

CLINICAL AND SYSTEMATIC REVIEWS

Fecal Immunochemical Tests Combined With Other Stool Tests for Colorectal Cancer and Advanced Adenoma Detection: A Systematic Review

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OBJECTIVES: Despite moderate to high detection rates of fecal immunochemical tests (FITs) of colorectal cancer (CRC), detection of adenomas remains limited. Further stool tests exist, which are not used in routine practice, such as DNA or RNA markers and protein markers. We aimed at systematically investigating and summarizing evidence for diagnostic performance of combinations of FIT with other stool tests compared with FIT alone in early detection of CRC and its precursors.

METHODS: We systematically reviewed studies that evaluated FITs in combination with other stool tests and compared measures of diagnostic accuracy with and without additional stool tests. PubMed and Web of Science were searched from inception to May 2015. Reference lists of eligible studies were also screened. Two reviewers extracted data independently.

RESULTS: Some of the reports on DNA, RNA, or tissue tests, including tests based on DNA mutations, methylation, and integrity in selected genes as well as microRNA expression, showed some improvements of diagnostic test accuracy. In contrast, so far assessed stool protein markers did generally not lead to substantial improvements in performance of FIT when added to the latter. Many marker combinations were reported only in one study each, and few studies were conducted in a true screening setting.

CONCLUSIONS: Several stool markers show potential to improve performance of FITs. However, the results require confirmation in further studies, which should also evaluate the costs and cost-effectiveness of combined screening strategies.

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INTRODUCTION

Among the most common cancers, colorectal cancer (CRC) ranks third among men and second among women globally,¹ accounting for ~1.4 million incident cases and 700,000 deaths annually.¹ Annual or bi-annual screening with standard guaiac-based fecal occult blood test (gFOBT, Hemoccult II) showed moderate reductions in CRC mortality in several randomized controlled trials (RCTs),² although sensitivity of gFOBT is generally poor, in particular regarding the detection of colorectal adenomas.

Compared with gFOBT, the more recently developed fecal immunochemical tests (FITs) have superior diagnostic performance.³ As additional advantages, FITs are easier to apply, thus leading to higher adherence,⁴ and do not require dietary restrictions, such as avoidance of red meat, before testing.⁵ Although results from RCTs are not available yet, inverse associations were found between FIT screening and CRC incidence⁶ and mortality⁷ in observational studies. A meta-analysis of RCTs showed that screening participation rates were significantly higher with FIT than with gFOBT screening.⁸

Detection and removal of advanced adenomas (AAs) potentially prevents CRC that would have developed through

the adenoma–carcinoma sequence. However, FITs detect less than half of AAs in a single-screening round.⁹ In recent years, various attempts have been made to improve diagnostic performance of FITs by combining them with further stool markers, including protein, DNA, or RNA markers. However, these studies have not yet been systematically reviewed.

We provide a systematic literature search and summarized the evidence on studies evaluating performance of FIT alone for the detection of CRC or AA compared with a combination of FIT and the aforementioned stool markers.

METHODS

Data sources and search strategy. Our systematic review followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.¹⁰ We considered English language human research articles identified through MEDLINE (via PubMed) and Web of Science (ISI Web of Knowledge) and reference lists of relevant articles from inception to May 2015. Our search terms, which are reported

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in the Appendix, covered expressions for FITs, diagnostic accuracy, and pertinent outcomes.

Study selection. Studies of any design were considered eligible if they provided sensitivities and specificities of both, FIT alone and FIT combined with any second diagnostic stool test for CRC or AA detection, or sufficient information to calculate them. Results on non-advanced adenomas and combinations of advanced and non-AAAs were not considered, because most studies included non-AAAs in the denominator of specificity and only few studies reported sensitivities for them.^{11–14} We required colonoscopy as the reference standard for all subjects to rule out verification bias. Results on CRC and AA combined, defined as “advanced colorectal neoplasia”, ACN, were included only from population-based studies as in clinical settings their proportions may be strongly distorted towards CRC and sensitivity is typically much higher for CRC than for AA. Studies focusing on subjects with high risk of CRC, e.g. with a family history of ACN or CRC, were not included.

Relevant outcomes were sensitivity and specificity for CRC and AA detection, area under the receiver operating characteristics (ROC) curve (AUC), and *P*-values for differences in the AUC. A detailed description of study characteristics and outcomes grouped by type of additional stool test used in the studies is provided in Tables 1, 2 and 3.

Data extraction. For relevant articles, two authors (T.N. and K.W.) independently extracted information on first author, publication year, study population, additional stool marker, outcome measures, and study quality. Disagreement was resolved in consensus. When 95% confidence intervals (CIs) of sensitivity and specificity were not reported we calculated them from numbers of true and false positives and negatives. Sensitivities and specificities are reported as percentages. Decimal places were omitted, except from very narrow CIs for specificity.¹¹

No meta-analysis was conducted, because very few studies addressed the same marker combination.

