***Asian Pacific Journal of Cancer Care***
Vol 11 No 1 (2026), 97-105
Original Research

# Comparative Diagnostic Accuracy of Fecal Occult Blood Test and Fecal Matrix Metalloproteinase-9 for Noninvasive Detection of Colorectal Cancer: A Cross-Sectional Study

| | |
|---|---|
| *Agus Hariyanto* | Division of Digestive Surgery, Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. |
| *Warsinggih Warsinggih* | Division of Digestive Surgery, Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia. Hasanuddin University Hospital, Makassar, Indonesia. |
| *Muhammad Luthfi Parewangi* | Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia. Department of Internal Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. |
| *M. Ihwan Kusuma* | Division of Digestive Surgery, Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia. |
| *Samuel Sampetoding* | Division of Digestive Surgery, Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia. |
| *Julianus Aboyaman Uwuratuw* | Division of Digestive Surgery, Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. |
| *Muhammad Faruk* | Hasanuddin University Hospital, Makassar, Indonesia. Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. |

**Introduction:** Colorectal cancer (CRC) ranks as the third most common malignancy and the second leading cause of cancer-related mortality globally. Early detection is vital to improving outcomes. The fecal occult blood test (FOBT) is widely used as a noninvasive screening tool but has limited diagnostic precision. Fecal matrix metalloproteinase-9 (MMP-9) has emerged as a promising biomarker, with limited regional validation in Southeast Asia. This study compared the diagnostic performance of FOBT and fecal MMP-9 against colonoscopy in detecting CRC.

**Materials and Methods:** A cross-sectional diagnostic accuracy study was conducted from July to September 2024 among 90 patients undergoing colonoscopy at Wahidin Sudirohusodo Hospital, Indonesia. All participants provided stool samples for FOBT and fecal MMP-9 quantification via enzyme-linked immunosorbent assay (ELISA). Colonoscopy with histopathology served as the reference standard. Sensitivity, specificity, predictive values, area under the ROC curve (AUC), Youden’s index, and 95% confidence intervals (CIs) were calculated.

**Results:** The mean participant age was 50.9 ± 15.6 years, with 51.1 % male. CRC was diagnosed in 37.8 % of cases. FOBT showed high sensitivity but low specificity (97.1 %, 95% CI 85.1–99.9; 32.7 %, 95% CI 20.6–46.7; AUC = 0.646 [95% CI 0.534–0.758]; Youden = 0.298). Fecal MMP-9, using an empirically derived ROC cut-off 0.153 ng/mL, yielded balanced performance (sensitivity 76.5 %, 95% CI 58.8–89.3; specificity 76.8 %, 95% CI 63.6–87.0; AUC = 0.835 [95% CI 0.751–0.918]; Youden = 0.533).

**Conclusion:** Fecal MMP-9 demonstrated greater overall accuracy compared with FOBT and may serve as a promising noninvasive biomarker to enhance colorectal cancer screening efficiency, particularly in resource-limited settings.

# Introduction

Colorectal cancer (CRC) represents a significant global health challenge, ranking as the third most common cancer and the second leading cause of cancer-related death worldwide. In 2020, CRC accounted for 10 % of all cancer cases and 9.4 % of cancer-related fatalities [1, 2]. While CRC incidence is higher in developed countries, mortality rates are disproportionately greater in developing nations [2, 3]. In Indonesia, the prevalence of CRC was reported at 8.6 % in 2020, with a mortality rate of 7.9 % [4]. The economic burden is also substantial, with global treatment costs for CRC being the second highest among all cancers [5, 6].

The prognosis for CRC is intrinsically linked to the stage at diagnosis. Early-stage disease can often be managed with surgery alone, whereas advanced, metastatic cancer necessitates a multidisciplinary approach and is associated with a grim 5-year survival rate of only 13 % [7]. Data from the Surveillance, Epidemiology, and End Results (SEER) program indicate that when detected at an early stage, the 5-year relative survival rate can be as high as 90.6 % [8]. This stark difference in survival underscores the critical importance of early detection. Consequently, leading health organizations, including the International Agency for Research on Cancer (IARC) [9], the U.S. Preventive Services Task Force (USPSTF) [10], and European Society for Medical Oncology (ESMO) [11] strongly recommend programmatic CRC screening. These guidelines endorse several strategies, prominently featuring noninvasive stool-based tests as a primary option to improve population-wide access and adherence, thereby reducing CRC-related mortality.

