Detection of Biomarkers In Fecal Immunochemical Test Residual Buffer to Enhance Colorectal Cancer Screening: A Systematic Scoping Review

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Background

- Fecal immunochemical tests (FIT) have limited sensitivity for neoplasia and colorectal cancer (CRC) detection

Annals of Internal Medicine

REVIEW

Performance Characteristics of Fecal Immunochemical Tests for Colorectal Cancer and Advanced Adenomatous Polyps

A Systematic Review and Meta-analysis

Thomas F. Imperiale, MD; Rachel N. Gruber, MS; Timothy E. Stump, MA; Thomas W. Emmett, MD; and Patrick O. Monahan, PhD

Background: Studies report inconsistent performance of fecal immunochemical tests (FITs) for colorectal cancer (CRC) and advanced adenomas.

Purpose: To summarize performance characteristics of FITs for CRC and advanced adenomas in average-risk persons undergoing screening colonoscopy (reference standard) and to identify factors affecting these characteristics.

Data Sources: Cochrane L… erence lists.

Study Selection: …cords to ide… rospective … and specifi… average-risk…

Data Extraction: … and evaluated study quality.

Data Synthesis: Thirty-one studies (120 255 participants; 18 FITs) were included; all were judged to have low to moderate risk of bias. Performance characteristics depended on the threshold for a positive result. A threshold of 10 µg/g resulted in sensitivity of 0.91 (95% CI, 0.84 to 0.95) and a negative likelihood ratio of 0.10 (CI, 0.06 to 0.19) for CRC, whereas a threshold of greater than 20 µg/g resulted in specificity of 0.95 (CI, 0.94 to 0.96) and a positive likelihood ratio of 15.49 (CI, 9.82 to 22.39).

For advanced adenomas, sensitivity was 0.40 (CI, 0.33 to 0.47) and the negative likelihood ratio was 0.67 (CI, 0.57 to 0.78) at 10 µg/g, and specificity was 0.95 (CI, 0.94 to 0.96) and the positive likelihood ratio was 5.86 (CI, 3.77 to 8.97) at greater than 20 µg/g. Studies had low to high heterogeneity, depending on the threshold. Although several FITs had adequate performance, sensitivity and specificity for CRC for 1 qualitative FIT were 0.90 and 0.91, respectively, at its single threshold of 10 µg/g; positive and negative likelihood ratios were 10.13 and 0.11, respectively. … sitivity and specificity for CRC, depending on the positivity threshold. Sensitivity of 1-time testing for advanced adenomas is low, regardless of the threshold.

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Purpose: To summarize performance characteristics of FITs for CRC and advanced adenomas in average-risk persons undergoing screening colonoscopy (reference standard) and to identify factors affecting these characteristics.

Thirty-one studies (120 255 participants;

Colorectal cancer (CRC) is a leading cause of death among digestive diseases and the second leading cause of cancer-related death in the United States (1). Despite the effectiveness and cost-effectiveness of screening (2–4), only 60% to 65% of the eligible population is current with screening (5), a rate that has fallen short of the goal of 80% by 2018 (2, 5, 6). This reflects concerns over the best test and strategy for screening. Colonoscopy is the most frequently used screening test in the United States (5), but several other countries use annual or biennial stool blood tests or a combination of stool testing and lower endoscopy (7, 8).

Although studies have shown that guaiac-based fecal occult blood testing reduces CRC incidence and mortality (9–13), it has several shortcomings, including low single-application sensitivity for CRC, poor detection of advanced adenomas (those with a diameter ≥1 cm, villous histologic characteristics, or high-grade dysplasia), the need for dietary and medication restrictions, and the requirement for more than 1 specimen. Use of the fecal immunochemical test (FIT) for human globulin is more sensitive and specific than guaiac-based fecal occult blood testing for CRC and advanced adenomas and has higher rates of participation and acceptance (14–16). However, studies evaluating FIT performance characteristics have shown inconsistent findings for CRC and advanced adenomas. A systematic review published in 2014 summarized performance characteristics for CRC (17) but not for advanced adenomas. The objectives of this systematic review and meta-analysis were to provide an updated summary of FIT performance for CRC, quantify FIT performance characteristics for advanced adenomas, and evaluate whether variation in reported performance characteristics among studies is a function of the threshold used to define a positive test result or the test brand.