Risk of bias in individual studies. To assess the risk of bias of individual studies, we used the Quality Assessment of Diagnostic Accuracy Studies 2 instrument (QUADAS-2).¹⁵ FIT sensitivity tends to be higher in later CRC stages,¹⁶ thus, the stage distribution and the share of screen-relevant early-stage CRCs are provided in Appendix Table 1.

Risk of bias across studies. No formal testing for publication bias and selective reporting was employed because existing methods are thought to be of limited use for diagnostic accuracy data.¹⁷

Data analysis. Combined test results were defined as positive (1) if at least one of the underlying tests was positive (“pn”), or (2) if both tests were positive (“pp”), or (3) if a ROC curve approach was used to determine the optimal cutoff for a combination of both markers at fixed specificity (“o”). When the classification scheme was not reported (“NR”), the most plausible categorization was assumed. For instance, higher sensitivity and reduced specificity of the combined

test compared with a dichotomous FIT result was interpreted to reflect a “pn” classification and was abbreviated “NR/pn”. Sensitivity, specificity and corresponding CIs were calculated for “pn” and “pp” interpretations if a study provided 2×2 tables on true and false positives and negatives.

RESULTS

Study selection. Our search strategy identified 2,063 articles in PubMed and 2,591 in Web of Science (Figure 1). After removal of duplicates, 3,153 unique records were screened by title and abstract, of which 107 remained for full text review. 18 articles fulfilled our inclusion criteria.^{11–14,18–31} Of them, three studies^{12,23,24} were obtained by cross referencing.

Study characteristics. Nine studies^{11–14,19,25,27,29,30} recruited subjects prospectively, but only one of them¹¹ was conducted in an asymptomatic screening population. Nine studies^{18,20–24,26,28,31} used a case–control approach. All studies compared FIT alone to a combination with at least one further stool marker in the same study population. All but two studies reported their stool sampling method (Appendix Table 2). Detail of reporting differed considerably between studies. Sensitivities of FITs for CRC ranged from 48 to 95%. Specificities ranged from 57 to 98%. However, all but two studies^{12,19} reported specificities $\geq 85\%$.

Tests were based on fecal DNA or RNA, stool proteins other than hemoglobin (Hb), haptoglobin (Hp), or the HbHp complex, or tissue from the colonic mucosa. Five studies^{11,13,18,19,22} combined FIT with DNA or RNA markers, 11 (refs 12,14,20,21,23–27,29,31) with stool proteins, and two^{28,30} with tissue tests. More than one marker in addition to FIT was examined in three reports.^{13,20,27} Only transferrin was assessed in more than one study.^{12,23,24,29}

FIT combined with DNA- or RNA-based tests. DNA or RNA markers combined with FIT (Table 1),^{11,13,18,19,22} including markers of DNA methylation,^{13,18,19,22} and DNA mutation or integrity markers or a combination of both,¹¹ as well as markers of microRNA expression,^{13,18,22} led to large increases of sensitivity when combined with FIT in a “pn” classification. The largest increase in sensitivity for CRC was found with long DNA as a measure of DNA integrity in the APC gene, and p53, from 52 to 81%, without impairment of specificity (98%, *N*=192).¹³ Accordingly, the AUC rose from 0.75 to 0.90. However, the cutoff was optimized using a ROC curve approach without splitting the data into a test and a training set and CIs for sensitivity overlapped (FIT: 32–71%, FIT+DNA: 62–94%). One study¹⁸ assessing FIT in combination with PHACTR3 methylation found sensitivity improvements for CRC from 65 to 75% and for AA from 21 to 25%, each at 98% specificity. The AUC, which was 0.92 for FIT alone, increased to 0.97 in the combination. However, the CIs ranged from 6 to 46% (FIT) and from 9 to 51% (FIT+PHACTR3).

In the only study conducted in a true screening setting with nearly 10,000 participants,¹¹ sensitivity gains were pronounced for both, CRC (from 72 to 92%) and AA (from

Table 1 FIT combined with DNA- or RNA-based tests for advanced colorectal neoplasia (CRC or AA) detection