The Fecal Occult Blood Test (FOBT) is a widely adopted noninvasive screening method designed to detect occult gastrointestinal bleeding, a potential sign of CRC. Its implementation has been associated with improved overall survival, largely by facilitating the detection of lesions at an earlier stage [12]. However, the clinical utility of FOBT is constrained by its reliance on detecting occult gastrointestinal bleeding a secondary and often inconsistent sign of CRC. Its low specificity, stemming from various non-malignant bleeding sources, leads to high false-positive rates, while its sensitivity may be compromised by non-bleeding tumors. These diagnostic limitations highlight the need for novel noninvasive markers that directly reflect the core biological processes of carcinogenesis rather than its downstream symptoms. This has shifted research focus toward biomarkers intrinsically linked to the molecular mechanisms of tumor progression. Among these, matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, are of particular interest. MMPs are crucial for degrading the extracellular matrix (ECM), a process fundamental to tumor invasion and metastasis [13, 14]. Specifically, elevated expression of MMP-9 has been consistently observed in CRC tissue samples and correlated with poor patient prognosis [15-17]. A preliminary study in Indonesia demonstrated that fecal MMP-9 had a sensitivity of 88.9 % and specificity of 76.7 % as a diagnostic biomarker [18].

Given the high prevalence of CRC in Indonesia and the limitations of existing screening methods, there is a pressing need to develop and validate superior noninvasive diagnostic tools. We hypothesized that fecal MMP-9 would demonstrate superior diagnostic accuracy compared with

FOBT for the detection of colorectal cancer in patients scheduled for colonoscopy. Therefore, the primary objective of this study was to directly compare the diagnostic accuracy of the conventional FOBT with that of fecal MMP-9 against the gold standard of colonoscopy at a tertiary care center in Indonesia.

# Materials and Methods

## Study Design and Setting

This was a cross-sectional diagnostic accuracy study conducted to compare the performance of two noninvasive tests against a reference standard. The research was conducted at the Digestive Surgery Subdivision of the Department of Surgery and the Human Molecular Research Center (HUMRC) Laboratory of the Faculty of Medicine, Hasanuddin University, which includes Dr. Wahidin Sudirohusodo General Hospital and Hasanuddin University Hospital in Makassar, Indonesia. The study period, encompassing participant recruitment and data collection, was from July to September 2024. The study protocol was approved by the relevant ethics review board, and all procedures were conducted in accordance with ethical guidelines.

## Study Population

The population for this study included all patients scheduled for a colonoscopy procedure at the participating hospitals. Participants were recruited from this accessible population using a consecutive sampling technique until the minimum required sample size was achieved. Inclusion criteria were: (1) patient age of at least 18 years, and (2) availability of complete baseline clinical and medical record data. Exclusion criteria were: (1) a known diagnosis of inflammatory bowel disease (e.g., Crohn’s disease, ulcerative colitis), (2) active infectious colitis or enteritis, (3) known sources of significant non-neoplastic gastrointestinal bleeding (e.g., severe hemorrhoids, diverticular bleeding), (4) current pregnancy, (5) a prior history of any malignancy or treatment with chemotherapy, and (6) refusal to provide written informed consent.

## Participant Flow and STARD Compliance

This study followed the Standards for Reporting of Diagnostic Accuracy Studies (STARD) 2015 guidelines to ensure methodological transparency. A flow diagram summarizing patient recruitment, inclusion, exclusion, and final analysis is presented in Figure 1.

**Figure 1. STARD-compliant Flow Diagram Summarizing Patient Recruitment, Inclusion and Exclusion Criteria, and Final Sample Analyzed for Diagnostic Accuracy of Fecal MMP-9 and FOBT Compared with Colonoscopy.**

Of 112 patients initially screened for eligibility, 14 were excluded due to incomplete stool samples or confounding gastrointestinal conditions, and 8 declined participation. Ultimately, 90 participants fulfilled all inclusion criteria and were analyzed. No participants were lost to follow-up or excluded post-enrollment.

## Sample Size Determination

The minimum sample size was calculated using the Lemeshow formula [19] for diagnostic test

studies, assuming a colorectal cancer prevalence of 26 % based on prior regional data, a 95% confidence level (Z = 1.96), and a desired precision (d) of 10 %. The resulting calculation yielded a minimum requirement of 78 participants. To compensate for possible nonresponse or unusable specimens, a 15 % contingency was added, producing a final target of 90 participants, which was achieved. This ensured adequate statistical power for estimating sensitivity and specificity with acceptable confidence intervals.

## Handling of Missing Data

All recruited participants provided complete colonoscopy, FOBT, and fecal MMP-9 data; thus, there were no missing diagnostic outcomes. Laboratory duplicates with indeterminate ELISA readings (< 5% of samples) were reanalyzed. No data imputation was required. Data completeness was verified prior to statistical analysis.

## Procedures and Data Collection

All patients who met the eligibility criteria were provided with a detailed explanation of the study's purpose and procedures, after which they provided written informed consent to participate. Prior to undergoing their scheduled colonoscopy, each participant was asked to provide a stool sample in a designated container. These samples were immediately stored at -20°C for a maximum of three months until analysis.

Relevant clinical and demographic data, including age, sex, and medical history, were extracted from the patients' medical records. All participants underwent a complete colonoscopy, which served as the gold standard for diagnosis. During the procedure, biopsies were taken from all suspicious lesions and sent for histopathological examination to confirm the diagnosis. Based on the combined colonoscopic and histological findings, patients were definitively diagnosed as having either CRC or non- CRC findings.

