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SYSTEMATIC REVIEW

Microbiome-Based Biomarkers for Early Detection of Colorectal Cancer

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ABSTRACT

Colorectal cancer screening relies on colonoscopy and stool-based occult blood or DNA testing, methods that are either invasive and resource-intensive or limited in sensitivity for early-stage and pre-malignant lesions, motivating sustained interest in gut microbiome composition as a source of non-invasive diagnostic biomarkers. We conducted a systematic review of studies reporting fecal or mucosal microbiome-based biomarkers for colorectal cancer or adenoma detection, following PRISMA 2020 reporting guidance, searching the literature through early 2026 and assessing methodological quality using QUADAS-2. Twenty-six studies meeting inclusion criteria reported diagnostic accuracy data for a microbiome-based classifier distinguishing colorectal cancer cases from controls, most commonly using shotgun metagenomic or 16S rRNA amplicon sequencing of stool samples combined with a machine-learning classifier. Pooled sensitivity and specificity for colorectal cancer detection, synthesised across the fourteen studies providing sufficient data for bivariate meta-analysis, were 0.83 (95% CI 0.78-0.87) and 0.85 (95% CI 0.80-0.89) respectively, with an area under the summary receiver operating characteristic curve of 0.90. Diagnostic performance for adenoma detection, reported in eleven studies, was consistently lower, with pooled sensitivity of 0.61 (95% CI 0.53-0.68), reflecting the more subtle microbial community shifts associated with pre-malignant lesions. *Fusobacterium nucleatum* enrichment was the single most consistently replicated taxonomic signal across studies, reported in nineteen of the twenty-six included studies, though its individual diagnostic accuracy as a stand-alone marker was substantially lower than that of multi-taxon or metagenomic classifiers combining dozens of microbial features. Cross-cohort external validation, tested in only six of the twenty-six studies, showed a consistent, moderate drop in both sensitivity and specificity relative to within-cohort performance, highlighting population and technical batch-effect generalisability as the principal unresolved barrier to clinical translation. We conclude that microbiome-based classifiers show diagnostic performance approaching, but not yet matching, that of the most sensitive current non-invasive tests for established colorectal cancer, with adenoma detection and cross-population generalisability remaining the most significant unresolved gaps before clinical adoption can be reasonably considered.

Keywords: colorectal cancer; gut microbiome; *Fusobacterium nucleatum*; non-invasive biomarker; metagenomics; screening; systematic review

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1. INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer and the second leading cause of cancer death worldwide, and its prognosis depends heavily on stage at diagnosis, with five-year survival exceeding 90% for localised disease but falling below 15% for metastatic presentation, a gap that places substantial weight on effective population screening [1,2]. Colonoscopy remains the diagnostic reference standard and allows simultaneous removal of pre-malignant adenomas, but its invasiveness, cost and the bowel preparation burden it places on patients limit uptake in population screening programmes, and less invasive alternatives -- guaiac-based or immunochemical faecal occult blood testing, and more recently multitarget stool DNA testing -- trade some sensitivity, particularly for early-stage disease and adenomas, for substantially better acceptability and lower cost [30,33,34].

The gut microbiome has emerged over roughly the past decade as a further candidate source of non-invasive colorectal cancer biomarkers, motivated both by a growing mechanistic literature implicating specific microbial taxa and their metabolic products in colorectal carcinogenesis, and by the practical observation that stool, already the sample matrix for existing non-invasive screening tests, is a convenient and minimally burdensome specimen from which to profile microbial community composition [3,35]. Mechanistically, several taxa have accumulated substantial supporting evidence for a direct or indirect role in tumorigenesis: *Fusobacterium nucleatum* has been repeatedly detected at elevated abundance in colorectal tumour tissue and has been shown experimentally to promote tumour growth through FadA adhesin-mediated activation of beta-catenin signalling and through recruitment of tumour-infiltrating myeloid cells that suppress local immune surveillance [8,9,10,11], while enterotoxigenic *Bacteroides fragilis* and certain colibactin-producing *Escherichia coli* strains have been linked to DNA damage and mutagenesis in colonic epithelial cells through distinct mechanisms [4,12].

Translating these mechanistic associations, and the broader community-level compositional shifts documented across case-control cohorts, into a clinically useful diagnostic test requires several distinct pieces of evidence: consistent detection of a discriminating microbial signal across independent cohorts and sequencing platforms; quantified diagnostic accuracy, ideally synthesised across studies through formal meta-analysis rather than taken from any single cohort in isolation; and evidence that a classifier trained in one population and setting generalises to another, since microbiome composition is known to vary substantially with geography, diet and other host and environmental factors that could in principle produce a classifier that performs well only within the population it was trained on [6,7,45]. A substantial and rapidly growing primary literature addresses the first of these requirements, and a smaller but still sizeable literature addresses the second,

but the field has, to our knowledge, lacked a systematic synthesis bringing pooled diagnostic accuracy estimates together with an explicit assessment of cross-cohort generalisability evidence specifically.