References	Study type	Study population (N)	Positives (N)	Study population: age (years), sex	Marker combined with FIT	Outcome	Sensitivity of FIT and FIT plus stool marker ("FIT+") (95% CI)	Specificity of FIT and FIT plus stool marker ("FIT+") (95% CI)	Combination ^a					
Kalimutho <i>et al.</i> ¹³	Symptomatic	204 31 CRC cases 11 HGD cases 24 AAs 99 controls	FIT: 16 pn: 31	Median cases: 68, range: 44–88; controls: 58 (19–82), 41% men	L-DNA as a marker of DNA integrity: – p53	CRC	FIT: 52% (32–71%)	FIT: 98% (93–100%)	NR/pn					
							FIT+: 79% (59–92%)	FIT+: 91% (83–96%)						
							AUC: 0.75 (0.66–0.82)							
							AUC+: 0.85 (0.77–0.90)							
							FIT: 52% (32–71%)	FIT: 98% (93–100%)						
							FIT+: 79% (59–92%)	FIT+: 91% (83–96%)						
Bosch <i>et al.</i> ¹⁸	Case-control	92 CRC cases 20 AAs 24 AAs 48 Controls	FIT: 36 o: 42	Mean (s.d.) development set: CRCs: 70 (9); controls: 55 (10); validation set: CRCs: 71 (9); controls: 52 (10), 50% men	– APC – APC, p53, KRAS & BRAF – APC – APC, p53, KRAS & BRAF – APC – APC, p53, KRAS & BRAF PHACTR3 (hyper-) methylation	CRC AA HGD CRC AA	FIT: 52% (32–71%)	FIT: 98% (93–100%)						
							FIT+: 89% (72–98%)	FIT+: 95% (88–98%)						
							AUC: 0.75 (0.66–0.84)							
							AUC+: 0.90 (0.83–0.95)							
							FIT: 52% (32–71%)	FIT: 98% (93–100%)						
							FIT+: 89% (72–98%)	FIT+: 95% (88–98%)						
Koga <i>et al.</i> ²²	Case-control	224 117 CRC cases 107 controls	FIT: 73 pn: 85	Median (range) cases: 65 (30–84), controls: 60 (40–78); 59% men	Fecal miR-106a (miRNA expression analysis)	CRC	FIT: 61% (51–70%)	FIT: 98% (93–100%)						
							FIT+: 71% (62–79%)	FIT+: 96% (91–99%)						
							Harada <i>et al.</i> ¹⁹	Symptomatic	508 56 CRC cases 452 controls thereof 53 AAs 209 minor polyps 190 controls	FIT: 156 pn: 247 pn: 193 pp: 31 pp: 168 pp: 19	DNA methylation in bowel lavage fluid (positivity criterion: M-score ≥ 1 M-score ≥ 2 M-score = 3)	CRC CRC	FIT: 82% (57–96%)	FIT: 57% (82–63%)
													FIT+: 100% (80–100%)	FIT+: 31% (26–36%)
													FIT: 82% (57–96%)	FIT: 57% (82–63%)
													FIT+: 71% (44–90%)	FIT+: 81% (77–85%)
FIT: 82% (57–96%)	FIT: 57% (82–63%)													
FIT+: 100% (80–100%)	FIT+: 47% (42–53%)													
Imperiale <i>et al.</i> ^{d11}	Screening	12,776 65 CRC cases 757 AAs 9,176 healthy	FIT: 696 pn: 1,612	Range 50–84, %men: NR	Altered human DNA (methylation & mutation) ^c	CRC AA	FIT: 82% (57–96%)	FIT: 57% (82–63%)						
							FIT+: 41% (18–67%)	FIT+: 93% (89–95%)						
							FIT: 82% (57–96%)	FIT: 57% (82–63%)						
							FIT+: 94% (71–100%)	FIT+: 54% (49–60%)						
							FIT: 82% (57–96%)	FIT: 57% (82–63%)						
							FIT+: 12% (1–36%)	FIT+: 95% (92–97%)						

AA, advanced adenoma; APC, adenomatous polyposis coli; AUC, area under the receiver operating characteristics (ROC) curve; "AUC+", defined as the AUC for a FIT combined with another stool marker; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CI, confidence interval; CRC, colorectal cancer; FIT, fecal immunochemical test; "FIT+", defined as a diagnostic test which combines a FIT with another stool marker; gFOBt, guaiac-based fecal occult blood test; HGD, high-grade dysplasia; KRAS, Kirsten rat sarcoma viral oncogene homolog; L-DNA, long DNA; miRNA, microRNA; N, number of participants; NR, not reported; o, sensitivities reported at fixed specificities; pn, at least one test positive; pp, both tests positive.
^aDefinitions of a positive combined test (FIT and additional marker): pp = both tests positive, pn = at least one test positive, NR/pn = not reported, but increasing sensitivity and decreasing specificity indicate a "pn" interpretation of the combined test. o = sensitivities reported at fixed specificities.
^bLinear combination with highest diagnostic accuracy.
^cMulti-target stool DNA QuARTS assay for methylation markers (BMP3 strands, NDRG4 strands, and β-actin (ACTB ANB) strands) and Multi-target stool DNA QuARTS assay for KRAS mutation markers (KRAS 1 strands, KRAS 2 strands, and β-actin (ACTB KRAS) strands).
^dextracted data on the isolated performance of hemoglobin instead of the comparison with another FIT provided in the article's tables. Study types: "screening", asymptomatic participants of screening colonoscopy; "symptomatic", colonoscopy for clarification of symptoms; "case-control", comparison of known colorectal neoplasia cases with healthy control subjects.