## FOBT

Patients were instructed to avoid red meat, high-dose vitamin C (>250 mg/day), NSAIDs, and anticoagulants seven days prior to stool collection. Stool samples were applied onto guaiac-impregnated test cards and developed with hydrogen peroxide [2]. Presence of occult blood in stool detected using guaiac-based immunoassay. A color change indicated a positive result. Results were categorized as positive or negative.

## Fecal MMP-9 Quantification

Fecal MMP-9 concentrations were measured using a quantitative Human MMP-9 Enzyme-Linked Immunosorbent Assay (ELISA) Kit (R&D Systems, Abingdon, UK, Cat. No. DMP900). Stored stool samples were thawed, and 1 gram of each sample was homogenized in 4 mL of cold Tris buffer. The homogenate was subjected to two rounds of centrifugation to obtain a clear final supernatant, which was used for the assay [17]. Fecal MMP-9 was measured according to the manufacturer's protocol and expressed in ng/mL.

The optimal MMP-9 cut-off (0.153 ng/mL) was derived empirically from ROC analysis using the Youden index (max [sensitivity + specificity − 1]) to maximize combined sensitivity and specificity. This threshold was consistent with prior studies (0.14–0.17 ng/mL).

### Colonoscopy

All patients underwent colonoscopy up to the terminal ileum. Suspicious lesions were biopsied and examined histopathologically. Findings were classified as normal, non-neoplastic lesions (e.g., diverticulosis, hyperplastic polyps), adenoma, or colorectal carcinoma.

### Blinding Procedures

To minimize diagnostic interpretation bias, all laboratory analyses were performed independently and blinded to colonoscopy and histopathological results. Similarly, the endoscopists and pathologists were blinded to FOBT and fecal MMP-9 outcomes. Data coding and statistical analyses were conducted after blinding was lifted upon completion of all laboratory and reference assessments.

### Statistical Analysis

All data were tabulated and analyzed using SPSS version 25.0 for Windows. Descriptive statistics were employed to summarize participant characteristics, with categorical data presented as frequencies and percentages, and numerical data as mean ± standard deviation (SD). Bivariate analysis was performed to assess the association between test results and CRC diagnosis. The relationship between the categorical FOBT result and CRC diagnosis was evaluated using the Chi-square test, while the non- parametric Mann-Whitney U test was used to compare median MMP-9 levels between the CRC and non-CRC groups due to non-normally distributed data. A p-value $<0.05$ was considered statistically significant.

Diagnostic performance was assessed by calculating sensitivity, specificity, predictive values (positive predictive value [PPV] and negative predictive value [NPV]), and area under the receiver operating characteristic (ROC) curve (AUC) with 95% confidence intervals. The comparison between the AUCs for FOBT and fecal MMP-9 was performed using the DeLong test in MedCalc (v.22.007). A two-sided p-value $< 0.05$ was considered statistically significant for all analyses.

### Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of Hasanuddin University (approval number: 856/UN4.6.4.5.31/PP36/2024, dated October 10, 2024). All participants provided written informed consent prior to enrollment in accordance with the Declaration of Helsinki.

## Results

### Patient Characteristics

A total of 90 patients scheduled for colonoscopy were enrolled in this study. The mean age was 50.9 ± 15.6 years (range 15–84 years), with 51.1 % male. Colonoscopy revealed CRC in 37.8 % (34/90) of participants. Among patients with CRC, the majority exhibited moderately differentiated tumors (52.9 %). Tumor location was most commonly in the rectum (41.2 %). These patient characteristics findings are summarized in Table 1.

| Characteristic | n (%) | Median MMP-9 (ng/mL) [IQR] | Correlation / p-value |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Age, years (mean ± SD, range) | 50.9 ± 15.6 (15–84) | | |
| Sex | | | |
| Male | 46 (51.1) | | |
| Female | 44 (48.9) | | |
| Colonoscopy findings | | | $p < 0.001^{\#}$ |
| Colorectal cancer (CRC) | 34 (37.8) | 0.25 (0.17–0.32) | |
| Non-CRC | 56 (62.2) | 0.09 (0.05–0.14) | |
| Tumor differentiation (CRC patients) | | | $p = 0.11^{\#}$ |
| Well differentiated | 13 (38.2) | 0.21 | |
| Moderately differentiated | 18 (52.9) | 0.24 | |
| Poorly differentiated | 3 (8.8) | 0.29 | |
| Tumor location | | | $p = 0.29^{\#}$ |
| Rectum | 14 (41.2) | 0.25 | |
| Sigmoid colon | 7 (20.6) | 0.27 | |
| Transverse colon | 5 (14.7) | 0.22 | |
| Ascending colon | 4 (11.8) | 0.2 | |
| Descending colon | 3 (8.8) | 0.23 | |
| Cecum | 1 (2.9) | 0.18 | |
| Tumor stage (AJCC $8^{th}$ edition) | | | $\rho = 0.41$, $p = 0.018^{*}$ |
| Stage I | 6 (17.6) | 0.14 | |
| Stage II | 9 (26.5) | 0.19 | |
| Stage III | 12 (35.3) | 0.26 | |
| Stage IV | 7 (20.6) | 0.32 | |

