

Manuscript title: Current state and future direction of screening tool for colorectal cancer  
Screening tool for CRC

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Response to the Reviewers' comments

May 22, 2019

Dear Reviewers and Editorial Staff of *World Journal of Meta-Analysis*,

First of all, thank you for giving us a valuable chance to revise our manuscript for publication in *World Journal of Meta-Analysis*.

I enclose our manuscript which was fully revised based upon valuable comments from the reviewers. The revised parts of our manuscript are colored in blue. Also, the answers on the reviewers' comments are included question by question. I hope that our manuscript may become more qualified than the previous version.

We would like to express our sincere gratitude for your thoughtful consideration and thorough scrutiny of our manuscript. Through the critique, we came to understand areas whereby we might improve the manuscript and now resubmit a revision and responses to the reviewers' critiques.

With regards,

Eun Ran Kim, MD. PhD

N.B. Texts written in blue with 12-point Book Antiqua fonts are verbatim of reviewers' comments. The author's responses are attached below each comment and the revised proportion of the manuscript is highlighted with yellow color.

## Review #1

In their paper “Current state and future direction of screening tool for colorectal cancer”, Hong and Kim report a review of the current tests that are being used for colorectal cancer (CRC) screening and on a series of tests that have been studied in the last years and which could eventually be useful for screening in the future. The Authors carried out a huge job in order to review the current literature on diagnostic test for CRC; however, I found not appropriate including in a paper focused on “screening tools for CRC” such a large amount of tests that showed performance levels that are not adequate for a screening tool. If, on one side, a screening test cannot be discarded due to an excessively low sensitivity (as repeated tests could yield a satisfying cumulative sensitivity), on the other side an excessively low specificity represents a tomb stone. Specificity levels lower than 92%-93% are not acceptable for a screening test within a mass screening programme, because the positivity rate would generate an excessive workload for diagnostic workups, and the false positive rate would be unacceptable too.

Response: We agree with Reviewer’s opinion and appreciated Reviewer’s excellent point.

### Major comments

Abstract: I would stress a bit more the necessity to prevent CRC through the diagnosis of advanced adenoma

Response: We agree with Reviewer’s opinion. According to Reviewer's recommendation, we have revised manuscript as follows:

The 5-year survival rate for patients with early-stage CRC is significantly better than that for patients with CRC detected at a late stage. The primary target for CRC screening and prevention is advanced neoplasia, which includes both CRC itself, as well as benign but histologically advanced adenomas that are at increased risk for

progression to malignancy. Prevention of CRC through detection of advanced adenomas is important<sup>[1-3]</sup>. It is, therefore, necessary to develop more efficient detection methods to enable earlier detection and therefore better prognosis.

## REFERENCES

- 1 Winawer SJ, Zauber AG. The advanced adenoma as the primary target of screening. *Gastrointestinal Endoscopy Clinics* 2002; **12**(1): 1-9
- 2 Kim DH, Pickhardt PJ, Taylor AJ, Leung WK, Winter TC, Hinshaw JL, Gopal DV, Reichelderfer M, Hsu RH, Pfau PR. CT colonography versus colonoscopy for the detection of advanced neoplasia. *New England journal of medicine* 2007; **357**(14): 1403-1412
- 3 Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, Dash C, Giardiello FM, Glick S, Levin TR. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA: a cancer journal for clinicians* 2008; **58**(3): 130-160

Introduction, last sentence of the first paragraph: the necessity of finding “new” screening tools suggests that the current ones are not adequate. This should be demonstrated.

Introduction, second paragraph: one further quality of screening tools that should be cited is acceptability by the target population. The performance parameters of a test are not enough.

Response: We agree with Reviewer’s opinion. According to Reviewer's recommendation, we have revised manuscript as follows:

[Introduction]

At present, the best available option for early detection and elimination of premalignant lesions is colonoscopy. However, it is invasive, expensive, and inconvenient for patients. Therefore, non-invasive and reliable methods for

diagnosing CRC are valuable due to colonoscopy risks: puncture of the colon, intraperitoneal bleeding, post-polypectomy, and infection. In particular, with regards to the detection of CRC precursor lesions, such as adenoma, the lack of sensitivity and specificity or an unacceptably wide range of the FOBT has hampered the clinical application in CRC screening<sup>[3]</sup>. Therefore, newer, non-invasive screening methods and biomarkers to permit identification of CRC and its precursors in easily accessible biospecimens are needed. Consequently, current screening methods have limitations, and it is necessary to find new screening methods that can detect CRC in the early phase to improve survival and quality of life for patients with CRC.