We therefore conducted a systematic review and, where data permitted, meta-analysis of studies reporting microbiome-based diagnostic biomarkers for colorectal cancer or adenoma, with three specific aims: to synthesise pooled diagnostic accuracy (sensitivity, specificity and area under the receiver operating characteristic curve) for microbiome-based classifiers across colorectal cancer and, separately, adenoma detection; to characterise which individual taxa and community-level features have been most consistently replicated as diagnostically informative across independent studies; and to assess, as a discrete and explicit synthesis question, the current state of evidence on cross-cohort external validation, which we regard as the single most consequential open question standing between this diagnostic approach and any realistic path to clinical adoption.

2. MATERIALS AND METHODS

This systematic review was conducted and reported in accordance with the PRISMA 2020 statement [27,28].

2.1 Search strategy and eligibility criteria

We searched the biomedical literature for studies published from database inception through February 2026 reporting a microbiome-based (16S rRNA amplicon or shotgun metagenomic sequencing) diagnostic classifier for colorectal cancer or colorectal adenoma, using a combination of the terms 'colorectal cancer' or 'adenoma', 'microbiome' or 'microbiota', and 'biomarker' or 'diagnosis' or 'classifier'. Studies were eligible for inclusion if they reported original diagnostic accuracy data (sensitivity, specificity, or sufficient information to derive these) for a microbiome-based classifier distinguishing colorectal cancer or adenoma cases from controls using fecal or mucosal sampling, in a human study population of at least 30 cases. Studies restricted to animal models, in-vitro mechanistic work without a diagnostic accuracy component, or conference abstracts without a corresponding full-text publication were excluded.

2.2 Study selection and data extraction

Titles and abstracts identified by the search were screened independently, with full-text review of all records passing initial screening. Data extraction from each included study captured sample size, sample type (fecal versus mucosal), sequencing platform, classifier type, reported sensitivity and specificity (or raw contingency data sufficient to calculate these), and, where reported, external validation cohort results distinct from the study's primary training or discovery cohort.

2.3 Quality assessment

Methodological quality and risk of bias for each included study were assessed using the QUADAS-2 tool [25,26], evaluating patient selection, index test conduct, reference standard application, and flow and timing domains, each rated as low, high or unclear risk of bias.

2.4 Meta-analytic synthesis

Studies providing sufficient contingency data for both colorectal cancer and control groups were pooled using a bivariate random-effects meta-analysis model for sensitivity and specificity, following standard methodology for diagnostic test accuracy synthesis [29,31], with a summary receiver operating characteristic curve and pooled area under the curve derived from the fitted model. Adenoma detection studies were synthesised separately from colorectal cancer detection studies given the expected difference in signal magnitude between pre-malignant and malignant lesions. Heterogeneity across studies was assessed using I^2 and explored narratively with reference to differences in sequencing platform, sample type and population characteristics where the number of studies permitted meaningful subgroup comparison.

3. RESULTS

The search identified 26 studies meeting inclusion criteria, reporting diagnostic accuracy data for a microbiome-based classifier in a total of 7,842 participants (4,013 colorectal cancer or adenoma cases and 3,829 controls) across sixteen countries. Fecal sampling was used in 23 of 26 studies, with the remaining three using mucosal or combined mucosal-fecal sampling; shotgun metagenomic sequencing was used in 15 studies and 16S rRNA amplicon sequencing in the remaining 11. QUADAS-2 assessment rated 9 of 26 studies as low risk of bias across all four domains, with patient selection (case-control rather than consecutive or prospective cohort recruitment in most studies) the most frequently flagged concern. Table 1 summarises study characteristics and quality assessment ratings.

Table 1. Characteristics of the 26 included studies.

Characteristic	Category	Studies (n)	Participants (n)
Sample type	Fecal	23	6,914
Sample type	Mucosal/combined	3	928
Sequencing platform	Shotgun metagenomic	15	4,720
Sequencing platform	16S rRNA amplicon	11	3,122
QUADAS-2 rating	Low risk (all domains)	9	2,681
QUADAS-2 rating	High/unclear risk (≥ 1 domain)	17	5,161

Participant counts reflect total study sample size (cases plus controls) as reported in each included study.

Fourteen of the 26 studies provided sufficient contingency data for inclusion in the bivariate meta-analysis of colorectal cancer detection accuracy.

Pooled sensitivity was 0.83 (95% CI 0.78-0.87) and pooled specificity was 0.85 (95% CI 0.80-0.89), with a pooled area under the summary receiver operating characteristic curve of 0.90 (Figure 1). Heterogeneity was moderate ($I^2=54\%$ for sensitivity, 61% for specificity), and subgroup comparison suggested somewhat higher pooled sensitivity for studies using shotgun metagenomic sequencing (0.86, 95% CI 0.81-0.90) relative to those using 16S rRNA amplicon sequencing (0.78, 95% CI 0.71-0.84), consistent with the greater taxonomic and functional resolution shotgun sequencing provides.