**Table 1. Patient Characteristics (n = 90).**

Notes: Spearman’s rank correlation used for tumor stage; # Mann–Whitney U test used for two-group comparisons; Median MMP-9 values derived from fecal ELISA measurements (cut-off 0.153 ng/mL); AJCC = American Joint Committee on Cancer.

Among CRC cases, fecal MMP-9 concentrations showed a moderate positive correlation with tumor stage (Spearman’s $\rho = 0.41$, $p = 0.018$), indicating higher levels in advanced stages. No significant association was observed with tumor location ( $p = 0.29$) or differentiation ($p = 0.11$). Median MMP-9 levels tended to increase from stage II (0.19 ng/mL) to stage IV (0.32 ng/mL), suggesting a potential link between tumor burden and biomarker expression.

## FOBT Findings

Overall, 78.9 % of patients (71/90) tested positive on FOBT. Among those with CRC, 97.1 % (33/34) were FOBT positive, compared with 67.9 % (38/56) of non-CRC cases ($p = 0.001$). The FOBT demonstrated a sensitivity of 97.1 % (95% CI, 85.1–99.9) and specificity of 32.7 % (95% CI, 20.6–46.7). The PPV and NPV were 47.9 % and 94.7 %, respectively (Table 2a).

| | a) Fecal occult blood test (FOBT) vs. colonoscopy | | |
|---|---|---|---|
| Colonoscopy result | FOBT Positive | | FOBT Negative |
| CRC | 33 | | 1 |
| Non-CRC | 38 | | 18 |
| Diagnostic Metric | | Estimate (95% CI) | |

| | | | |
|---|---|---|---|
| Sensitivity | | 97.1 % (85.1–99.9) | |
| Specificity | | 32.7 % (20.6–46.7) | |
| PPV | | 47.9 % (36.1–59.9) | |
| NPV | | 94.7 % (73.9–99.1) | |
| | (b) Fecal MMP-9 (cut-off = 0.153 ng/mL) vs. colonoscopy | | |
| Colonoscopy result | MMP-9 Positive | | MMP-9 Negative |
| CRC | 26 | | 8 |
| Non-CRC | 11 | | 45 |
| Diagnostic Metric | | Estimate (95% CI) | |
| Sensitivity | | 76.5 % (58.8–89.3) | |
| Specificity | | 76.8 % (63.6–87.0) | |
| PPV | | 66.7 % (49.0–81.4) | |
| NPV | | 84.3 % (71.4–93.0) | |

**Table 2. Cross-tabulation of FOBT and Fecal MMP-9 Results Against Colonoscopy Findings.**

Note: CRC = colorectal cancer; PPV = positive predictive value; NPV= negative predictive value.

## Fecal MMP-9 Findings

The fecal MMP-9 concentrations were non-normally distributed. Patients with CRC exhibited significantly higher median MMP-9 levels (0.25 ng/mL; IQR: 0.17– 0.32) compared to those without CRC (0.09 ng/mL; IQR: 0.05–0.14; $p < 0.001$, Mann-Whitney U test). Using a cut-off value of 0.153 ng/mL, 41.1 % (37/90) of patients were classified as MMP-9 positive. Among CRC cases, 76.5 % (26/34) were correctly identified, and 45 of 56 non-CRC cases were true negatives. This corresponded to a sensitivity of 76.5 % (95% CI, 58.8–89.3) and specificity of 76.8 % (95% CI, 63.6–87.0). The PPV and NPV were 66.7 % and 84.3 %, respectively (Table 2b).

## Comparative Diagnostic Performance

The diagnostic accuracy of both noninvasive tests is summarized in Table 3.

| Variable | FOBT | Fecal MMP-9 |
|---|---|---|
| | | (cut-off 0.153 ng/mL) |
| Sensitivity (% [95% CI]) | 97.1 (85.1–99.9) | 76.5 (58.8–89.3) |
| Specificity (% [95% CI]) | 32.7 (20.6–46.7) | 76.8 (63.6–87.0) |
| Positive Predictive Value (% [95% CI]) | 47.9 (36.1–59.9) | 66.7 (49.0–81.4) |
| Negative Predictive Value (% [95% CI]) | 94.7 (73.9–99.1) | 84.3 (71.4–93.0) |
| AUC (95% CI) | 0.646 (0.534–0.758) | 0.835 (0.751–0.918) |
| Youden’s Index | 0.298 (29.8 %) | 0.533 |

**Table 3. Diagnostic Performance Metrics of FOBT and Fecal MMP-9 for Colorectal Cancer Detection.**

Note: AUC: Area under the receiver operating characteristic (ROC) curve; Youden’s Index = sensitivity + specificity − 1; The MMP-9 cut-off (0.153 ng/mL) was empirically derived from ROC analysis using the Youden criterion;

FOBT demonstrated high sensitivity but poor specificity, whereas fecal MMP-9 showed a more balanced diagnostic profile. Youden’s index was 0.298 for FOBT and 0.533 for fecal MMP-9, indicating superior combined sensitivity and specificity for the latter.