The following qualities are what an ideal screening method would possess: it should show high sensitivity and specificity, it must be safe and cost-effective to be widely used, it must be simple to measure, and readings must be consistent among patients of all genders and races. Acceptability of screening method is also important in target population<sup>[9]</sup>. In this review, we summarize the current status of screening tools for colorectal cancer and discuss the future direction of colon cancer screening, including metabolism and proteomics.

## REFERENCES

- 3 Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, Dash C, Giardiello FM, Glick S, Levin TR. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA: a cancer journal for clinicians* 2008; **58**(3): 130-160
- 9 Markowitz SD, Bertagnolli MM. Molecular basis of colorectal cancer. *New England journal of medicine* 2009; **361**(25): 2449-2460

Chapter 1.1: as the FIT is intended to be repeated, I find not adequate to refer to single-sample sensitivity and specificity, even if there are no comparisons between repeated FITs vs. colonoscopy. However, some evidence has started being published. See for instance PMID 29101260

Response: We agree with Reviewer's opinion and appreciated Reviewer's excellent

point. According to Reviewer's recommendation, We have revised manuscript as follows:

[Chapter 1.1] [Paragraph 2]

Studies also used different versions of FITs tests to analyse their outcomes. As the FIT is intended to be repeated, the results for single-sample sensitivity and specificity alone are not sufficient. Although some evidence has started being published recently, the data for the long-term performance of FIT is still lacking<sup>[21]</sup>; thus, these studies should not be the basis for determining the performance qualities of FITs because they do not yet have adequate data.

## REFERENCES

21 Zorzi M, Hassan C, Capodaglio G, Narne E, Turrin A, Baracco M, Dal Cin A, Fiore A, Martin G, Repici A. Divergent long-term detection rates of proximal and distal advanced neoplasia in fecal immunochemical test screening programs: A retrospective cohort study. *Ann Intern Med* 2018; **169**(9): 602

Chapter 1.1: The reported price of FIT refers to the US. In general, much of this paper is centred on the US (e.g., only the ACS guidelines are cited in paragraph 1). Therefore, the Authors should more clearly refer this manuscript to the US setting, e.g. in the title, abstract and tables.

Response: According to Reviewer's recommendation, we have revised manuscript as follows:

[Abstract]

In the present, evidence-based review, the authors summarize the current state as recognized by the recent guideline recommendation from the American Cancer Society, US Preventive Services Task Force and the U.S. Multi-Society Task Force and discuss future direction of screening tools for colorectal cancer<sup>[4-6]</sup>.

**Table 1. Characteristics of Colorectal Cancer Screening Tests Currently in Use in the US.**

## REFERENCES

- 4 Wolf AM, Fontham ET, Church TR, Flowers CR, Guerra CE, LaMonte SJ, Etzioni R, McKenna MT, Oeffinger KC, Shih YCT. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. *CA: a cancer journal for clinicians* 2018; **68**(4): 250-281
- 5 Force UPST. Screening for colorectal cancer: US Preventive Services Task Force recommendation statement. *Annals of internal medicine* 2008; **149**(9): 627
- 6 Rex DK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, Levin TR, Lieberman D, Robertson DJ. Colorectal cancer screening: recommendations for physicians and patients from the US Multi-Society Task Force on Colorectal Cancer. *The American journal of gastroenterology* 2017; **112**(7): 1016

Chapter 1.1: sentence "...the sensitivity of FIT could improve to 77% when the specificity was as high as that of mt-sDNA (86.6%)". A specificity lower than – say – 90% is not acceptable in a mass screening programme.

Response: According to Reviewer's recommendation, we have revised manuscript as follows:

[Chapter 1.1] [Paragraph 5]

Although the sensitivity of FIT could improve to 77% when the specificity of FIT was as high as that of mt-sDNA (86.6%), the sensitivity of FIT is significantly lower than that of mt-sDNA and did not show sufficient specificity for screening program.

