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, 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...
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-As were not considered, because most studies included non-AAs 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”, ACNs, 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 sensitivities and specificities of FITs for CRC ranged from 48 to 95%. Specificities ranged from 57 to 98%. However, all but two studies1,12,13,15 reported specificities ≥ 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 studies11,13,18,19,22 combined FIT with DNA or RNA markers, 11 (refs. 12,14,20,21,23–27,29,31) with stool proteins, and two28,30 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 |
|------------|------------|----------------------|--------------|-----------------------------------|-------------------------|---------|------------------------------------------------|------------------------------------------------|-------------|
| Kalimutho et al. | Symptomatic | 204 31 CRC cases 11 HGD cases 24 AAs 99 controls | FIT: 16 | 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+ 79% (59–92%); AUC: 0.75 (0.66–0.82) | FIT+: 98% (93–100%); AUC+: 0.85 (0.77–0.90) | NR/pn |
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| References | Study type | Study population (N) | FIT (N) | 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 |
|------------|------------|----------------------|---------|--------------------------|---------|---------------------------------------------------------------|---------------------------------------------------------------|------------|
| Miyoshi et al.23 | Case-control | 36 Polyp cases + 40 Controls | FIT: 51 | Mean CRCs: 65, Polyp cases: 54, Controls: 49 | Transferrin CRC | FIT: 67% (62–79%) | FIT+: 99% (96–100%) | pn |
| Miyoshi et al.24 | Case-control | 26 Polyp cases + 33 Controls | FIT: 57 | Mean CRCs: 66, Controls: 50, Polyp cases: 54 | Transferrin CRC | FIT: 68% (55–80%) | FIT+: 99% (96–100%) | pn |
| Mizuno et al.25 | Symptomatic | 40 CRC cases + 41 Controls | FIT: 31 | Mean CRCs: 64, Controls: 52 | Stool DAF CRC | FIT: 73% (65–85%) | FIT+: 95% (89–96%) | pn |
| Yokoyama et al.26 | Case-control | 31 CRC cases + 58% Men | FIT: 15 | Mean CRCs: 64, Controls: 52 | Carbonic anhydrase II CRC | FIT: 48% (30–67%) | FIT+: 100% (87–100%) | NR / o |
| Sieg et al.27 | Symptomatic | 43 CRC cases + 40 Controls | FIT: 27 | Mean CRCs: 65, Controls: 53, Men: NR | Albumin CRC | FIT: 99% (84–99%) | FIT+: 97% (95–99%) | pn |
| Mizuno et al.28 | Case-control | 100 CRC cases + 58% Men | FIT: 82 | Mean CRCs: 65, Controls: 36, 60% Men | Stool DAF CRC | FIT: 75% (65–82%) | FIT+: 88% (79–93%) | pn |
| Kaf et al.29 | Case-control | 353 AAs + 47% Men | FIT: 118 | Mean (s.d.) CRCs: 68 (12), Controls: 63 (8), 44% men | Calgranulin C (S100A12) CRC | FIT: 82% (73–89%) | FIT+: 95% (91–97%) | o |
| Sheng et al.30 | Symptomatic | 26 CRC cases + 40 Controls | FIT: 50 | >20, 64% Men | Transferrin CRC | FIT: 75% (59–87%) | FIT+: 95% (91–97%) | o |
| Jin et al.31 | Symptomatic | 2144 CRCs | FIT: 96 | Mean 67, range 31–91, 76% men | Transferrin CRC | FIT: 80% (64–94%) | FIT+: 95% (91–97%) | o |
| Parente et al.32 | Symptomatic | 21 CRC cases + 47 AAs | FIT: 55 | Range 50–80, 56% men | Calprotectin CRC | FIT: 60% (47–74%) | FIT+: 89% (84–92%) | o |
| Kim et al.33 | Case-control | 30 CRC cases + 51 Controls | FIT: 85 | Mean (s.d.) development set CRCs: 63 (10), controls: 50 (10), validation set: mean (s.d) CRCs: 63 (12), Controls: 49 (11), Men: NR | Calgranulin B (S100A9) CRC | FIT: 80% (70–87%) | FIT+: 90% (82–95%) | NR / o |

AA, advanced adenoma; ACN, advanced colorectal neoplasia (≥ AA or CRC); AUC, area under the receiver operating characteristics (ROC) curve; "AUC+", defined as the AUC for a FIT combined with another stool marker; CN, confidence interval; CRC, colorectal cancer; DAF, decay-accelerating factor; 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; N, number of participants; NR, not reported.

* "p" means 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, o, sensivities reported at fixed specificities; NR/o, not reported, but specificity was unchanged and thus probably fixed.

** Sieg et al. report specificities defined as "false-positive results if a normal colon mucosa and no other reason of gastrointestinal bleeding were found", thus overestimating specificity. Specificities (95% CI) defined as true negatives/(true negatives + all false-positives) were 88% (84–91%) (FIT) and 84% (80–88%) (FIT+).**

*Includes 16 subjects with high-risk adenomas (villosus, moderate-severe dysplasia, multiple adenoma or adenoma ≥ 1 cm) and 20 subjects with ulcerative colitis. Study types: "symptomatic", colonoscopy for clarification of symptoms; "case-control", comparison of known colorectal neoplasia cases with healthy control subjects.
Table 3

| References     | Study type        | Study population (N) | FIT combined with stool marker ("FIT+") (95% CI) | Sensitivity of FIT and stool marker combination ("FIT++") (95% CI) |
|----------------|-------------------|----------------------|-------------------------------------------------|---------------------------------------------------------------|
| Vironen et al. | Symptomatic       | 36 CRC cases (31–94) | FIT: 72% (55–95%) NR | FIT: 85% (73–92%) FIT: 58% (47–70%) |
| Sheng et al.   | Case-control      | 41 CRC cases (28–34) | FIT: 76% (60–88%) Fecal cytology (epithelial cells) | FIT: 85% (73–93%) FIT: 51% (35–67%) |