ROC analysis revealed that fecal MMP-9 had a significantly higher area under the curve (AUC = 0.835, 95% CI 0.751–0.918) than FOBT (AUC = 0.646, 95% CI 0.534–0.758; p = 0.004, DeLong test), confirming its superior discriminative performance for colorectal cancer detection.

A combined ROC plot (Figure 2) illustrates this comparison. The fecal MMP-9 curve consistently lies above the FOBT curve across all decision thresholds.

**Figure 2. Combined Receiver Operating Characteristic (ROC) Curves Comparing the Diagnostic Performance of Fecal Matrix Metalloproteinase-9 (MMP-9) and Fecal Occult Blood Test (FOBT) for Colorectal Cancer Detection. The fecal MMP-9 curve (AUC = 0.835) demonstrates superior discriminative ability compared with FOBT (AUC = 0.646). The empirically derived operating points are indicated: MMP-9 (sensitivity 76.5%, specificity 76.8 %) and FOBT (sensitivity 97.1 %, specificity 32.7 %). The diagonal dashed line represents the line of no discrimination.**

# Discussion

This study compared the diagnostic accuracy of FOBT and fecal MMP-9 for colorectal cancer detection in an Indonesian cohort. The main finding is that fecal MMP- 9 demonstrated statistically superior overall diagnostic accuracy compared to FOBT. While FOBT exhibited very high sensitivity (97.1%), its clinical utility was severely compromised by extremely low specificity (32.7%). In contrast, fecal MMP-9 provided a more effective trade-off between sensitivity and specificity, with a sensitivity of 76.5%, a specificity of 76.8%, and a significantly higher Area Under the ROC Curve (AUC) of 0.835 compared to 0.646 for FOBT (p = 0.004). These findings strongly suggest that fecal MMP-9 represents a more robust and accurate noninvasive biomarker for CRC detection in this clinical setting.

CRC remains the third most commonly diagnosed malignancy worldwide and the second leading cause of cancer-related mortality [3, 7]. Effective screening is therefore the cornerstone of mortality reduction. While international guidelines vary, identifying early- stage cancers or precursor lesions is the universal goal. In many low- and middle-income countries (LMICs) like Indonesia, where access to colonoscopy is constrained, affordable and accurate noninvasive tests are of paramount importance.

The high sensitivity (97.1 %) of FOBT observed in our study aligns with its established utility in detecting bleeding tumors. However, its markedly low specificity (32.7 %) is a significant drawback, consistent with meta- analyses reporting high false-positive rates for guaiac-based tests [20, 21]. Such false positives stem from various non-malignant conditions and lead to numerous unnecessary colonoscopies, straining limited healthcare resources [22].

In contrast, fecal MMP-9 yielded superior and more balanced diagnostic metrics (AUC 0.835, sensitivity 76.5 %, specificity 76.8 %). The higher Youden index for fecal MMP-9 corroborates its superior diagnostic balance compared to FOBT. This performance is consistent with previous international reports, suggesting its robustness across different populations [17, 18]. The superior diagnostic performance of fecal MMP-9 observed in this study is consistent with its direct role in CRC pathogenesis, a mechanism established in the introduction. Unlike FOBT, which detects a non-specific, secondary sign of cancer (bleeding), fecal MMP-9 appears to directly reflect the underlying processes of tumor invasion and inflammatory remodeling [13, 23]. This pathophysiological link is strongly supported by our key finding that fecal MMP-9 concentrations positively correlated with advancing tumor stage ($\rho = 0.41$, $p = 0.018$), a result that aligns with previous reports from tissue and plasma studies demonstrating higher MMP-9 expression in more advanced disease [24, 25].

Moreover, the potential utility of MMP-9 may extend to the detection of precancerous lesions. Prior studies have documented elevated MMP-9 expression in high-risk adenomatous polyps, suggesting

its role as a biomarker for early neoplastic transformation, not just established carcinoma [26-31]. Taken together, our findings reinforce that fecal MMP-9 is not merely a static marker of cancer presence but a dynamic indicator of the biological processes driving tumor progression.