Chapter 1.3.1: the description of the mSEPT9 test is not clear. The Authors state that "sensitivity for cancer is lower than that of FIT", but the last paragraph starts with "in comparison with suggested screening tests, the aforementioned studies showed increases in sensitivity...". They also state that detection of advanced adenoma is impossible and subsequently they report a sensitivity and specificity parameters for advanced adenoma... In general, given the long series of disadvantages of this test (listed in the second paragraph), it is not clear why the Authors dedicate so much space to it.

Response: We agree with Reviewer's opinion. we have deleted unnecessary content as follows:

The majority of studies regarding this test have compared advanced neoplasia detection rates by mSEPT9 to a conventional test for screening. As stated by the USPSTF, a prospective study by Lin et al. screening for CRC in a population with average risk and validated by colonoscopy showed that mSEPT9 had a sensitivity of 48% and a specificity of 91%<sup>[7, 63]</sup>. A higher sensitivity (68%) but decreased specificity (80%) was observed for advanced adenomas and cancer when identical samples from the aforementioned study were tested again but with a more recent model of the test<sup>[64]</sup>. Another study on US participants that used the more recent model of the test in screening for CRC showed sensitivity (73%) and specificity (82%) values that were close to that of the previously mentioned test<sup>[65]</sup>. In comparison with suggested screening tests, the aforementioned studies showed increases in sensitivity but significant reduction in specificity.

Chapter 1.4: this chapter could be deleted, as the main limitations of all test have been reported in the previous chapters. Further, it is misleading to refer to CEA and Ca19-9 in a paper focusing on screening tools.

Response: According to Reviewer's recommendation, we eliminate chapter 1.4 as follows:

[Chapter 1.4]

#### **1.4 Limitations of Current Screening Tools**

At present, the best available option for early detection and elimination of premalignant lesions is colonoscopy. However, it is invasive, expensive, and inconvenient for patients. However, non invasive and reliable methods for diagnosing CRC are valuable due to colonoscopy risks: puncture of the colon, intraperitoneal bleeding, post polypectomy, and infection. In particular, with regards to the detection of CRC precursor lesions, such as adenoma, established non-invasive tests, such as FOBTs, are highly specific but have limited sensitivity. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 are frequently used

tumor markers in the clinic. Yet, such tumor markers cannot be used solely for screening or diagnosis because they have a low sensitivity for CRC, and their blood levels could be elevated by benign diseases, such as inflammatory bowel diseases and pneumonia and even smoking. Furthermore, it is evident that there are no non-invasive screening tools for the detection of precancerous lesions, in particular, colorectal adenoma. Consequently, newer, non-invasive screening methods and biomarkers to permit identification of CRC and its precursors in easily accessible biospecimens are needed. Developing cheaper, simple, and accurate diagnostic tools for detecting advanced adenoma and early stage CRC is crucial for complete recovery and reduction in medical expenses.

Chapter 2.1.3. Sentence “They proposed that ... mRNA expression... may be useful as a CRC screening test”. The specificity of the test was 74%, which is absolutely unacceptable for a screening test.

Response: According to Reviewer's recommendation, we have revised manuscript as follows:

[Chapter 2.1.3]

The result showed a sensitivity of 74.1% and specificity of 74.1%. Although it did not show sufficient specificity to be used as a screening test, they proposed that the profile of miRNA expression may be useful as a CRC screening test from stool specimens.

Chapter 2.1.4. Opposite concepts are reported. TFPI2 gene methylation and SDC2 gene obtained outstanding performances (spec 93% sens 89% and spec 95% and sens 87%, respectively). But in the last paragraph the Authors state that “...studies have failed to produce a ... biomarker that could be used for ... screening”. Please clarify.

Response: According to Reviewer's recommendation, we have revised manuscript as follows:

[Chapter 2.1.3] [Paragraph 2]

Despite the wide variety of molecular techniques, More research is needed to produce a new molecular biomarker or biomarker panel that could be used for a broad range



of screening.