See also:
Editorial comment 342
Web-Only
Supplement
CME/MOC activity

Imperiale et al, *Ann Intern Med* 2019 Vol. 170 Issue 5 Pages 319-329

FIT Threshold	Informative Studies	Outcome	Sensitivity	Specificity
20 µg/gm	14	Colorectal Cancer	0.75 (0.61, 0.86)	0.95 (0.92, 0.96)
20 µg/gm	15	Advanced Adenoma	0.25 (0.20, 0.31)	0.95 (0.93, 0.96)

Imperiale et al, *Ann Intern Med* 2019 Vol. 170 Issue 5 Pages 319-329

Background

- Fecal immunochemical tests (FIT) have limited sensitivity for neoplasia and colorectal cancer (CRC) detection

- There is growing interest in using FIT residual buffer for biomarker detection to maximize the neoplasia predictive information from a FIT specimen
  - No loss in adherence if using a single sample

Aim

To systematically review studies on the use of FIT residual buffer for biomarker detection to enhance FIT performance

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Scoping Review:

An iterative review process to understand a field that has not previously been described

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Domain	Subcategories
Biomarker Stability	Short term/long term
Neoplasia Detection	Protein, DNA/RNA, microbiome

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Systematic scoping review: Use of the faecal immunochemical test residual buffer to enhance colorectal cancer screening

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Summary

Background: The faecal immunochemical test (FIT) is an inexpensive and convenient modality to screen for colorectal cancer. However, its one-time sensitivity for detecting colorectal cancer and cancer precursors is limited. There is growing interest in using the non-haemoglobin contents of FIT residual buffer to enhance colonic neoplasia detection.

Aim: To establish from the literature a framework to catalogue candidate biomarkers within FIT residual buffer for non-invasive colorectal cancer screening.

Methods: The search strategy evaluated PubMed, Scopus, Web of Science, Embase, and Google Scholar for all publications through 25 October 2023, with search terms including FIT, buffer, OC-sensor, biomarkers, microbiome, microRNA (miR), colon, rectum, screening, neoplasm, and early detection. Studies employing home-based collection samples using quantitative FIT first processed for haemoglobin were included. One author reviewed all articles; a second author completed a 20% full-text audit to ensure adherence to eligibility criteria.