From an implementation perspective, the fecal MMP-9 assay is technically feasible for integration into existing laboratory workflows. The ELISA platform used in this study requires minimal specialized equipment beyond what is already available in most diagnostic laboratories. The per-test reagent cost is modest potentially comparable to or slightly higher than that of conventional fecal occult blood testing but could decrease further with large-scale procurement and automation. Given its noninvasive nature and higher specificity, fecal MMP-9 testing could complement or sequentially follow FOBT in tiered screening algorithms, thereby reducing unnecessary colonoscopies. If validated in population-based cohorts, MMP-9 could be integrated into national colorectal cancer screening programs as a cost-effective adjunct biomarker that enhances early detection and optimizes resource utilization.

Fecal MMP-9 could also complement existing fecal immunochemical test (FIT) based screening algorithms. FIT primarily detects occult blood, which reflects mucosal bleeding rather than the underlying molecular changes associated with tumorigenesis. In contrast, MMP-9 indicates extracellular matrix degradation and inflammatory remodeling, processes that may occur earlier and independently of bleeding. Combining MMP- 9 with FIT could therefore improve overall sensitivity while maintaining specificity, particularly for detecting non-bleeding or proximal lesions that FIT may miss. A sequential strategy where MMP-9 testing follows a positive FIT result or vice versa could optimize cost- effectiveness and reduce unnecessary colonoscopies.

In the Indonesian context, where colonoscopy resources are scarce, FOBT remains widely used due to its accessibility. However, the low specificity observed here would lead to unnecessary referrals, straining limited endoscopic capacity. Fecal MMP-9 offers a more balanced diagnostic profile, potentially reducing false positives and conserving healthcare resources. Integrating fecal MMP- 9 into screening programs could yield dual benefits: (1) improved early detection of CRC, and (2) better triage of patients requiring colonoscopy. Furthermore, combined testing strategies may enhance performance. Previous modeling studies [17, 18, 20, 21, 30, 32-34] suggest that combining highly sensitive but non-specific tests (e.g., FOBT) with more specific biomarkers (e.g., MMP-9) can improve overall predictive accuracy while maintaining cost-effectiveness.

From a public health policy perspective, our findings suggest that fecal MMP-9 could be strategically integrated into national CRC screening programs to address the limitations of current tests. Given the scarcity of colonoscopy resources in Indonesia, a two-step or tiered screening algorithm presents a pragmatic implementation model. In this approach, a low-cost, high-sensitivity test like the Fecal Immunochemical Test (FIT) could be retained for initial mass screening. Individuals who test positive would then undergo a reflex test with the more specific fecal MMP-9 assay. This strategy would leverage MMP-9's higher specificity to act as an effective "gatekeeper" for colonoscopy, significantly reducing the number of unnecessary invasive procedures prompted by false-positive initial screens. By improving patient triage, such a program would optimize the allocation of limited endoscopic resources, ensuring that patients at the highest risk are prioritized. Before this can be adopted as policy, however, health economic analyses are needed to formally model the cost-effectiveness of a tiered screening approach and provide the evidence base required to guide national health policy decisions.

The strengths of this study include its direct head-to-head comparison against the gold standard and the use of blinding to reduce observer bias. However, the study is not without limitations. First, the single-center design and modest sample size may limit generalizability. Second, while laboratory protocols were standardized, the potential for biomarker degradation during sample handling and storage is a key pre-analytical variable that could influence results and limit inter-assay reproducibility. Third, this study did not assess advanced adenomas separately from invasive carcinoma, which may underestimate the utility of MMP-9 in early lesion detection. However, this

study also has several limitations. First, the single-center design and modest sample size may limit generalizability. Second, while laboratory protocols were standardized, biomarker stability during stool handling and storage could influence results. Third, this study did not assess advanced adenomas separately from invasive carcinoma, which may underestimate the utility of MMP-9 in early lesion detection. Fourth, the potential for selection bias, specifically spectrum bias, inherent in our recruitment design. Participants were enrolled from a hospital-based cohort of patients already scheduled for colonoscopy, a group that likely includes a higher proportion of symptomatic individuals or those with known risk factors compared to the general population. The resulting high prevalence of CRC in our sample (37.8%) is not representative of a true screening population. This may lead to an overestimation of diagnostic accuracy metrics, particularly the positive predictive value, and could limit the direct generalizability of our findings to a community- based, asymptomatic screening context. Future validation studies in a true screening cohort are essential to confirm the performance characteristics reported here. Finally, no cost-effectiveness analysis was performed, an essential consideration for implementation in LMICs.

Future studies should include multicenter trials with community-based participants across diverse Indonesian regions to validate the performance and reproducibility of fecal MMP-9 as a diagnostic tool. Comparative analyses with FIT and other emerging stool-based biomarkers, such as multi-target stool DNA tests, would further establish its relative value. Large-scale cost-effectiveness studies are needed to inform health policy decisions regarding integration into national CRC screening programs. Additionally, longitudinal studies could explore whether fecal MMP-9 has prognostic significance, such as predicting recurrence or progression.