Table 1. Colonoscopy: please consider including acceptability and participation among the disadvantages. The same applies to CTC and FS

Response: We appreciated Reviewer's excellent point. According to Reviewer's recommendation, We have revised Table 1 and added a reference as follows:

### Direct Visualization Screening Tests

Colonoscopy <sup>A, B, C</sup>	Every 10 y	<ul style="list-style-type: none"> <li>Non-RCT evidence of incidence and mortality reduction</li> <li>Prospective cohort study with mortality end point</li> </ul>	<ul style="list-style-type: none"> <li>Requires less frequent screening</li> <li>Screening, diagnosis, treatment and prevention through polypectomy can be done at the same-session.</li> <li>Gross visualization of the entire colon</li> </ul>	<ul style="list-style-type: none"> <li>Pain and discomfort</li> <li>lower tolerability and compliance than FS<sup>[119]</sup></li> <li>Possibility of bowel perforation/bleeding and cardiopulmonary complications from anesthesia</li> <li>Requires full bowel cleansing</li> <li>Performance varies upon adequacy of bowel prep, the cecal intubation rate, withdrawal time, and detection rate</li> <li>Lower sensitivity for neoplasia in the proximal and distal colon</li> </ul>
CTC <sup>A, B, C</sup>	Every 5 y	<ul style="list-style-type: none"> <li>Test characteristic studies</li> <li>Extrapolation from RCTs of sigmoidoscopy demonstrating mortality reduction</li> </ul>	<ul style="list-style-type: none"> <li>Rapid, non-invasive imaging method</li> <li>Well-tolerated by patients</li> <li>Does not require anesthesia</li> <li>Better tolerability and acceptance than colonoscopy and FS<sup>[120]</sup></li> </ul>	<ul style="list-style-type: none"> <li>Exposure to low-dose radiation</li> <li>Requires full bowel cleansing</li> <li>A second bowel cleansing will be required before repeat colonoscopy for positive test</li> </ul>
FS <sup>A, B, C</sup>	Every 5 y	<ul style="list-style-type: none"> <li>RCTs with mortality end points:</li> </ul>	<ul style="list-style-type: none"> <li>Does not require anesthesia</li> <li>Requires more limited bowel cleansing</li> <li>Better acceptance than colonoscopy<sup>[119]</sup></li> </ul>	<ul style="list-style-type: none"> <li>Pain and discomfort</li> <li>Does not examine the proximal Colon</li> <li>Requires enema prior to procedure</li> <li>Abnormal findings require second colonoscopy</li> </ul>

### REFERENCES

119 Senore C, Ederle A, Fantin A, Andreoni B, Bisanti L, Grazzini G, Zappa M, Ferrero F, Marutti A, Giuliani O. Acceptability and side-effects of colonoscopy and sigmoidoscopy in a screening setting. *Journal of medical screening* 2011; **18**(3): 128-134

120 Taylor SA, Halligan S, Saunders BP, Bassett P, Vance M, Bartram CI. Acceptance by patients of multidetector CT colonography compared with barium enema examinations, flexible sigmoidoscopy, and colonoscopy. *American journal of roentgenology* 2003; **181**(4): 913-921

## Minor

Chapter 1.: please add a reference when referring to the ACS guideline

Response: According to Reviewer's recommendation, We have added a reference as follows:

[Chapter 1]

The American Cancer Society (ACS) Guideline recommends stool-based tests and structural examinations as options for colorectal cancer screening<sup>[4]</sup>.

## REFERENCES

4 Wolf AM, Fontham ET, Church TR, Flowers CR, Guerra CE, LaMonte SJ, Etzioni R, McKenna MT, Oeffinger KC, Shih YCT. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. *CA: a cancer journal for clinicians* 2018; **68**(4): 250-281

Chapter 1.1: please add a reference when referring to the “multiple consistent RCTs” on gFOBT

Response: According to Reviewer's recommendation, We have added a reference for evidence of gFOBT as follows

[Chapter 1.1]

The use of this stool testing for CRC screening has been supported by multiple consistent randomized clinical trials<sup>[10]</sup>.

## REFERENCES

10 Lin JS, Piper MA, Perdue LA, Rutter CM, Webber EM, O'Connor E, Smith N, Whitlock EP. Screening for colorectal cancer: updated evidence report and systematic review for the US Preventive Services Task Force. *Jama* 2016; **315**(23): 2576-2594

Chapter 1.1: please replace “However, the use of FOBT..” with “However, the use of

gFOBT..”

Response: According to Reviewer's recommendation, we have revised manuscript as follows:

“However, the use of FOBT..” → “However, the use of gFOBT..”