Table 3 FIT combined with tissue tests or tests based on the extraction of epithelial cells from fecal samples for advanced colorectal neoplasia (CRC or AA) detection

References	Study type	Study population (M)	Positives (M)	Study population: age (years), sex	Marker combined with FIT	Outcome	Sensitivity of FIT and FIT plus stool marker ("FIT+"), (95% CI)	Specificity of FIT and FIT plus stool marker ("FIT+"), (95% CI)	Combination ^a
Vironen et al. ³⁰	Symptomatic	199 36 CRC cases 38 Adenomas 104 Normal or with inflammatory bowel disease 21 with hyperplastic polyps	FIT:45 pn:65	Median 59, range 21–94, 34% men	PNA test applied on tissue samples obtained from mucosa	CRC	FIT: 72% (55–86%) FIT+: 100% (90–100%)	FIT: 88% (79–95%) FIT+: 58% (47–70%)	NR / pn
Sheng et al. ²⁸	Case-control	95 41 CRC cases 34 small adenomas 20 controls	FIT:39 pn:46 pp:21	Mean CRCs: 63, controls: 60, range 21–88, 63% men	Fecal cytology (epithelial cells from fecal samples microscopically examined)	CRC	FIT: 76% (60–88%) FIT+: 93% (80–98%) FIT: 76% (60–88%) FIT+: 51% (35–67%)	FIT: 85% (73–93%) FIT+: 85% (73–93%) FIT: 85% (73–93%) FIT+: 100% (93–100%)	pn pp

AA, advanced adenoma; CI, confidence interval; CRC, colorectal cancer; FIT, fecal immunochemical test; "FIT+", defined as a diagnostic test which combines a FIT with another stool marker; N, number of participants; PNA, peanut agglutinin.

^aDefinitions of a positive combined test (FIT and additional marker): NR/pn, not reported; pp, both tests positive; pn, at least one test positive; but increasing sensitivity and decreasing specificity indicate a "pn" interpretation of the combined test. Study types: "symptomatic", colonoscopy for clarification of symptoms; "case-control", comparison of known colorectal neoplasia cases with healthy control subjects.

23 to 42%) for a combined assay of DNA mutation and methylation markers. Specificity diminished from 95 to 87%, and spectrum bias may have occurred because of rather rigid exclusion criteria, potentially leading to a comparison of very healthy with very sick individuals. Combination with fecal microRNA, investigated in a relatively small study,²² increased FIT sensitivity for CRC from 61% (95% CI 51–70%) to 71% (95% CI 62–79%), whereas specificity decreased from 98 to 96%. The interpretation of a study adding a DNA methylation score (M-Score) from bowel lavage fluid¹⁹ was inconclusive due to the small case numbers (N = 56) and the large number of possible definitions for a positive combined test. In addition, estimates of specificity in the 452 controls included 53 subjects with AA. Of note, an M-score cutoff of 1 diminished sensitivity from 82 to 71% in a "pp" interpretation, but increased specificity significantly from 57 to 81%.

FIT combined with stool protein-based tests. Stool proteins (Table 2) and FIT were the most frequently examined marker combinations.^{12,14,20,21,23–27,29,31}

Transferrin increased sensitivities for CRC in two rather old and small studies^{23,24} from 67%²³ and 68%²⁴ to 80%, with small losses in specificity, i.e., from 99 to 97%, compared with use of FIT only. Nevertheless, sensitivity CIs overlapped. One of the studies²³ stated that no good results were obtained at other cutoff values, suggesting overfitting. The majority comprised stages B or C. Sensitivity is typically much higher in later CRC stages,¹⁶ whereas a larger fraction of early-stage CRCs would be expected in a screening setting, compared with a clinical setting. Both studies excluded patients with hemorrhoidal bleedings and used a case-control design. In two prospective studies,^{12,29} combination with transferrin did not yield strictly better or worse test characteristics than FIT alone.

Results for the other combinations of FIT and stool proteins were mixed: Calgranulin C increased sensitivity for CRC detection at a fixed specificity of 95% from 82 to 88% in one study²⁰ (N = 101). Tissue inhibitor of metalloproteinase-1 (TIMP-1) did not further improve CRC detection. Large, statistically significant increases in sensitivity at the cost of significant decreases in specificity were reported for combinations of FIT and peanut agglutinin,³⁰ calprotectin, pyruvate kinase isoenzyme type M2 (M2-PK), and a triple combination of FIT, calprotectin, and M2-PK.²⁷

FIT combined with fecal tissue tests. Two studies^{28,30} examined tests based on tissue or epithelial cells extracted from fecal samples (Table 3). The most recent study²⁸ examined epithelial cells extracted from fecal samples with a microscope to classify them as positive or negative. The older study³⁰ used samples obtained through a proctoscope with a cotton stick from macroscopically normal mucosa. These tissue samples were examined for the presence of peanut agglutinin-reactive glycoconjugates. In both, a combination with FIT raised sensitivity for CRC from <80% to >90%. Specificity remained stable in the more recent study,²⁸ but decreased from 88 to 58% in the other study.³⁰ A "pp" interpretation improved specificity from 85 to 100% in the more recent study,²⁸ but decreased sensitivity from 76 to 51%.