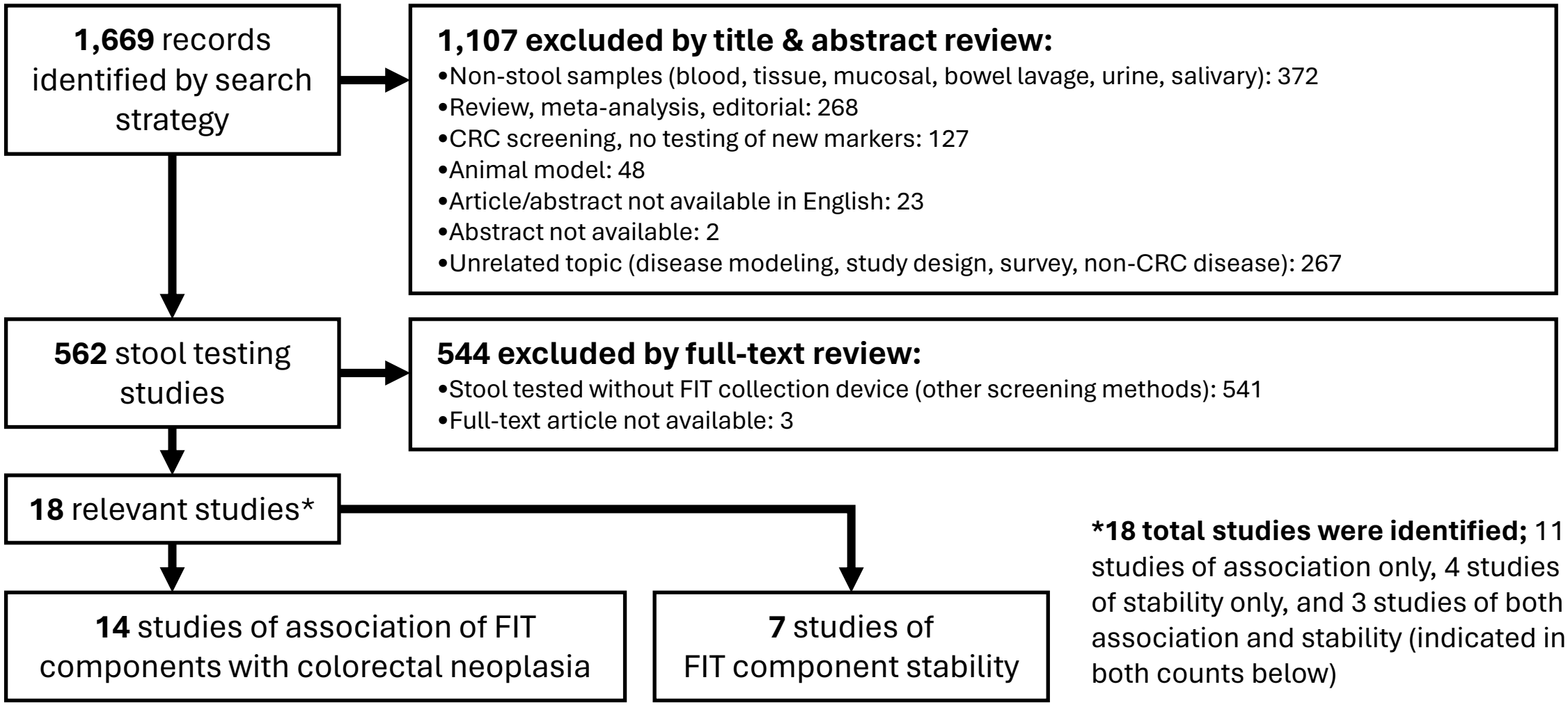
Results: A broad search yielded 1669 studies and application of eligibility criteria identified 18 relevant studies. Multiple protein, DNA/RNA, and microbiome biomarkers (notably haptoglobin, miR-16, miR-27a-3p, miR-92a, miR-148a-3p, miR-223, miR-421, let-7b-5p, and *Tyzzerella 4*) were associated with colorectal neoplasia. Furthermore, studies highlighted the short-term stability of biomarkers for clinical use and long-term stability for research purposes.

Conclusions: This scoping review summarises the framework and progress of research on stability of biomarkers in FIT residual buffer and their associations with colorectal neoplasia to guide opportunities for further confirmatory studies to enhance colorectal cancer screening.