[Chapter 1.1] [Paragraph 1]

The use of this stool testing for CRC screening has been supported by multiple consistent randomized clinical trials. However, the use of gFOBT is complicated by its poor sensitivity and specificity as the test shows false negatives when a patient uses antioxidants, like vitamin C, whereas false positives occur when a patient has upper GI bleeding from NSAIDS intake, or consumes red meat or dietary peroxidase from certain vegetables and fruits<sup>[4]</sup>.

Chapter 1.2: sentence “colonoscopy is usually performed with anaesthesia”. To which area are the Authors referring to? Please add a reference.

Response: According to Reviewer's recommendation, we have revised manuscript and added a reference as follows:

[Chapter 1.2]

Unlike FS and CTC, colonoscopy is often performed with anesthesia; hence, the patient must be accompanied by a caretaker<sup>[27]</sup>.

## REFERENCES

27 Wernli KJ, Brenner AT, Rutter CM, Inadomi JM. Risks associated with anesthesia services during colonoscopy. *Gastroenterology* 2016; **150**(4): 888-894

Chapter 1.3.1: did FDA specifically approve this test for individuals who “continuously rejected undergoing other types of screening tests”? Please clarify this point.

Response: According to Reviewer's recommendation, we have revised manuscript to

clarify the meaning and added a reference as follows:

[Chapter 1.3.1] [Paragraph 1]

The FDA recently cleared a blood test that identifies a CRC biomarker, mSEPT9<sup>[63]</sup>.

## REFERENCES

63 US Food and Drug Administration (FDA). Epi ProColon. Silver Spring, MD: FDA;2016. [https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma\\_template.cfm?id=p130001](https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma_template.cfm?id=p130001). Accessed February 6, 2018.

Chapter 2.1.1. What does “From 1997 to 2010...” mean? Did Liang take 13 years to carry out his meta-analysis? Please clarify.

Response: According to Reviewer's recommendation, we have revised manuscript as follows:

[Chapter 2.1.1]

Liang et al. have performed meta-analysis study between 1997 and 2010 to correlate APC polymorphisms and CRC risk.

Chapter 2.2.1.2. Sentence “from those ten, the formula of one was not identified, while that of the six were identified”. What about the last three? Anyway, this detail seems superfluous.

Response: According to Reviewer's recommendation, we have deleted unnecessary content as follows:

[Chapter 2.2.1.2] [Paragraph 1]

Among the studies, the assay with the highest sensitivity used ten distinct metabolites. ~~From those ten, the chemical formula of one was not identified, while that of six were identified.~~ However, no additional categorization was done for the latter<sup>[108]</sup>.

Table 1. Add “in the US” in the title.

Response: According to Reviewer's recommendation, we have revised the title of Table 1 as follows:

Characteristics of Colorectal Cancer Screening Tests Currently in Use

→ Characteristics of Colorectal Cancer Screening Tests Currently in Use **in the US.**

**Table 1. Characteristics of Colorectal Cancer Screening Tests Currently in Use **in the US.****

Table 1. HSgFOBT: I am not aware of consistent evidence of incidence reduction. Please add references

Response: According to Reviewer's recommendation, We have added a reference for evidence of HSgFOBT as follows:

gFOBT with high sensitivity <sup>A, B</sup> (HSgFOBT)	Every year	<ul style="list-style-type: none"><li>• Good RCT evidence for incidence and mortality reduction<sup>[114-118]</sup></li><li>• Varies in test performance characteristics by version of the test</li></ul>	<ul style="list-style-type: none"><li>• Inexpensive compared with structural examinations and mt-sDNA</li><li>• Can be done at home</li><li>• Does not require bowel preparation or anesthesia</li></ul>	<ul style="list-style-type: none"><li>• High nonadherence to yearly tests (especially without reminder systems)</li><li>• Less effective for advanced adenoma detection</li><li>• Difficulty in determining test performance among the many cleared tests</li><li>• Requires <u>multiple</u> samples</li><li>• Requires dietary and medication</li><li>• <u>Higher false-positive rate</u> than to more colonoscopies</li></ul>
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## REFERENCES

- 114 Hardcastle JD, Chamberlain JO, Robinson MH, Moss SM, Amar SS, Balfour TW, James PD, Mangham CM. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *The Lancet* 1996; **348**(9040): 1472-1477
- 115 Kronborg O, Fenger C, Olsen J, Jørgensen OD, Sørensen O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *The Lancet* 1996; **348**(9040): 1467-1471

