Review Article

Biomarkers Beyond Colonoscopy: A Review of Stool and Blood Tools for Colorectal Cancer Management

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Article History
Submitted: 05/10/2025; Accepted: 09/10/2025: Published: 15/11/2025

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ABSTRACT

Colonoscopy remains the gold standard for colorectal cancer (CRC) detection, but its invasiveness, cost, and limited accessibility highlight the need for non-invasive biomarkers. To evaluate stool- and blood-based biomarkers as tools for CRC screening, surveillance, and disease monitoring, comparing their diagnostic accuracy, clinical utility, and limitations. A narrative review of recent evidence (2014–2024) on stool (FIT, multitarget DNA) and blood-based biomarkers (SEPT9, circulating tumour DNA, multi-analyte assays) was conducted, focusing on sensitivity, specificity, and real-world application. FIT demonstrates high specificity but modest sensitivity for advanced adenomas. Multitarget stool DNA improves sensitivity for CRC but at the expense of specificity. Blood-based assays, including SEPT9 methylation and ctDNA, show utility in non-invasive detection, minimal residual disease monitoring, and relapse prediction. However, challenges include cost, variability across populations, and infrastructural requirements. Stool- and blood-based biomarkers represent valuable adjuncts to colonoscopy, offering scalable, patient-friendly options for CRC management. Future directions include multi-omics platforms, artificial intelligence integration, and strategies to enhance accessibility in low-resource settings.

Keywords: Colorectal cancer, Biomarkers, Circulating tumour DNA, Fecal immunochemical test, Minimal residual disease, Non-invasive diagnostics, Precision oncology, SEPT9, Screening, Stool DNA

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the second leading cause of cancer-related mortality worldwide, accounting for nearly 2 million new cases and 1 million deaths annually [1]. The global burden is projected to rise by 60% by 2040, largely driven by ageing populations and adoption of Westernized lifestyles in low- and middle-income countries (LMICs)[2]. Despite advances in treatment, survival strongly depends on stage at diagnosis, with five-year survival exceeding 90% for localized disease but dropping below 15% in advanced stages [3].

Colonoscopy remains the gold standard for CRC screening, enabling both detection and removal of premalignant adenomas. However, its uptake is hindered by invasiveness, cost, limited availability, and patient aversion [4]. Population-based colonoscopy programs are well established in high-income countries but remain scarce in LMICs due to resource constraints and infrastructural challenges [5]. These limitations underscore the need for non-invasive, cost-effective, and widely applicable

Article Access
Website: www.wjmbs.org
10.5281/zenodo.17980436

How to cite this article
Ugwu IV, Gbaa ZL, Ngbea JA, Umobong EO, Omolabake BI, Tsegha LJ, Otene SA, Gbaa FA. Biomarkers Beyond Colonoscopy: A Review of Stool and Blood Tools for Colorectal Cancer Management. West J Med & Biomed Sci. 2025;6(4):324-333. DOI:10.5281/zenodo.17980436

alternatives.

Stool- and blood-based biomarkers have emerged as promising tools for CRC detection and monitoring. Stool tests such as the faecal immunochemical test (FIT) and stool DNA assays are widely evaluated, offering moderate-to-high sensitivity for CRC and advanced adenomas [6-8]. Blood-based biomarkers, including circulating tumor DNA (ctDNA), methylated DNA assays (e.g., SEPT9), circulating tumour cells (CTCs), and protein markers such as carcinoembryonic antigen (CEA), are increasingly integrated into screening and disease monitoring algorithms [9-11].

Beyond screening, these biomarkers play a role in Minimal Residual Disease (MRD) monitoring, enabling early detection of recurrence and guiding adjuvant therapy decisions [12]. Furthermore, advances in multi-target stool assays, next-generation sequencing, and liquid biopsy platforms are enhancing diagnostic performance and clinical applicability [13,14].

TYPES OF BIOMARKERS IN COLORECTAL CANCER

Biomarkers for colorectal cancer (CRC) are broadly classified into stool-based and blood-based assays. These non-invasive modalities provide alternatives to colonoscopy, offering improved patient compliance and potential for population-level screening. Each class has unique diagnostic advantages and limitations.