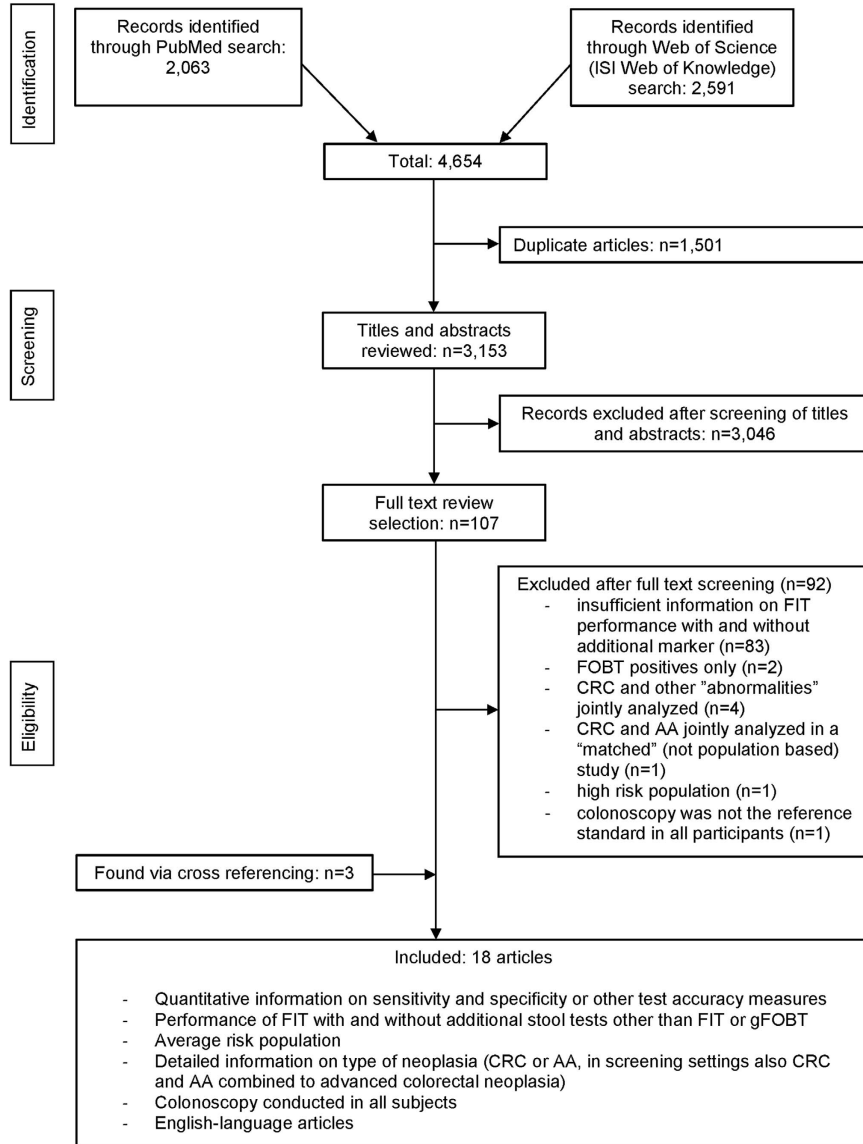


Figure 1 Flow diagram of study selection.

Assessment of risk of bias across studies. Appendix Table 3 summarizes the results of our assessment of risk of bias across studies. Only one study examined a screening population, i.e., seemingly healthy participants instead of prospectively recruited, but symptomatic individuals or known CRC cases compared with healthy controls. Other criteria were fulfilled by most of the included studies. Many studies did not report on age and sex distribution among cases and healthy individuals. The share of early-stage CRCs ranged between 43 and 78%. One study²⁷ did not provide information on CRC stage distribution.

DISCUSSION

Main findings. Overall, improvements in FIT performance might be possible by combining FITs with other stool tests, in particular with DNA- or RNA-based tests. Two small

studies^{13,18} reported strong improvements in the AUC for CRC detection when adding DNA- or RNA-based tests to a FIT, but they were conducted in clinical settings. In the only study based on a screening population,¹¹ increases in sensitivity were achieved, albeit at the cost of some loss of specificity. The combinations with stool protein-based tests so far assessed did not yield strong improvements in FIT performance. The studies combining FIT with fecal tissue based tests^{28,30} differed considerably in their methods and results. Overall evidence is limited by the fact that most studies were conducted in clinical settings, and most markers or marker combinations were evaluated in a single study only without external validation. Owing to relatively small sample sizes, resulting in overlapping confidence intervals in most comparisons of FITs alone and combined tests, reported changes in sensitivity and specificity are unlikely to be statistically significant. Evidence on adenoma detection remains very limited.

Comparison to other studies. Several reviews have summarized performance of FITs and of defined other potential early detection markers.^{32,33} One systematic review³³ of biomarkers for early detection of CRC and polyps concluded that DNA markers, volatile organic compounds, and panels of DNA or microRNA were promising marker candidates. To our knowledge, evidence on the performance of combinations of FIT with other stool markers has not previously been summarized.

Two studies^{34,35} suggested that fluorescence long DNA (FL-DNA) might be a suitable tool for risk stratification: they indicated that coincidence of high FL-DNA values and a positive FIT corresponds to a strong increase in the probability of having CRC, whereas a low FL-DNA and a negative FIT together indicate a lower CRC risk than a negative FIT alone.