Methods

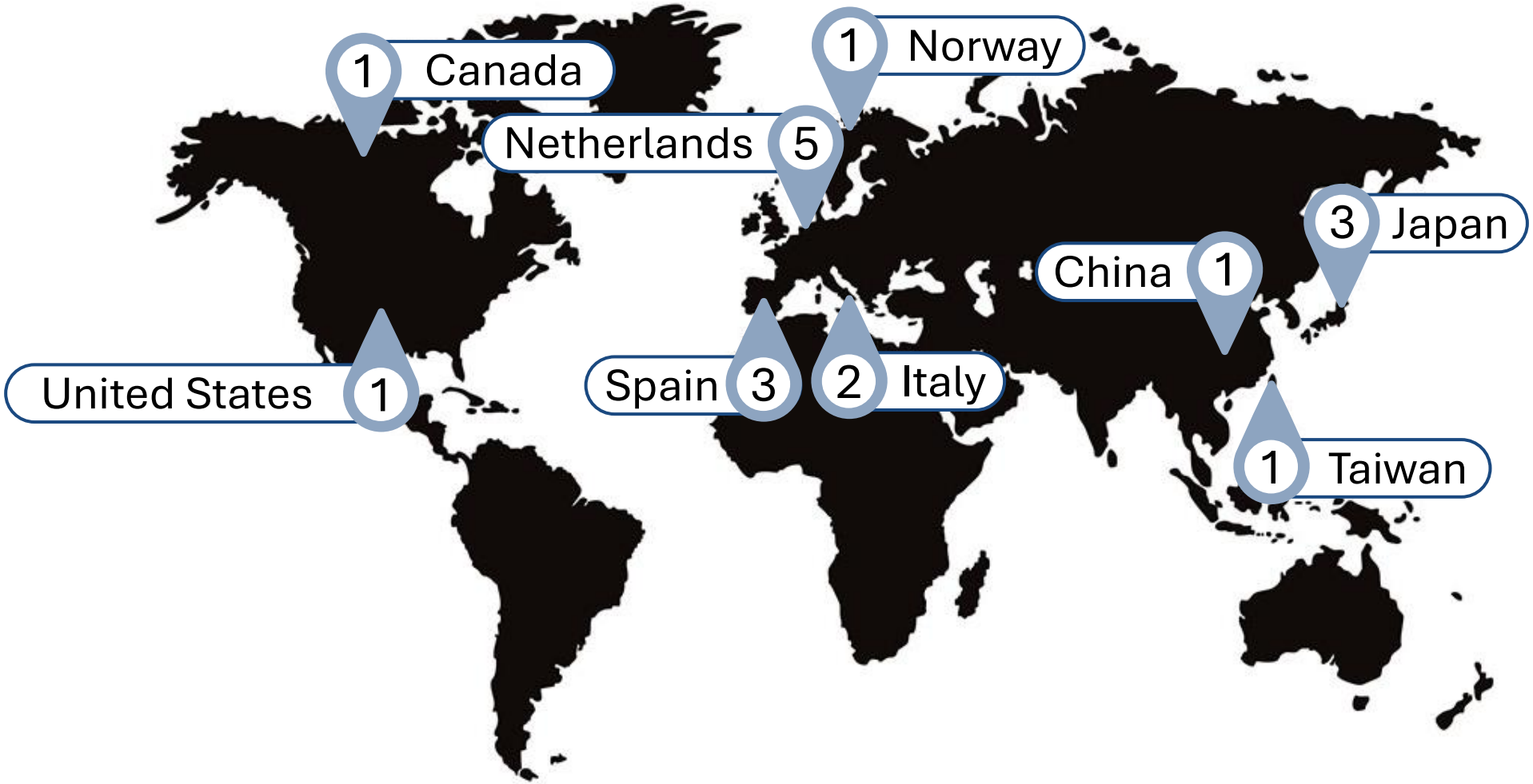
- **Databases:** PubMed, Scopus, Web of Science, Embase, and Google Scholar searched for all historical publications through October 25, 2023
- **Search terms** included FIT, buffer, OC-sensor, biomarkers, microbiome, microRNA, screening, and neoplasm
- **Eligibility:** Studies that employed home-based collected samples using quantitative FIT first processed for Hgb, before testing of additional biomarkers; available in English
- **Study selection:** One author reviewed all articles; a second author completed a 20% full-text audit