Stool-Based Biomarkers

Faecal Occult Blood Test (FOBT): The guaiac-based faecal occult blood test (gFOBT) was among the earliest stool screening methods. It detects peroxidase activity of haemoglobin in stool but suffers from low sensitivity, dietary interference, and poor detection of advanced adenomas. Consequently, its role has diminished in favour of immunochemical methods [15].

Faecal Immunochemical Test (FIT): Specifically targets human haemoglobin, improving both sensitivity and specificity compared with gFOBT. Meta-analyses report sensitivities of 70–80% for CRC and higher specificity for advanced adenomas [16,17]. FIT is now widely adopted in organized screening programs in high-income countries due to its low cost, ease of use, and reproducibility.

Stool DNA and Methylation Assays: Multitarget stool DNA testing, exemplified by Cologuard® (which combines KRAS mutations, aberrant methylation of NDRG4 and BMP3, and FIT), has shown superior sensitivity for CRC detection compared with FIT alone [18]. The pivotal study reported a sensitivity of 92.3% for CRC versus 73.8% for FIT [19]. Methylation assays targeting genes such as SDC2, VIM, and SEPT9 in stool samples further enhance detection of early-stage lesions [20,21].

Microbiome-Based Signatures: Alterations in gut microbiota composition, including enrichment of *Fusobacterium nucleatum* and *Bacteroides fragilis*, have been associated with CRC pathogenesis [22]. Panels combining microbial markers with FIT improve diagnostic accuracy, though clinical implementation remains in early stages [23].

Blood-Based Biomarkers

Circulating tumour DNA (ctDNA): Reflects tumour-derived fragments released into circulation and enables highly sensitive detection of somatic mutations, methylation changes, and copy number variations [24]. Studies demonstrate its utility in early detection, recurrence monitoring, and minimal residual disease (MRD) surveillance [25].

Circulating Tumour Cells (CTCs): These are intact tumor cells shed into the bloodstream. Although promising as prognostic indicators, their clinical use is limited by low abundance, technical detection challenges, and lack of standardization [26].

Methylated DNA Assays: The methylated SEPT9 assay is the most extensively studied blood-based biomarker for CRC screening. Large prospective studies demonstrated sensitivities ranging from 68% to 79% with specificities of 80–90% [27,28]. While inferior to colonoscopy, SEPT9 offers a non-invasive alternative for individuals unwilling or unable to undergo stool-based testing.

Protein Biomarkers: Carcinoembryonic antigen (CEA) remains widely used for CRC surveillance rather than screening due to low sensitivity for early-stage disease [29]. Other proteins, such as CA19-9 and novel panels combining multiple proteins, are under

investigation but have yet to achieve clinical adoption [30].

Extracellular Vesicles and MicroRNAs: Exosomes and microRNAs (miRNAs) are gaining recognition as stable, minimally invasive biomarkers. Specific miRNA signatures, including miR-21, have shown diagnostic and prognostic potential [31]. However, their clinical integration is limited by technical and cost challenges.

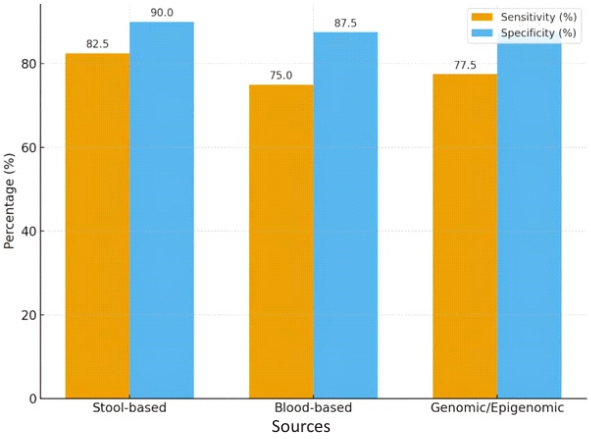
Diagnostic Performance of Biomarkers

The diagnostic accuracy of non-invasive biomarkers for colorectal cancer (CRC) has been extensively investigated, with particular focus on stool-based and blood-based modalities.