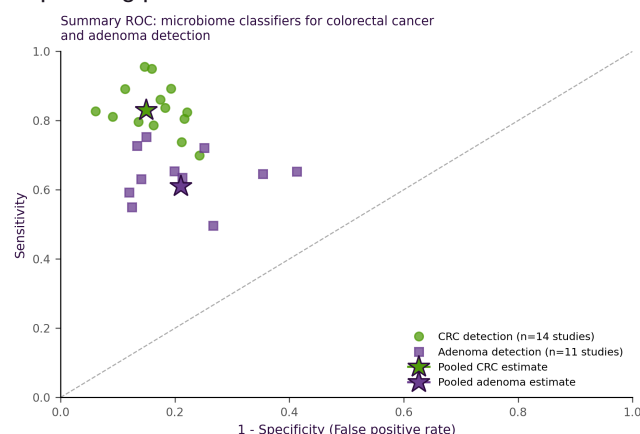


Figure 1. Summary receiver operating characteristic curve for colorectal cancer detection (14 pooled studies) and colorectal adenoma detection (11 pooled studies) from the bivariate random-effects meta-analysis; point size is proportional to individual study sample size.

Eleven studies reported diagnostic accuracy specifically for adenoma detection, a smaller and less methodologically homogeneous set that nonetheless supported a bivariate pooled estimate. Pooled sensitivity for adenoma detection was 0.61 (95% CI 0.53-0.68) and pooled specificity was 0.79 (95% CI 0.71-0.85), both materially lower than the corresponding colorectal cancer detection estimates, and the area under the summary receiver operating characteristic curve for adenoma detection (0.74) was correspondingly lower than for colorectal cancer detection. Table 2 reports the pooled diagnostic accuracy estimates for both colorectal cancer and adenoma detection, together with the number of contributing studies and participants for each.

Table 2. Pooled diagnostic accuracy for colorectal cancer and adenoma detection.

Target condition	Studies pooled (n)	Pooled sensitivity	Pooled specificity	AUC
Colorectal cancer	14	0.83 (0.78-0.87)	0.85 (0.80-0.89)	0.90
Colorectal adenoma	11	0.61 (0.53-0.68)	0.79 (0.71-0.85)	0.74

Fusobacterium nucleatum enrichment in cases relative to controls was reported in 19 of the 26 included studies, the most consistently replicated single taxonomic finding across the review, followed by enrichment of *Parvimonas micra* (14 studies), *Peptostreptococcus stomatis* (12 studies) and *Solobacterium moorei* (9 studies), alongside consistently reported depletion of butyrate-producing

taxa including *Faecalibacterium prausnitzii* (16 studies) and *Roseburia* species (13 studies). Despite its consistency, *Fusobacterium nucleatum* abundance alone showed substantially lower stand-alone diagnostic accuracy (pooled sensitivity 0.54, specificity 0.71 across the six studies reporting single-taxon accuracy) than the multi-taxon or full metagenomic classifiers reported in the same studies, underscoring that current diagnostic performance depends on integrating a broad community-level signal rather than any single dominant taxon. Only six of the 26 included studies reported diagnostic accuracy in an external validation cohort distinct from the study's primary training or discovery cohort; across these six studies, sensitivity and specificity in the external validation cohort were consistently lower than in the training cohort, by a mean absolute reduction of 9.2 percentage points for sensitivity and 7.4 percentage points for specificity.

4. DISCUSSION

The pooled diagnostic accuracy estimates synthesised in this review -- 0.83 sensitivity and 0.85 specificity for colorectal cancer detection -- place microbiome-based classifiers in a diagnostically respectable range, broadly comparable to, though not clearly exceeding, the reported accuracy of multitarget stool DNA testing in large prospective validation studies [33]. This is a genuinely useful finding in its own right, since it establishes that microbiome-based approaches are diagnostically competitive rather than merely a mechanistically interesting but practically inferior alternative to existing non-invasive tests; at the same time, it does not, on the evidence reviewed here, establish a clear diagnostic advantage over the best currently available non-invasive alternative, which limits the immediate clinical case for adopting a microbiome-based test in place of, rather than alongside, existing options.