PRISMA Diagram

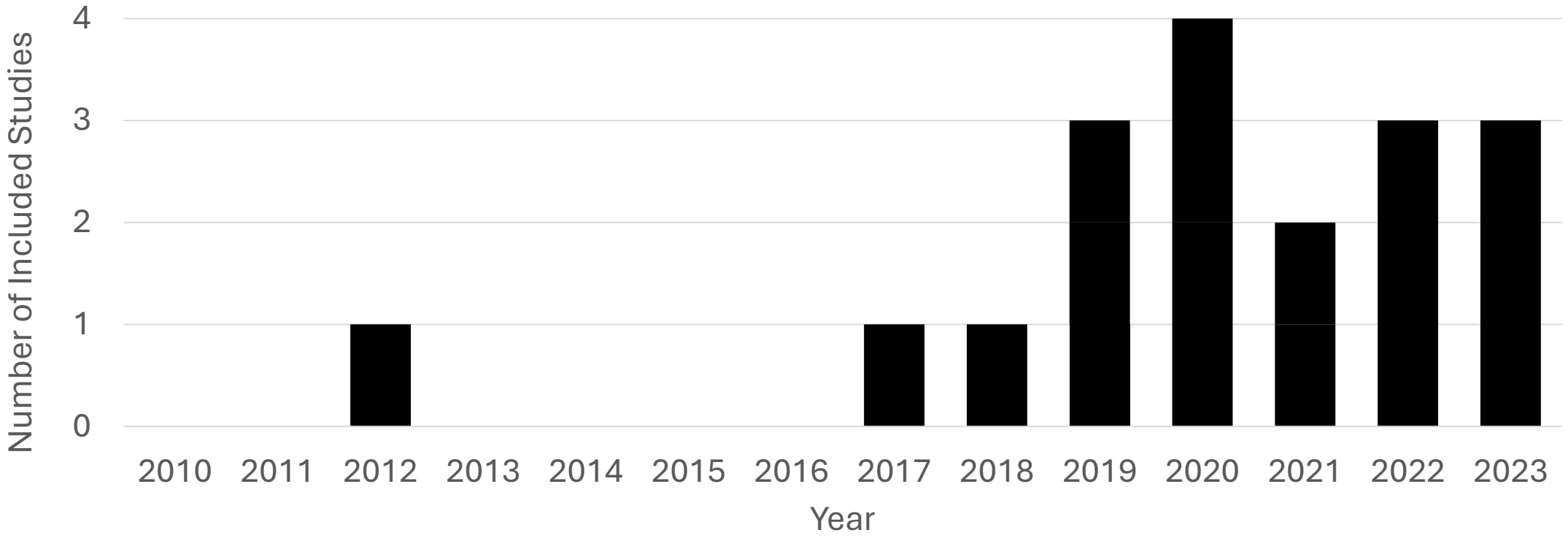


***18 total studies were identified;** 11 studies of association only, 4 studies of stability only, and 3 studies of both association and stability (indicated in both counts below)

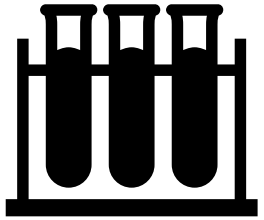
Distribution of studies by location



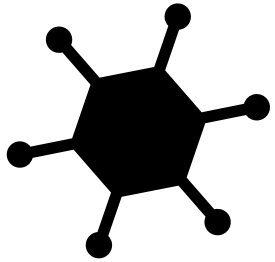
Studies by year



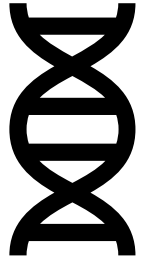
Major categories of findings



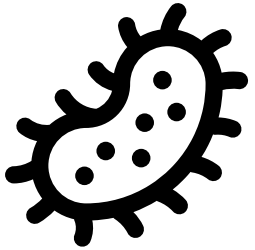
Stability of biomarkers in FIT residual buffer



Non-Hgb protein biomarker associations

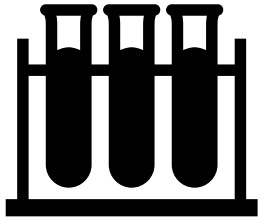


DNA/RNA biomarker associations



Microbiome biomarker associations

Major categories of findings



Stability of biomarkers in FIT residual buffer

Non-Hgb protein biomarker associations

DNA/RNA biomarker associations

Microbiome biomarker associations

Stability

Stability was present across all biomarker types, for short- and long-term durations; storage conditions and durations varied

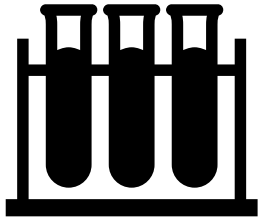
	Number of studies	Short-term stability	Long-term stability
Proteins	1	3 days (room temp) 3 weeks (4°C)	
DNA/RNA	3	1 week (-5°C)	Up to 1 year (-40°C) 3-5 years (-80°C)
Microbiome	4	6 days (-20°C)	Up to 1 year (-40°C) 3-5 years (-80°C) 0-6 years (-80°C)