- 116 Lindholm E, Brevinge H, Haglind E. Survival benefit in a randomized clinical trial of faecal occult blood screening for colorectal cancer. *British Journal of Surgery: Incorporating European Journal of Surgery and Swiss Surgery* 2008; **95**(8): 1029-1036
- 119 Mandel JS, Church TR, Ederer F, Bond JH. Colorectal cancer mortality: effectiveness of biennial screening for fecal occult blood. *Journal of the National Cancer Institute* 1999; **91**(5): 434-437
- 120 Hewitson P, Glasziou PP, Irwig L, Towler B, Watson E. Screening for colorectal cancer using the faecal occult blood test, Hemoccult. *Cochrane Database of Systematic Reviews* 2007(1)

Table 3. Evidence of efficacy of blood based biomarkers. What do 85.7% and 52.1% refer to? And 71.0% and 75.0%?

Response: According to Reviewer's recommendation, We have added a reference for evidence of Table 3 as follows:

Sample Types	Evidence of Efficacy	Advantage	Disadvantage
<b>Blood-based biomarkers</b>	<ul style="list-style-type: none"> <li>• <u>A combination of 8 metabolites</u> (99.3% sensitivity, 93.8% specificity, and AUC 0.996)<sup>[96]</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Easily accessible</li> <li>• Less affected by diet than urine</li> </ul>	<ul style="list-style-type: none"> <li>• Affected by smoking status</li> <li>• More invasive than urine and stool</li> </ul>
(serum, plasma, and dried blood spot)	<ul style="list-style-type: none"> <li>• <u>Gastrointestinal tract acid 446</u> (83.3% sensitivity, 84.8% specificity, 85.7%, and 52.1% , respectively)<sup>[98, 99]</sup></li> <li>• <u>Decanoic acid</u> (87.87% sensitivity, 80.0% specificity, 71.0%, and 75.0%, respectively)<sup>[100, 101]</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Less diurnal variation and Less inter- and intra-subject variability than urine</li> <li>• Stable over a 4-months period frozen at -80 °C except at room temperature</li> </ul>	<ul style="list-style-type: none"> <li>• Analysis can be more complex than urine</li> </ul>
<b>Urine</b>	<ul style="list-style-type: none"> <li>• <u>Cross-validated panel of seven metabolites</u> (97.5% sensitivity, 100% specificity, and AUC 0.998)<sup>[106]</sup></li> <li>• 10 different metabolites (100% sensitivity, 80% specificity but small sample size)<sup>[105]</sup></li> <li>• N1, N12-Diacetylspermine<sup>[107, 108]</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Easily accessible</li> <li>• less invasive than blood</li> </ul>	<ul style="list-style-type: none"> <li>• More affected by diet than serum samples</li> <li>• More diurnal variation and More inter- and intra-subject variability than serum</li> <li>• A full day storing at room temperature or on cool packs altered metabolite concentration</li> <li>• More than 2 freeze and thaw cycles affected the metabolic profile significantly</li> </ul>

<b>Stool</b>	<ul style="list-style-type: none"> <li>• A three metabolite panel (AUC 1.0 but very small sample size)<sup>[109]</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Easily accessible</li> <li>• less invasive than blood</li> </ul>	<ul style="list-style-type: none"> <li>• Inconvenient to collect of stool samples</li> <li>• Low compliance</li> </ul>
	<ul style="list-style-type: none"> <li>• A metabolomics panel (AUC 0.94)<sup>[110]</sup></li> </ul>		