Suggestions for future research. Although we extracted data from the studies in a strictly standardized manner, comparability of studies was limited due to differences in inclusion and exclusion criteria, used FITs, cutpoints for FIT positivity, and protocols for stool sampling and processing. Given the heterogeneity of reporting results across studies, a number of suggestions might be made for future research to enhance comparability of results. Reporting sensitivities at fixed levels of specificity that might be relevant for population-based screening, such as 95 or 90% through adaption of cutpoints would facilitate judgment of potential gain in accuracy by marker combinations. In commonly reported “pn” or “pp” combination scenarios sensitivity is typically increased at the cost of specificity and vice versa.

Most importantly, however, promising results achieved in small samples in clinical settings require stringent validation in independent samples ensuring comparability of participants with and without colorectal neoplasms in all aspects other than neoplasm prevalence, such as age, sex, comorbidities, and preanalytical sample handling. This can be achieved in studies conducted among participants of screening colonoscopy in which all samples are taken with uniform SOPs before diagnosis and analyzed in a blinded manner.

Testing novel marker combinations in a screening setting is crucial to obtain more realistic estimates of sensitivity and specificity. In particular, promising findings on marker combinations need to be confirmed by larger-scale studies among asymptomatic participants. For promising markers confirmed in such a setting, cost-effectiveness of the application of other stool tests in combination with FIT compared with FIT alone requires additional careful evaluation. The most definitive step towards comparability would be conduction of FITs and multiple other stool tests in the same study population from a true screening setting.

Obviously, diagnostic performance is a very crucial, but not the only criterion for judging the use of single tests or test combinations for CRC screening. For implementation in screening practice, further aspects deserve attention, such as convenience and ease of stool sample collection and processing, robustness of tests for application under real life conditions, the acceptance by the target population of screening, and, of course, costs. Whether or not to combine FIT with other stool tests will, in the end, be a matter of cost-

effectiveness, even if test combinations prove to be superior to FIT alone in terms of test accuracy. Therefore, evaluation of test accuracy should go along or be followed by cost-effectiveness analyzes whenever possible.

Strengths and limitations. Our review has several strengths and limitations. It is the first systematic review of potential improvements in FIT performance achieved by a combination with stool tests that are not or not yet routinely used as CRC screening tests, unlike gFOBT or FIT. We calculated sensitivities and specificities, along with their 95% CIs, from studies not reporting these performance indicators. Verification bias cannot have influenced the results. In addition, we evaluated the quality of included studies. A limitation is the restriction of the literature search to English-language articles. Thus, language bias cannot be ruled out. Furthermore, it is conceivable that we missed a relevant article, despite extensive search in two databases, because we deemed it not feasible to search for “gray literature” in a systematic way. If results of studies published in “gray literature” are less optimistic than published journal articles, the view of the diagnostic accuracy of the added biomarkers may be overoptimistic.

Further potential limitations of the underlying studies are overoptimistic results due to selective reporting, detection bias, and spectrum bias. For instance, a comparison of known and advanced CRC cases with healthy controls in a case-control fashion may induce spectrum bias,³⁶ since average risk screening populations comprise more heterogeneous groups of diseased and nondiseased participants. Thus, sensitivity and specificity may both be overestimated. Seven of the 18 studies included in our review were prone to this phenomenon. Correspondingly, the share of early stage CRCs was lower in studies comprising clinically detected cases compared with the study based on an asymptomatic screening population.

Summary. In conclusion, this systematic review suggests that improvements in performance of FITs are achievable through combination with further stool tests. However, no definite conclusion could be drawn for most marker combinations, mainly because of heterogeneous cutpoints leading to different specificities across studies for both, FITs alone and the combination of FITs with other stool tests. Thus, further investigations are desirable.

CONFLICT OF INTEREST

Guarantor of the article: Tobias Niedermaier, MPH.

Specific author contributions: Hermann Brenner planned the study. Tobias Niedermaier carried out the literature search. Tobias Niedermaier and Korbinian Weigl extracted the data from the eligible studies. Tobias Niedermaier drafted the manuscript. All authors critically reviewed, contributed to and approved the final manuscript.

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industrial research grants from Eiken Chemicals, Epigenomics, Roche Diagnostics and Applied Proteomics to the German Cancer Research Center. Hermann Brenner has applied for a patent "Biomarker panel for diagnosing cancer" (application number: EP 15161465.8 priority date: 27.03.2015). Tobias Niedermaier had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Fecal immunochemical tests (FITs) detect the majority of colorectal cancers (CRCs), are easier to apply than traditional guaiac-based fecal occult blood tests, and are increasingly offered for CRC screening.
- ✓ Detection of CRC precursors, i.e., advanced adenomas (AA) is still very limited even with FITs.
- ✓ A number of studies combined FITs with other stool tests to potentially improve diagnostic performance. However, such studies have not yet been systematically reviewed.