Stability of biomarkers

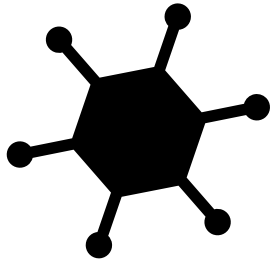
Birkeland (2023) n=266	DNA/RNA: 23 miRNA specifically analyzed	1 year at -40°C - 3-5 years at -80°C
	Microbiome: 7+ phyla	1 year at -40°C - 3-5 years at -80°C
Bosch (2012) n=31	DNA/RNA: PHACTR3 (DNA methylation marker), ACTB	1 week at -5°C
Chénard (2020) n=500	Microbiome: OTU count, composition, diversity	-80°C (no duration reported)
Grobbee (2020) n=200	Microbiome: 4 species	6 days at -20°C
Hiraoka (2019) n=304	Protein: calprotectin	3 days at room temp. - 3 weeks at 4°C
Pardini (2023) n=114	DNA/RNA: 25 differentially expressed miRNA	-80°C (no duration reported)
Rounge (2018) n=117	Microbiome: alpha diversity, composition, abundance, Bray-Curtis dissimilarity index	0-6 years at -80°C

- **Protein (calprotectin), DNA methylation markers, and miRNA consistently shown to be stable** when comparing FIT and stool samples.
- **Microbiome markers had variable results**, with some studies with comparable results and some with decreased markers in FIT samples.

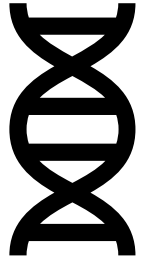
Major categories of findings



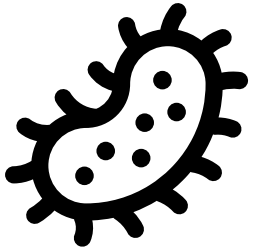
Stability of biomarkers in FIT residual buffer



Non-Hgb protein biomarker associations



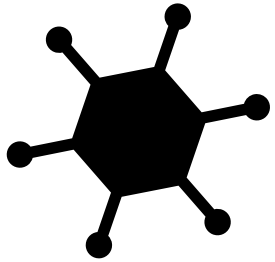
DNA/RNA biomarker associations



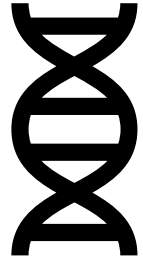
Microbiome biomarker associations

Major categories of findings

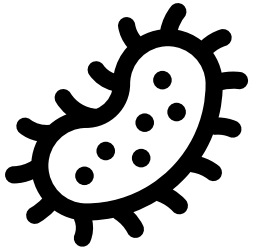
Stability of biomarkers in FIT residual buffer



Non-Hgb protein biomarker associations



DNA/RNA biomarker associations



Microbiome biomarker associations

Protein biomarkers: individual markers

de Klaver (2021)	**n=1,201**
α2-macroglobulin, calprotectin, C3 complement, **haptoglobin**, hemopexin, lactotransferrin, myeloperoxidase, and serpin family F member 2	Increased vs. control (p<0.001)
Hirata (2020)	**n=525**
transferrin	Sensitivity 63.5% was not different than F-Hgb sensitivity 67.3%. Specificity 74.6% was not different than F-Hgb specificity 90.6%
Komor (2020)	**n=743**
haptoglobin	Increased vs. control (p=9.7e-5). pAUC 63.2% less than FIT pAUC 73.7% (p=0.004).

- **All listed biomarkers (except transferrin) were increased** in CRC samples.
- **Haptoglobin** was found to be significantly increased in CRC and AA/high-risk adenomas by multiple studies (de Klaver, Komor).

Footnote: Bolded text in the table indicates biomarkers that were evaluated in multiple studies. Some studies (combined biomarkers/models and biomarkers with negative findings) are not included in this table; these biomarkers are included in the full-text manuscript and review.