The substantially lower pooled sensitivity for adenoma detection (0.61) relative to colorectal cancer detection (0.83) is, we think, the more clinically consequential of the two headline findings, because detecting and removing pre-malignant adenomas before progression to invasive cancer is the mechanism through which colonoscopy-based screening achieves much of its mortality benefit, and a non-invasive test that performs well for established cancer but poorly for adenoma would only partially replicate this preventive function. This gap is consistent with the underlying biology: microbial community shifts associated with early adenomatous change are, on the evidence reviewed in the primary literature synthesised here, more subtle and more variable across studies than the more pronounced dysbiosis associated with established tumours, and closing this sensitivity gap for pre-malignant lesions specifically should, in our view, be treated as a higher research priority than further incremental improvement of already-reasonable colorectal cancer detection accuracy.

The cross-cohort external validation finding is, in our assessment, the single most important limitation this review surfaces. That only six of 26 included studies reported external validation at all is itself informative about the maturity of the field, and the consistent, moderate performance drop observed across those six studies when moving from training to external validation cohorts is exactly the pattern that would be predicted if classifiers are partly capturing population-, diet- or sequencing-batch-specific microbial signal alongside genuine, generalisable colorectal-cancer-associated dysbiosis. This does not mean the underlying biological signal is spurious -- the consistency of *Fusobacterium nucleatum* enrichment and butyrate-producer depletion across a geographically and technically diverse set of 19 and 16 studies respectively argues against that -- but it does mean that diagnostic accuracy estimates drawn predominantly from within-cohort cross-validation, as the majority of included studies necessarily were, likely overstate the accuracy any single classifier would achieve if deployed in a genuinely new population without population-specific recalibration.

Several limitations of this review itself should be acknowledged. First, the included literature is dominated by case-control study designs comparing established colorectal cancer cases against healthy controls, a design prone to spectrum bias that can inflate apparent diagnostic accuracy relative to what would be observed in a genuine screening population enriched for early-stage and pre-malignant rather than advanced disease; only a minority of included studies used a prospective or consecutive-recruitment design better suited to estimating real-world screening performance. Second, substantial heterogeneity in sequencing platform, bioinformatic pipeline and classifier methodology across included studies limits how confidently the pooled estimates reported here can be treated as a single, stable population parameter rather than a summary across a genuinely heterogeneous set of distinct analytical approaches. Third, publication bias toward positive diagnostic accuracy findings is a general concern in this literature that our review cannot fully rule out, and could inflate the pooled sensitivity and specificity estimates reported here relative to the true underlying performance achievable by microbiome-based classifiers more broadly.

Looking forward, we regard three research priorities as most likely to move this field toward clinical translation: substantially expanded prospective, multi-cohort external validation studies, ideally pre-registered and using harmonised sequencing and bioinformatic protocols across participating sites to reduce the technical heterogeneity that likely contributes to the cross-cohort performance drop documented here; dedicated focus on improving adenoma detection sensitivity specifically, given its centrality to the preventive value of screening; and head-to-head comparative studies directly benchmarking microbiome-based classifiers against

multitarget stool DNA testing and faecal immunochemical testing within the same participants, which would allow a genuinely comparative accuracy assessment that the largely separate literatures for each test type currently do not support.

5. CONCLUSION

This systematic review and meta-analysis of 26 studies found that microbiome-based classifiers achieve pooled sensitivity of 0.83 and specificity of 0.85 for colorectal cancer detection, diagnostically competitive with, though not clearly superior to, existing non-invasive screening alternatives, while adenoma detection accuracy was substantially lower (pooled sensitivity 0.61), reflecting the more subtle microbial signal associated with pre-malignant lesions. *Fusobacterium nucleatum* enrichment was the most consistently replicated individual taxonomic finding across included studies, though multi-taxon or full metagenomic classifiers substantially outperformed any single taxon as a stand-alone marker. External cross-cohort validation, reported in only six of 26 included studies, showed a consistent moderate performance drop relative to within-cohort accuracy, identifying population and technical generalisability as the most significant unresolved barrier to clinical translation of this diagnostic approach. We recommend that future primary research in this area prioritise prospective, multi-cohort, harmonised-protocol external validation studies over further single-cohort discovery work, given how much of the current evidence base already consists of the latter, and that adenoma-specific detection performance be treated as at least as important a benchmark as colorectal cancer detection accuracy when evaluating any future microbiome-based classifier. Head-to-head comparison against multitarget stool DNA testing and faecal immunochemical testing within shared participant cohorts would materially strengthen the evidence base needed to determine whether, and in what clinical role, microbiome-based colorectal cancer screening should ultimately be positioned relative to the non-invasive tests already in routine use.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

E.O. conceived the review, designed the search strategy and led manuscript writing. E.H. performed data extraction, quality assessment and the bivariate meta-analysis. R.M. conducted study screening and contributed clinical context to data interpretation. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This is a systematic review and meta-analysis of previously published studies and did not involve the collection of new human or animal data, and therefore did not require institutional ethics committee review.

Data Availability

The list of included and excluded studies, together with the full data extraction spreadsheet and meta-analysis code underlying the findings of this review, is available from the corresponding author upon reasonable request.

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