**Marker combined with FIT**

**Outcome**

**Sensitivity of FIT and stool marker combination ("FIT++") (95% CI)**

- FIT: 72% (55–95%)
- FIT: 85% (73–92%)
- FIT: 58% (47–70%)

**Combination**

| Reference       | Combination | Sensitivity (95% CI) |
|-----------------|-------------|----------------------|
| Niedermaier et al. | FIT combined with stool protein-based tests. Stool proteins (Table 2) and FIT were the most frequently examined marker combinations,1,12,14,20,21,23–27,29,31 |
| Transferrin increased sensitivities for CRC in two rather old and small studies23,24 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,1,12,29 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 study30 (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 stool protein-based tests.** Stool proteins (Table 2) and FIT were the most frequently examined marker combinations,1,12,14,20,21,23–27,29,31

**FIT combined with fecal tissue tests.** Two studies28,30 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%.

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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 study 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 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, 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 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 studies34,35 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 cutoffs 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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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])
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*)
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 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 (%) |
|--------------------|-----------|----------|-----------|----------|-----------------|
| Kalimutho et al.    | 44b       | 37b      | 19b       | 0b       | 81b             |
| Imperiale et al.    | 40        | 30       | 30        | 0        | 70              |
| Myoshi et al.       | 26        | 42       | 29        | 3        | 68              |
| Yokoyama et al.     | 23        | 65       | 35        | 65       |                 |
| Koga et al.         | 23        | 43       | 35        | 66       |                 |
| Harada et al.       | 33        | 28       | 31        | 8        | 61              |
| Bosch et al.        | 14        | 50b      | 31b       | 8b       | 61b             |
| Sheng et al.        | 27b       | 32b      | 14b       | 27b      | 59b             |
| Karl et al.         | 22        | 33       | 45        | 0        | 55              |
| Miyoshi et al.      | 22        | 33       | 35        | 10       | 55              |
| Mizuno et al.       | 23        | 25       | 39        | 13       | 48              |
| Sieg et al.         | 37        | 10       | 37        | 16       | 47              |
| Jin et al.          | 20        | 20       | 50        | 10       | 40              |
| Vironen et al.      | 14        | 10       | 61        | 15       | 24              |
| Parente et al.      | NR        | NR       | NR        | NR       |                 |

*Stages 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 et al.       | 1 g of stool collected 1 day before colonoscopy, immediately stored at 4 °C and transferred to – 20 ºC at the day of colonoscopy without stabilization buffer | OC-sensor, Eiken Chemical Co., Tokyo, Japan    |
| Harada et al.      | 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 et al.   | Single spontaneous stool sample (whole-bowel movement)     | OC FIT-CHEK, Polymedco (Cortland Manor, NY, USA) |
| Jin et al.         | Not reported                                               | Hemosure Inc., Irwindale, CA, USA              |
| Kalimutho et al.   | By patients, transported with an ice bag, stored at – 20 ºC immediately on receipt with fecal stabilization buffer using a stool collection tube | MP Biomedical, LLC                             |
| Karl et al.        | 2 different portions of ~ 1 g of feces from one bowel movement | RIDASCREEN Hemoglobin-Haptoglobin              |
| Kim et al.         | 0.1 g collected before bowel preparation                     | OC-sensor, Eiken Chemical Co.                  |
| Koga et al.        | Naturally evacuated samples from CRC patients before undergoing surgical resection. Samples from healthy volunteers a few weeks after screening colonoscopy | OC-Hemochack, Eiken Chemical                  |
| Miyoshi et al.     | By patients, immediately stored at 4 ºC 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 et al.     | By patients, immediately stored at 4 ºC 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 et al.25 Spontaneous stool sample (1–5 g) OC-Hemodia; Eiken Chemical Co. Ltd.
Mizuno et al.26 Spontaneous stool sample (1–5 g) OC-Hemodia; Eiken Chemical Co. Ltd.
Parente et al.27 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
Sheng et al.29 Not reported Not reported, WHPM, Inc.
Sheng et al.28 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
Sieg et al.14 By patients, 1 ml from two different sites of one stool, immediately stored in the deep-freeze
Vironen et al.30 By patients, over 3 days before the outpatient appointment Hemolex (Orion Diagnostica, Espoo, Finland)
Yokoyama et al.31 Sampling method and amount not reported. Samples stored at −70 °C

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 et al.18 | ☹ | ☹ | ☹ | ☹ | ☹ | ☹ | ☹ |
| Harada et al.19 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Imperiale et al.11 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Jin et al.12 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Kalimutho et al.13 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Kari et al.20 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Kim et al.21 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Koga et al.22 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Miyoshi et al.23 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Miyoshi et al.24 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Mizuno et al.25 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Mizuno et al.26 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Parente et al.27 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Sheng et al.29 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Sheng et al.28 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Sieg et al.14 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Vironen et al.30 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |
| Yokoyama et al.31 | ☹ | ☹ | ☹ | ? | ☹ | ☹ | ☹ |

☻ Low risk; ☐ high risk; ? unclear risk.

*Study was prospective in design, but comprised symptomatic patients.