Protein biomarkers: combined/models

de Klaver (2021) n=1,201	**Multitarget FIT model: calprotectin, serpin family F member 2, hemoglobin**	AN: mtFIT sensitivity 42.9% greater than FIT (p=0.025) AA: mtFIT sensitivity 37.8% greater than FIT (p=0.006)
Hirata (2020) n=525	**Two Hgb & transferrin combined assays: assay A and assay B** **Two Hgb (two-step cutoff) & transferrin assays: assay C and assay D**	CRC: assay B and assay D sensitivity greater than FIT (p<0.001, p=0.046) CRC: assay A and assay C specificity greater than FIT (p<0.001, p=0.0011)

- **Model with calprotectin, serpin family F member 2, and Hgb** (de Klaver) found increased AA and AN sensitivity.
- **Models with transferrin and Hgb** (Hirata) found 2 with increased sensitivity and 2 with increased specificity.

DNA/RNA biomarkers: individual markers

Birkeland (2023)	**n=185**
miR-148a-3p and **let-7b-5p**	Increased vs. control (p<0.05)
miR-4451 and miR-11399	Decreased vs. control (p<0.05)
Bosch (2012)	**n=33**
PHACTR3 methylation	Of samples with reference gene ACTB detected, increased vs. control (p=0.0084)
Duran-Sanchon (2020)	**n=767**
miR-25-3p, **miR-27a-3p**, miR-29a-3p, miR-34a-5p, miR-130b-3p, miR-221-3p, and **miR-421**	Increased vs. control (p<0.04 - p<0.001; AUC=0.69 - AUC=0.77)
Koga (2017)	**n=150**
miR-16, **miR-92a**, miR-106a, miR-142-3p, **miR-223**, and miR-451	Increased vs. control (p<0.05)
Pardini (2023)	**n=185**
miR-21-5p, **miR-148a-3p**, miR-320a-3p, miR-607-5p, miR-12114, let-7a-5p, **let-7b-5p**, and let-7i-5p	Increased or decreased vs. control (adjusted p<0.05)

Footnote: Bolded text in the table indicates biomarkers that were evaluated in multiple studies. Some studies (combined biomarkers/models and biomarkers with negative findings) are not included in this table; these biomarkers are included in the full-text manuscript and review.

- **All listed microRNA were significantly increased or decreased** in CRC or AA samples.
- **miR-16, miR-27a-3p, miR-92a, miR-148a-3p, miR-223, miR-421, let-7b-5p** were significantly associated across multiple studies (some individual or combined biomarkers).

Microbiome biomarkers: individual markers

Grobbee (2020)	**n=200**
Total bacterial load (universal bacterial 16S)	Increased vs. control (p=0.006)
Khannous-Lleiffe (2022)	**n=1,059**
4 microbiome species	Among the most significant findings, increased vs. control (p=2e-4 - p=0.001).
Zhang (2021)	**n=1,432**
13 microbiome genera and species	For advanced adenoma, *Tyzzerella 4* performed best (AUC = 0.578)

- **Universal bacterial 16S and numerous other species, genera, and families** were significantly increased or decreased in CRC.
- **Polyketide synthase+ *Escherichia coli*** (de Klaver)**, 66 species** (Birkeland)**, and 46 genera** (Grobbee) were found to have no association. (not included on this slide)

Footnote: Bolded text in the table indicates biomarkers that were evaluated in multiple studies. Some studies (combined biomarkers/models and biomarkers with negative findings) are not included in this table; these biomarkers are included in the full-text manuscript and review.

Major areas for future research and progress

1. **Validation and expansion of biomarker studies** with FIT biobank samples, to expand breadth of findings
2. Exploration of **findings from studies not included in eligibility criteria** (studies with stool spiked by lab personnel, studies that did not use *residual* FIT buffer)
3. Evaluation of conclusions from **newly published findings**, since review publication and other active studies identified through literature evaluation
4. **Standardization of data reporting** (consistent FIT and biomarker test characteristics) and FIT devices utilized

Results recently published in AP&T

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DDW Poster Presentation
Monday May 20th (Poster 1176)