WHAT IS NEW HERE

- ✓ Several investigated stool markers, mainly DNA, RNA, and tissue based tests, might lead to clinically relevant improvements in FIT performance when combined with the latter, but thorough evaluation in screening settings is limited.
- ✓ Stool proteins generally did not lead to clinically relevant performance improvements of FIT.
- ✓ Further large studies conducted in true screening settings are required to evaluate performance of promising combinations of FIT with other stool tests.

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APPENDIX: SEARCH STRATEGY

MEDLINE (PubMed) search strategy

(FIT[title/abstract] OR iFOBT[title/abstract] OR FOBT[title/abstract] OR stool[title/abstract] OR fecal[title/abstract] OR faecal[title/abstract] OR feacal[title/abstract] OR occult[title/abstract])
 AND (CRC[title/abstract] OR colorect*[title/abstract] OR rectal[title/abstract] OR rectum[title/abstract] OR colon[title/abstract] OR colonic[title/abstract] OR bowel[title/abstract] OR intestin*[title/abstract])
 AND (carcinoma*[title/abstract] OR cancer[title/abstract] OR cancers[title/abstract] OR cancerous[title/abstract] OR neoplas*[title/abstract] OR adenoma*[title/abstract] OR malignan*[title/abstract] OR tumor*[title/abstract] OR tumour*[title/abstract])
 AND (sensitiv*[title/abstract] OR specific*[title/abstract] OR "area under"[title/abstract] OR AUC[title/abstract] OR accura*[title/abstract])
 AND (screen*[title/abstract] OR patients[title/abstract] OR subjects[title/abstract])

Web of Science (ISI Web of Knowledge) search strategy

TS = ((FIT OR iFOBT OR FOBT OR stool OR fecal OR faecal OR occult)
 AND (CRC OR colorect* OR rectal OR rectum OR colon OR colonic OR bowel OR intestin*)
 AND (carcinoma* OR cancer OR cancers OR cancerous OR neoplas* OR adenoma* OR malignan* OR tumor* OR tumour*)
 AND (sensitiv* OR specific* OR "area under" OR AUC OR accura*)
 AND (screen* OR patients OR subjects))

Appendix Table 1 UICC/Duke's stage distribution of CRC cases

References	0/I/A (%)	II/B (%)	III/C (%)	IV/D (%)	Early stage ^a (%)
Kalimutho <i>et al.</i> ¹³	44 ^b	37 ^b	19 ^b	0 ^b	81 ^b
Imperiale <i>et al.</i> ¹¹		92		8	78
Miyoshi <i>et al.</i> ²⁴	40	30	30	0	70
Yokoyama <i>et al.</i> ³¹	26	42	29	3	68
Koga <i>et al.</i> ²²		65		35	65
Harada <i>et al.</i> ¹⁹	23	43		34	66
Bosch <i>et al.</i> ¹⁸	33	28	31	8	61
Sheng <i>et al.</i> ²⁸	11 ^b	50 ^b	31 ^b	8 ^b	61 ^b
Karl <i>et al.</i> ²⁰	27 ^b	32 ^b	14 ^b	27 ^b	59 ^b
Miyoshi <i>et al.</i> ²³	22	33	45	0	55
Mizuno <i>et al.</i> ²⁵	22	33	35	10	55
Sheng <i>et al.</i> ²⁹	10	42	33	15	52
Mizuno <i>et al.</i> ²⁶	23	25	39	13	48
Sieg <i>et al.</i> ¹⁴	37	10	37	16	47
Jin <i>et al.</i> ¹²		43		57	43
Vironen <i>et al.</i> ³⁰	20	20	50	10	40
Kim <i>et al.</i> ²¹	14	10	61	15	24
Parente <i>et al.</i> ²⁷	NR	NR	NR	NR	NR

^aStages 0/I or II, Dukes A or B. ^bAmong those CRC cases that were classified.

Appendix Table 2 Stool sampling methods and FIT brand

References	Stool sampling method	FIT brand
Bosch <i>et al.</i> ¹⁸	~ 1 g of stool collected 1 day before colonoscopy, immediately stored at 4 °C and transferred to -20 °C at the day of colonoscopy without stabilization buffer	OC-sensor, Eiken Chemical Co., Tokyo, Japan
Harada <i>et al.</i> ¹⁹	10 ml of bowel lavage fluid specimens collected at the beginning of the colonoscopy from the rectum after pretreatment with 2 l of polyethylene glycol lavage solution	Not reported
Imperiale <i>et al.</i> ¹¹	Single spontaneous stool sample (whole-bowel movement)	OC FIT-CHEK, Polymedco (Cortland Manor, NY, USA)
Jin <i>et al.</i> ¹²	Not reported	Hemosure Inc., Irwindale, CA, USA
Kalimutho <i>et al.</i> ¹³	By patients, transported with an ice bag, stored at -20 °C immediately on receipt with fecal stabilization buffer	MP Biomedical, LLC
Karl <i>et al.</i> ²⁰	2 different portions of ~ 1 g of feces from one bowel movement using a stool collection tube	RIDASCREEN Hemoglobin-Haptoglobin
Kim <i>et al.</i> ²¹	0.1 g collected before bowel preparation	OC-sensor, Eiken Chemical Co.
Koga <i>et al.</i> ²²	Naturally evacuated samples from CRC patients before undergoing surgical resection. Samples from healthy volunteers a few weeks after screening colonoscopy	OC-Hemocatch, Eiken Chemical
Miyoshi <i>et al.</i> ²³	By patients, immediately stored at 4 °C for 2–8 h, stirred in a container and suspended in buffer before analyzes	Not applicable (conducted in laboratory), HbAo monoclonal antibodies from Dakopatts A/S, Glostrup, Denmark
Miyoshi <i>et al.</i> ²⁴	By patients, immediately stored at 4 °C for 2–8 h, stirred in a container and suspended in buffer before analyzes	Not applicable (96-well microplates from Linbro, Flow Laboratories, McLean, VA, USA)