## REFERENCES

- 96 Nishiumi S, Kobayashi T, Kawana S, Unno Y, Sakai T, Okamoto K, Yamada Y, Sudo K, Yamaji T, Saito Y. Investigations in the possibility of early detection of colorectal cancer by gas chromatography/triple-quadrupole mass spectrometry. *Oncotarget* 2017; **8**(10): 17115
- 98 Hata T, Takemasa I, Takahashi H, Haraguchi N, Nishimura J, Hata T, Mizushima T, Doki Y, Mori M. Downregulation of serum metabolite GTA-446 as a novel potential marker for early detection of colorectal cancer. *British journal of cancer* 2017; **117**(2): 227
- 99 Ritchie SA, Tonita J, Alvi R, Lehotay D, Elshoni H, Myat S, McHattie J, Goodenowe DB. Low-serum GTA-446 anti-inflammatory fatty acid levels as a new risk factor for colon cancer. *International journal of cancer* 2013; **132**(2): 355-362
- 100 Uchiyama K, Yagi N, Mizushima K, Higashimura Y, Hirai Y, Okayama T, Yoshida N, Katada K, Kamada K, Handa O. Serum metabolomics analysis for early detection of colorectal cancer. *Journal of gastroenterology* 2017; **52**(6): 677-694
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- 106 Cheng Y, Xie G, Chen T, Qiu Y, Zou X, Zheng M, Tan B, Feng B, Dong T, He P. Distinct urinary metabolic profile of human colorectal cancer. *Journal of proteome research* 2011; **11**(2): 1354-1363
- 107 Nakajima T, Katsumata K, Kuwabara H, Soya R, Enomoto M, Ishizaki T, Tsuchida A, Mori M, Hiwatari K, Soga T. Urinary polyamine biomarker panels with machine-learning differentiated colorectal cancers, benign disease, and healthy controls. *International journal of molecular sciences* 2018; **19**(3): 756
- 108 Hiramatsu K, Takahashi K, Yamaguchi T, Matsumoto H, Miyamoto H, Tanaka S, Tanaka C, Tamamori Y, Imajo M, Kawaguchi M. N1, N12-Diacetylspermine as a sensitive and specific novel marker for early-and late-stage colorectal and breast cancers. *Clinical cancer research* 2005; **11**(8): 2986-2990
- 109 Phua LC, Chue XP, Koh PK, Cheah PY, Ho HK, Chan ECY. Non-invasive fecal metabonomic detection of colorectal cancer. *Cancer biology & therapy* 2014; **15**(4): 389-397

110 Amiot A, Dona AC, Wijeyesekera A, Tournigand C, Baumgaertner I, Lebaleur Y, Sobhani I, Holmes E. 1H NMR spectroscopy of fecal extracts enables detection of advanced colorectal neoplasia. *Journal of proteome research* 2015; **14**(9): 3871-3881

Table 3. Advantages and disadvantages: Please specify all sentences beginning with “Less” or “more”. For instance: “Less affected by diet” than???

Response: According to Reviewer's recommendation, We have specified all the sentences beginning with “Less” or “more” as follows:

Sample Types	Evidence of Efficacy	Advantage	Disadvantage
<b>Blood-based biomarkers</b>  (serum, plasma, and dried blood spot)	• <u>A combination of 8 metabolites</u> (99.3% sensitivity, 93.8% specificity, and AUC 0.996) <sup>[96]</sup>	• Easily accessible  • Less affected by diet <b>than urine</b>	• Affected by smoking status  • More invasive than urine and stool
	• <u>Gastrointestinal tract acid 446</u> (83.3% sensitivity, 84.8% specificity, 85.7%, and 52.1% , respectively) <sup>[98, 99]</sup>	• Less diurnal variation and Less inter- and intra-subject variability <b>than urine</b>	• Analysis can be more complex than urine
	• <u>Decanoic acid</u> (87.87% sensitivity, 80.0% specificity, 71.0%, and 75.0%, respectively) <sup>[10, 101]</sup>	• Stable over a 4-months period frozen at -80 °C except at room temperature	
<b>Urine</b>	• <u>Cross-validated panel of seven metabolites</u> (97.5% sensitivity, 100% specificity, and AUC 0.998) <sup>[106]</sup>	• Easily accessible  • less invasive than blood	• More affected by diet <b>than serum samples</b>  • More diurnal variation and More inter- and intra-subject variability <b>than serum</b>
	• 10 different metabolites (100% sensitivity, 80% specificity but small sample size) <sup>[105]</sup>  • N1, N12-Diacetylspermine <sup>[107, 108]</sup>		• A full day storing at room temperature or on cool packs altered metabolite concentration  • More than 2 freeze and thaw cycles affected the metabolic profile significantly
<b>Stool</b>	• A three metabolite panel (AUC 1.0 but very small sample size) <sup>[109]</sup>	• Easily accessible  • less invasive than blood	• Inconvenient to collect of stool samples
	• A metabolomics panel (AUC 0.94) <sup>[110]</sup>		• Low compliance