Stool-Based Biomarkers: The faecal immunochemical test (FIT) is widely adopted for CRC screening due to its specificity for human haemoglobin. Meta-analyses report sensitivities ranging from 70% to 80% for CRC, though sensitivity for advanced adenomas is lower at approximately 25% to 40% [32]. Multitarget stool DNA (mt-sDNA), which combines molecular markers of DNA methylation and mutation with haemoglobin immunoassay, demonstrates significantly higher sensitivity than FIT (92% vs. 74%), albeit with lower specificity (87% vs. 95%) [33]. Real-world implementation studies have further confirmed the utility of mt-sDNA, particularly in detecting early-stage disease [34]]. However, both FIT and mt-sDNA face reduced sensitivity in proximal colon lesions [35].

Blood-Based Biomarkers: Circulating tumour DNA (ctDNA) assays have emerged as a transformative tool in CRC detection and monitoring. Ultra-deep sequencing approaches have demonstrated sensitivities exceeding 80% for stage II–III CRC [36]. In addition, ctDNA outperforms carcinoembryonic antigen (CEA), particularly in detecting minimal residual disease (MRD) and predicting relapse [37]. Multi-analyte blood tests that integrate ctDNA, protein biomarkers, and epigenetic alterations further improve detection, achieving sensitivities of 70% to 85% for CRC at high specificity [38]. Methylated SEPT9 DNA is the most studied blood-based marker, with a pooled sensitivity of 68% to 75% and specificity of 80% to 90%, making it a potential adjunct to stool-based testing [39]. (Figure 1 and 2)

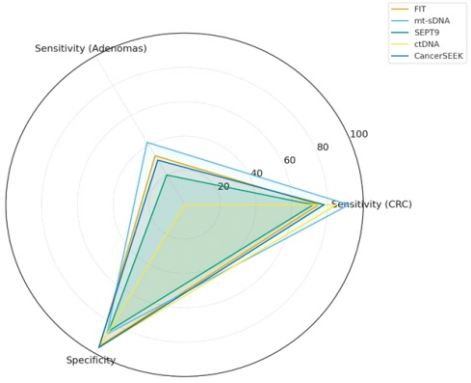
Comparative Effectiveness: Direct comparative studies suggest that stool-based tests remain more sensitive for early detection of CRC, while blood-based biomarkers offer advantages in surveillance and MRD monitoring [40]. Combining both stool and blood modalities in a multimodal strategy may enhance diagnostic yield and address the limitations of single-modality testing [41]. (Figure 1 and 2).



Grouped Bar Chart comparing sensitivity (CRC & adenomas) and specificity across stool- and blood-based biomarkers for colorectal cancer.
It clearly shows:

- *mt-sDNA has the highest CRC sensitivity but lower specificity.*
- *FIT is very specific but weaker for adenomas.*
- *ctDNA and CancerSEEK score high in specificity but show trade-offs in adenoma sensitivity.*

Figure 1: Sensitivity and Specificity of Stool and Blood Based Biomarkers for CRC



Radar Chart comparing stool- and blood-based biomarkers for colorectal cancer.

- *Which shows the overall diagnostic profile of each test at a glance.*
- *mt-sDNA has the broadest coverage (strong CRC & adenoma sensitivity, decent specificity).*
- *FIT is highly specific but weaker on adenomas. ctDNA and CancerSEEK stand out for specificity but miss adenomas.*

Figure 2: Performance profile for CRC Biomarkers.