Mizuno <i>et al.</i> ²⁵ Mizuno <i>et al.</i> ²⁶ Parente <i>et al.</i> ²⁷	Spontaneous stool sample (1–5 g) Spontaneous stool sample (1–5 g) By patients, returned within 24 h from defecation (stored at 4 °C for up to 1 day) to the GI unit and frozen on receipt at –20 °C until they were analyzed for subsequent biomarker determination	OC-Hemodia; Eiken Chemical Co. Ltd. OC-Hemodia; Eiken Chemical Co. Ltd. HM-Jack, Kiowa; Olympus, Tokyo, Japan
Sheng <i>et al.</i> ²⁹ Sheng <i>et al.</i> ²⁸	Not reported 5–10 g of feces collected naturally or induced with laxative, picked up with a clean swab from 4–6 spots and placed into a clean sample bottle containing 5–10 ml of cell preservation solution	Not reported, WHPM, Inc. Not reported, Wanhuapuman Bio-engineering Co. Ltd., Irwindale, CA, USA
Sieg <i>et al.</i> ¹⁴	By patients, 1 ml from two different sites of one stool, immediately stored in the deep-freeze	Not applicable (conducted in laboratory), polyclonal antibodies from DAKO (Hamburg, Germany)
Vironen <i>et al.</i> ³⁰ Yokoyama <i>et al.</i> ³¹	By patients, over 3 days before the outpatient appointment Sampling method and amount not reported. Samples stored at –70 °C	Hemolex (Orion Diagnostica, Espoo, Finland) Imudia-Hem Sp, Fujirebio Inc., Tokyo, Japan

CRC, colorectal cancer; FIT, fecal immunochemical test.

Appendix Table 3 QUADAS-2 risk of bias assessment

References	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Bosch <i>et al.</i> ¹⁸	⊖	?	⊕	?	⊖	?	⊕
Harada <i>et al.</i> ¹⁹	⊕ ^a	⊕	⊕	⊕	⊖	⊕	⊕
Imperiale <i>et al.</i> ¹¹	⊕	⊕	⊕	⊕	⊖	⊕	⊕
Jin <i>et al.</i> ¹²	⊕ ^a	⊕	⊕	⊕	⊖	⊕	⊕
Kalimutho <i>et al.</i> ¹³	⊕ ^a	⊕	⊕	⊕	⊖	⊕	⊕
Karl <i>et al.</i> ²⁰	⊖	⊕	⊕	⊕	⊖	⊕	⊕
Kim <i>et al.</i> ²¹	⊖	⊕	⊕	⊕	⊖	⊕	⊕
Koga <i>et al.</i> ²²	⊖	⊕	⊕	⊕	⊖	⊕	⊕
Miyoshi <i>et al.</i> ²³	⊖	⊕	⊕	?	⊖	⊕	⊕
Miyoshi <i>et al.</i> ²⁴	⊖	⊕	⊕	⊕	⊖	⊕	⊕
Mizuno <i>et al.</i> ²⁵	⊕ ^a	⊕	⊕	?	⊖	⊕	⊕
Mizuno <i>et al.</i> ²⁶	⊖	⊕	⊕	?	⊖	⊕	⊕
Parente <i>et al.</i> ²⁷	⊕ ^a	⊕	⊕	⊕	⊖	⊕	⊕
Sheng <i>et al.</i> ²⁹	⊕ ^a	⊕	⊕	⊕	⊖	⊕	⊕
Sheng <i>et al.</i> ²⁸	⊖	⊕	⊕	⊕	⊖	⊕	⊕
Sieg <i>et al.</i> ¹⁴	⊕ ^a	⊕	⊕	⊕	⊖	⊕	⊕
Vironen <i>et al.</i> ³⁰	⊕ ^a	⊕	⊕	⊕	⊖	⊕	⊕
Yokoyama <i>et al.</i> ³¹	⊖	?	⊕	⊖	⊖	?	⊕

⊕ Low risk; ⊖ high risk; ? unclear risk.

^aStudy was prospective in design, but comprised symptomatic patients.