Zhang J. Plasma choline-containing phospholipids: potential biomarkers for colorectal cancer progression. Metabolomics 2013; 9: 202-212 [DOI: 10.1007/s11306-012-0439-3]

Zhao Z, Xiao Y, Elson P, Tan H, Plummer SJ, Berk M, Angg PNG, Lavercy IC, Achkart JP, Li L, Casey G, Xu Y. Plasma lysophosphatidylcholine levels: potential biomarkers for colorectal cancer. J Clin Oncol 2007; 25: 2696-2701 [PMID: 17662074 DOI: 10.1200/jco.2006.08.5571]

Jing Y, Wu X, Gao P, Fang Z, Wu J, Wang Q, Li C, Zhao Z, Cao Y. Rapid differentiating colorectal cancer and colorectal polyp using dried blood spot mass spectrometry metabolic approach. JUmbM Life 2017; 69: 347-354 [PMID: 28322027 DOI: 10.1002/ubm.1617]

Hiramatsu K, Takahashi K, Yamaguchi T, Matsumoto H, Miyamoto H, Tanaka S, Tanaka C, Tanamaro Y, Imajo M, Kagawuchi M, Toi M, Mori T, Kato K, Wakahisa S, N.,(12)-Diacetylspermine as a sensitive and specific novel marker for early- and late-stage colorectal and breast cancers. Clin Cancer Res 2005; 11: 2986-2990 [PMID: 15837752 DOI: 10.1158/1078-0432.CCR-04-2275]

Deng L, Ismond K, Liu Z, Constabile J, Wang H, Alatise OJ, Weiser MR, Kingham TP, Chang D. Urinary Metabolomics to Identify a Unique Biomarker Panel for Detecting Colorectal Cancer: A Multicenter Study. Cancer Epidemiol Biomarkers Prev 2019; 28: 1283-1291 [PMID: 31159393 DOI: 10.1158/1055-9965.EPI-18-1291]

Wang Z, Lin Y, Liang J, Huang Y, Ma C, Liu X, Yang J. NMR-based metabolomics identifies potential urinary biomarkers for early colorectal cancer detection. Oncotarget 2017; 8: 105819-105831 [PMID: 29286295 DOI: 10.18632/oncotarget.22402]

Rozalski R, Gackowski D, Siomek-Gorecka A, Starczak M, Modrzejewska M, Banaszekiewicz Z, Olimski R. Urinary 5-hydroxymethyluracil and 8-oxo-7,8-dihydroguanine as potential biomarkers in patients with colorectal cancer. Biomarkers 2015; 20: 287-291 [PMID: 26329254 DOI: 10.1089/biom.2015.008680]

Hsu WY, Chen CJ, Huang YC, Tsai FJ, Jeng LB, Lai CC. Urinary nucleosides as biomarkers of breast, colon, lung, and gastric cancer in Taiwanese. PLoS One 2013; 8: e81701 [PMID: 24367489 DOI: 10.1371/journal.pone.0081701]

Cheng Y, Xie G, Chen T, Qiu Y, Zou X, Zheng M, Tan B, Feng B, Dong T, He P, Zhao L, Zhao A, Xu LX, Zhang Y, Jia W. Distinct urinary metabolic profile of human colorectal cancer. Clin Cancer Res 2012; 11: 1354-1363 [PMID: 22148915 DOI: 10.1158/1078-0432.CCR-10-0889]

Feng B, Zheng MH, Zheng YF, Lu AG, Li JW, Wang ML, Ma JJ, Xu GW, Li Y, Zha ZG. Normal and modified urinary nucleosides represent novel biomarkers for colorectal cancer diagnosis and surgery monitoring. J Gastroenterol Hepatol 2005; 20: 1913-1919 [PMID: 16336453 DOI: 10.1111/j.1440-1746.2005.03883.x]

Zhang YF, Yang J, Zhao XJ, Feng B, Kong HW, Chen YJ, Li S, Zheng MH, Xu GW. Urinary nucleosides as biological markers for patients with colorectal cancer. World J Gastroenterol 2005; 11: 3871-3876 [PMID: 15991285 DOI: 10.3748/wjg.v11.i25.s387]

Suehiro Y, Sakai K, Nishioka M, Hashimoto S, Takami T, Higaki S, Shinoy Y, Hazama S, Oka M, Nagano H, Sakaide I, Yamasaki T. Highly sensitive stool DNA testing of Fusobacterium nucleatum as a marker for detection of colorectal tumours in a Japanese population. Ann Clin Biochem 2017; 54: 88-91 [PMID: 27126270 DOI: 10.1177/0004563216643970]

Wong SH, Kwong TN, Chow TC, Luk AKC, Dai RZW, Nakatsu G, Lam TYT, Zhang L, Wu JCY, Chan FKL, Ng SMS, Wong MCS, Ng SC, Wu WK, Yu J, Sung JYJ. Quantitation of faecal Fusobacterium improves faecal immunochemical test in detecting advanced colorectal neoplasia. Gut 2017; 66: 1441-1448 [PMID: 27797940 DOI: 10.1136/gutjnl-2016-312766]

Liang Q, Chiu J, Chen Y, Huang Y, Higashimori A, Fang J, Brim H, Ashktorab H, Ng SC, Ng SMS, Zheng S, Chan FKL, Sung JYJ, Yu J. Fecal Bacteria Act as Novel Biomarkers for Noninvasive Diagnosis of Colorectal Cancer. Clin Cancer Res 2017; 23: 2061-2070 [PMID: 27697996 DOI: 10.1158/1078-0432.CCR-16-1599]

Eklid V, Lifgren-Burstöm A, Zingmark C, Edin S, Larsson P, Karling P, Alexeoy E, Rutegård J.
McFerran E, O'Mahony JF, Fallis R, McVear D, Zauber AG, Kee F. Evaluation of the Effectiveness and
Cost-Effectiveness of Personalized Surveillance After Colorectal Adenomatous Polypectomy. *Epidemiol Rev* 2017; 39: 148-160 [PMID: 28402402 DOI: 10.1093/epirev/mxx002]

157 Lo SH, Halloran S, Snowball J, Wardle J, von Wagner C. Colorectal cancer screening uptake over three biennial invitation rounds in the English bowel cancer screening programme. *Gut* 2015; 64: 282-291 [PMID: 24812001 DOI: 10.1136/gutjnl-2013-306144]

158 Hirst Y, Stoffel S, Baio G, McGregor L, von Wagner C. Uptake of the English Bowel (Colorectal) Cancer Screening Programme: an update 5 years after the full roll-out. *Eur J Cancer* 2018; 103: 267-273 [PMID: 30196989 DOI: 10.1016/j.ejca.2018.07.135]

159 Palmer CK, Thomas MC, von Wagner C, Raine R. Reasons for non-uptake and subsequent participation in the NHS Bowel Cancer Screening Programme: a qualitative study. *Br J Cancer* 2014; 110: 1705-1711 [PMID: 24619071 DOI: 10.1038/bjc.2014.125]

160 Reynolds LM, Bissett IP, Consdine NS. Emotional predictors of bowel screening: the avoidance-promoting role of fear, embarrassment, and disgust. *BMC Cancer* 2018; 18: 518 [PMID: 29720112 DOI: 10.1186/s12885-018-4423-5]