RESULTS:

- FIT remains the most accessible test, with excellent specificity but limited adenoma detection.
- mt-sDNA provides superior sensitivity, especially for early CRC, but at the cost of specificity.
- SEPT9 methylation assays offer a convenient blood-based alternative but have limited adenoma detection.
- ctDNA assays excel in MRD detection and prognosis, rather than population-level screening.
- Multi-analyte blood tests hold promise for early detection but require further validation

CLINICAL UTILITY AND COMPARATIVE EFFECTIVENESS

The integration of stool- and blood-based biomarkers into colorectal cancer (CRC) management depends not only on diagnostic accuracy but also on their utility in **screening, surveillance, and minimal residual disease (MRD) monitoring.**

Screening Applications: FIT remains the most widely implemented biomarker-based screening tool globally due to its low cost, ease of administration, and high specificity [42]. Its effectiveness in population-level screening programs is well documented, although its sensitivity for advanced adenomas remains suboptimal [43]. Multitarget stool DNA (mt-sDNA) testing offers higher sensitivity for early-stage CRC and advanced adenomas, making it particularly valuable for patients at increased risk or those unwilling to undergo colonoscopy [44]. However, its reduced specificity and cost have limited widespread adoption, especially in low- and middle-income countries (LMICs) [45].

Surveillance and Risk Stratification: Blood-based biomarkers such as methylated SEPT9 and ctDNA hold promise in stratifying surveillance intensity after polypectomy or curative resection. The SEPT9 assay, while not sufficiently sensitive for standalone screening, may be used in individuals declining stool-based testing [46]. In contrast, ctDNA has demonstrated superior predictive value compared to carcinoembryonic antigen (CEA) in detecting recurrence, often identifying relapse months before radiographic evidence [47,48]. This positions ctDNA as a valuable adjunct in personalized surveillance strategies.

Minimal Residual Disease (MRD) Monitoring: ctDNA is emerging as a key tool in guiding adjuvant therapy decisions. Studies show that postoperative ctDNA positivity correlates strongly with disease recurrence, providing a rationale for intensifying therapy in high-risk patients while sparing low-risk individuals from unnecessary toxicity [49,50]. Such approaches exemplify precision oncology in CRC management.

Comparative Effectiveness: Direct head-to-head comparisons suggest that stool-based biomarkers are more effective for **population-level screening**, while blood-based biomarkers offer greater utility in **treatment monitoring and MRD assessment.** Combining these modalities in a tiered approach may optimize outcomes: stool-based tests for initial detection, followed by ctDNA for treatment guidance and surveillance [51]. Cost-effectiveness analyses indicate that while FIT remains the most economically viable in LMICs, incorporation of ctDNA into high-resource settings could significantly improve outcomes when balanced against recurrence-related healthcare costs [52].

CHALLENGES AND LIMITATIONS

Despite the promising role of stool- and blood-based biomarkers in colorectal cancer (CRC)

management, several challenges limit their widespread adoption and integration into clinical practice.

Variability in Sensitivity and Specificity: Although FIT and multitarget stool DNA (mt-sDNA) demonstrate high sensitivity for CRC, their performance in detecting advanced adenomas and serrated lesions remains suboptimal [53,54]. Blood-based tests, including methylated SEPT9 and circulating tumor DNA (ctDNA), show variable sensitivity in early-stage disease, which is the critical window for intervention [55]. Such variability across populations and disease stages hampers universal applicability.

Cost and Accessibility: The economic burden of advanced biomarker testing is a significant barrier, particularly in low- and middle-income countries (LMICs). FIT remains cost-effective for population-level screening [56]. whereas mt-sDNA and ctDNA assays are substantially more expensive and require advanced laboratory infrastructure [57]. This disparity risks widening global inequities in CRC outcomes.

Standardization and Regulatory Approval: There is currently no universal consensus on assay protocols, cutoff values, or reporting standards for stool- and blood-based biomarkers [58]. Inconsistent methodologies across studies limit comparability and hinder clinical translation. Regulatory approvals have been achieved for tests like mt-sDNA and SEPT9 in selected countries [59], but broader harmonization is needed.

False Positives and Overdiagnosis: Biomarker-based screening can lead to increased false positives compared to colonoscopy, especially with mt-sDNA, which lowers specificity relative to FIT [54]. This may result in unnecessary colonoscopies, anxiety, and higher healthcare costs. Furthermore, the long-term implications of biomarker-driven overdiagnosis of indolent lesions remain poorly defined.