In conclusion, fecal MMP-9 demonstrated superior diagnostic accuracy compared with FOBT and may serve as a promising noninvasive biomarker to enhance colorectal cancer screening efficiency, particularly in resource-limited settings. Larger multicenter studies are warranted to confirm its clinical utility.

# Acknowledgments

None

### *Conflicts of interest/Competing interests*

Authors declare that they have no conflicts of interest.

### *Availability of data and material*

The data sets used and/or analyzed during the current study are available from the corresponding authors per reasonable request.

### *Authors' contributions*

AH (Concept, Design, Resources, Materials, Data Collection and Processing, Analysis and Interpretation, Literature Search, Writing Manuscript), WS (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), MLP (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), MIK (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), SS (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), JAU (Concept, Design, Supervision, Analysis and Interpretation, Literature Search), and MF (Concept, Design, Analysis and Interpretation, Critical Review). All authors read and approved

the final version of the manuscript.

*Ethics approval*

This study was approved by Ethics Committee of Dr Wahidin Sudirohusodo Hospital Faculty of Medicine, Hasanuddin University (approval no. 856/UN4.6.4.5.31/ PP36/2024).

*Consent to participate*

Written informed consent was obtained from all participants, and the trial was conducted in accordance with the Declaration of Helsinki.

*Consent for publication*

Written informed consent was obtained from all participants, and the trial was conducted in accordance with the Declaration of Helsinki.

# References

# References

1. Sawicki T, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE. A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. *Cancers.* 2021; 13(9)DOI
2. Chowdhury MR, Hone KGMS, Prévost K, Balthazar P, Avino M, Arguin M, Beaudoin J, et al. Optimizing Fecal Occult Blood Test (FOBT) Colorectal Cancer Screening Using Gut Bacteriome as a Biomarker. *Clinical Colorectal Cancer.* 2024; 23(1)DOI
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians.* 2021; 71(3)DOI
4. WHO. Cancer Indonesia 2020 Country Profile. 2020. DOI
5. National Cancer Institute. Financial Burden of Cancer Care. Cancer Trends Prog Rep. 2022;Updated Ap.
6. Chen S, Cao Z, Prettner K, Kuhn M, Yang J, Jiao L, Wang Z, et al. Estimates and Projections of the Global Economic Cost of 29 Cancers in 204 Countries and Territories From 2020 to 2050. *JAMA Oncology.* 2023; 9(4)DOI
7. Mendis S, Hong W, Ananda S, Faragher I, Jones I, Croxford M, Steel M, et al. Biology and Clinical Implications of Fecal Occult Blood Test Screen-Detected Colorectal Cancer. *JNCI Cancer Spectrum.* 2022; 6(1)DOI
8. Daly M, Paquette I. Surveillance, Epidemiology, and End Results (SEER) and SEER-Medicare Databases: Use in Clinical Research for Improving Colorectal Cancer Outcomes. *Clinics in Colon and Rectal Surgery.* 2019; 32(01)DOI
9. Lauby-Secretan B, Vilahur N, Bianchini F, Guha N, Straif K. The IARC Perspective on Colorectal Cancer Screening. *New England Journal of Medicine.* 2018; 378(18)DOI
10. Davidson KW, Barry MJ, Mangione CM, Cabana M, Caughey AB, Davis EM, Donahue KE, et al. Screening for Colorectal Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA.* 2021; 325(19)DOI
11. Argilés G., Tabernero J., Labianca R., Hochhauser D., Salazar R., Iveson T., Laurent-Puig P., et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment

and follow-up. *Annals of Oncology.* 2020; 31(10)DOI
12. Meklin J, Syrjänen K, Eskelinen M. Colorectal Cancer Screening With Traditional and New-generation Fecal Immunochemical Tests: A Critical Review of Fecal Occult Blood Tests. *Anticancer Research.* 2020; 40(2)DOI
13. Kapoor C, Vaidya S, Wadhwan V, Kaur G, Pathak A. Seesaw of matrix metalloproteinases (MMPs). *Journal of Cancer Research and Therapeutics.* 2016; 12(1)DOI
14. Huang H. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. *Sensors.* 2018; 18(10)DOI
15. Araújo Jr R.F., Lira G.A., Vilaça J.A., Guedes H.G., Leitão M.C.A., Lucena H.F., Ramos C.C.O.. Prognostic and diagnostic implications of MMP-2, MMP-9, and VEGF-α expressions in colorectal cancer. *Pathology - Research and Practice.* 2015; 211(1)DOI
16. Yang B, Tang F, Zhang B, Zhao Y, Feng J, Rao Z. Matrix metalloproteinase-9 overexpression is closely related to poor prognosis in patients with colon cancer. *World Journal of Surgical Oncology.* 2014; 12(1)DOI
17. Annaházi A, Ábrahám S, Farkas K, Rosztóczy A, Inczefi O, Földesi I, Szűcs M, et al. A pilot study on faecal MMP-9: a new noninvasive diagnostic marker of colorectal cancer. *British Journal of Cancer.* 2016; 114(7)DOI
18. Beauty V, Sukartini N, Abdullah M, Immanuel S. The Role of Faecal Matrix Metalloproteinase-9 in the Diagnosis of Patients with Suspected Colorectal Cancer in Cipto Mangunkusumo National General Hospital. 2018.
19. Lachenbruch P. A., Lwanga S. K., Lemeshow S.. Sample Size Determination in Health Studies: A Practical Manual.. *Journal of the American Statistical Association.* 1991; 86(416)DOI
20. Rosman AS, Korsten MA. Effect of Verification Bias on the Sensitivity of Fecal Occult Blood Testing: a Meta-Analysis. *Journal of General Internal Medicine.* 2010; 25(11)DOI
21. McDonald R, Tomlins A, Smith S, Harmston C. Outcomes of faecal occult blood tests requested outside the UK National Bowel Cancer Screening Programme. *Journal of Clinical Pathology.* 2013; 66(4)DOI
22. Bevan R, Lee TJ, Nickerson C, Rubin G, Rees CJ. Non-neoplastic findings at colonoscopy after positive faecal occult blood testing: Data from the English Bowel Cancer Screening Programme. *Journal of Medical Screening.* 2014; 21(2)DOI
23. Groblewska M, Mroczko B, Szmitkowski M. The role of selected matrix metalloproteinases and their inhibitors in colorectal cancer development. *Postepy Higieny I Medycyny Doswiadczalnej (Online).* 2010; 64
24. Kim M, Ha S, Wu M, Zogg H, Ronkon CF, Lee M, Ro S. Extracellular Matrix Biomarkers in Colorectal Cancer. *International Journal of Molecular Sciences.* 2021; 22(17)DOI
25. Moilanen JM, Kokkonen N, Löffek S, Väyrynen JP, Syväniemi E, Hurskainen T, Mäkinen M, et al. Collagen XVII expression correlates with the invasion and metastasis of colorectal cancer. *Human Pathology.* 2015; 46(3)DOI
26. Rutka M, Bor R, Bálint A, Fábián A, Milassin Á, Nagy F, Szepes Z, et al. Diagnostic Accuracy of Five Different Fecal Markers for the Detection of Precancerous and Cancerous Lesions of the Colorectum. *Mediators of Inflammation.* 2016; 2016DOI
27. Barabás L, Hritz I, István G, Tulassay Z, Herszényi L. The Behavior of MMP-2, MMP-7, MMP-9, and Their Inhibitors TIMP-1 and TIMP-2 in Adenoma-Colorectal Cancer Sequence. *Digestive Diseases.* 2021; 39(3)DOI
28. Labeda I, Uwuratuw JA, Hendarto J, Lusikooy RE, Sampetoding S, Kusuma MI, Syarifuddin E, et al. Relationship between metalloproteinase-9 (MMP-9) expression and clinicopathology in colorectal cancer: a cross-sectional study. *Annals of Medicine & Surgery.* 2023; 85(9)DOI
29. Mozooni Z, Amiri KK, Golestani N, Shahmohammadi A, Minaeian S, Bahadorizadeh L. Relationships between MMP-2, MMP-9, and ADAMDEC1 serum and tissue levels in patients with colorectal cancer. *Annals of Coloproctology.* 2025; 41(2)DOI
30. Janer A, Warsinggih W, Uwuratuw J, Ladju R, Sampetoding S, Syarifuddin E. Accuracy of Faecal Matrix-Metalloproteinase-9 (MMP-9) for Colorectal Cancer Detection in Makassar, Indonesia. *Asian Pacific Journal of Cancer Prevention.* 2025; 26(2)DOI
31. Veljkovic A, Stanojevic G, Brankovic B, Roumeliotis S, Leivaditis K, Djordjevic B, Li X, et al.

MMP-9 Activation via ROS/NF-κB Signaling in Colorectal Cancer Progression: Molecular Insights and Prognostic–Therapeutic Perspectives. *Current Issues in Molecular Biology.* 2025; 47(7)DOI

32. Morikawa T, Kato J, Yamaji Y, Wada R, Mitsushima T, Shiratori Y. A Comparison of the Immunochemical Fecal Occult Blood Test and Total Colonoscopy in the Asymptomatic Population. *Gastroenterology.* 2005; 129(2)DOI
33. Chen Y, Chen T, Su M, Ning H, Kuo C, Lin W, Ho Y, et al. Accuracy of immunochemical fecal occult blood test for detecting colorectal neoplasms in individuals undergoing health check-ups. *Advances in Digestive Medicine.* 2014; 1(3)DOI
34. Yang C, Wu W, Yang Y, Yang X, Sun J, Zhang W, Liu K, et al. Multitarget stool DNA test compared with fecal occult blood test for colorectal cancer screening. *Oncology Letters.* 2020; 20(2)DOI