Technological and Logistical Barriers: Blood-based biomarker assays such as ctDNA require highly sensitive sequencing platforms and bioinformatics expertise [60]. The need for ultra-deep sequencing and standardization of pre-analytical variables (sample collection, storage, and processing) adds complexity and limits scalability outside specialized centers [61].

Patient and Provider Acceptance: While non-invasive tests are generally more acceptable to patients than colonoscopy, awareness and trust in novel biomarkers remain limited [62]. Clinician hesitancy due to insufficient long-term outcome data further restricts adoption.

FUTURE DIRECTIONS

The growing body of evidence supporting stool- and blood-based biomarkers for colorectal cancer (CRC) underscores their potential to transform screening and disease monitoring. However, their integration into clinical practice requires overcoming current limitations through innovation, validation, and system-level adaptation.

Multimodal and Combined Approaches: No single biomarker currently achieves the diagnostic accuracy of colonoscopy. Combining stool- and blood-based assays, or integrating them with imaging and clinical risk scores, may yield higher sensitivity and specificity than standalone modalities [63]. Emerging models suggest that hybrid approaches could optimize resource use and minimize false positives [64].

Technological Advances: Next-generation sequencing (NGS) and digital PCR are enhancing ctDNA detection at ultra-low variant allele frequencies, improving sensitivity for early-stage CRC and minimal residual disease (MRD) [65]. Integration of machine learning to interpret multi-omics signatures (DNA, RNA, proteins, metabolites) is a promising frontier for biomarker-driven precision screening [66].

Cost Reduction and Accessibility: Economic feasibility is a major barrier, especially in low- and middle-income countries (LMICs). Scaling production, simplifying assays, and adopting tiered pricing models could improve affordability [67]. Public–private partnerships will be critical to ensure equitable global access [68].

Standardization and Regulatory Frameworks: Consensus on analytical methods, reporting thresholds, and interpretation guidelines is essential for broad clinical implementation. International

collaborative consortia should drive harmonization efforts and accelerate regulatory approvals for novel biomarkers [69].

Integration into Screening Programs: Stool-based tests such as FIT should remain the cornerstone of mass CRC screening programs due to their accessibility. However, mt-sDNA and ctDNA may be incorporated as second-tier or complementary tests for individuals with inconclusive results, high risk, or poor colonoscopy compliance [70]. Such stratified strategies could optimize detection while preserving cost-effectiveness.

Longitudinal and Outcome-Oriented Studies: Large-scale prospective trials are required to evaluate biomarker-guided screening and MRD monitoring in terms of survival outcomes, recurrence reduction, and healthcare cost savings [71]. Establishing long-term evidence will enhance provider confidence and facilitate adoption in routine care.

CONCLUSION

Stool- and blood-based biomarkers provide non-invasive tools that complement colonoscopy for colorectal cancer screening, surveillance, and monitoring. FIT and stool DNA tests improve population-level detection, while blood assays like SEPT9 and ctDNA expand applications in precision oncology. Despite their promise, challenges of cost, variable performance, and limited access—especially in low-resource settings—remain. Future integration of multi-omics and AI-driven platforms could make biomarker-based strategies more accurate, accessible, and personalized, ultimately enhancing early detection and patient outcomes.

Recommendations

Expand population screening with FIT or multitarget stool DNA as cost-effective, non-invasive options.

Adopt blood-based biomarkers (e.g., SEPT9, ctDNA) to complement stool tests, especially for patients reluctant to undergo colonoscopy.

Prioritize local validation of biomarker performance across diverse populations.

Strengthen infrastructure in low- and middle-income countries to improve access to advanced biomarker testing.

Promote standardization of assay protocols and regulatory frameworks for global comparability.

Encourage research investment in multi-omics and AI-driven biomarker platforms for improved accuracy and personalization.

Conflict of interest

There are no conflicts of interest.

Funding sources For this review article, we did not receive any grants or funding.

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