

Format for ANSWERING REVIEWERS

December 3, 2013

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 5840-review.doc).

Title: Circulating free nucleic acid analysis for colorectal cancer diagnosis

Author: Giulia De Maio, Claudia Rengucci, Wainer Zoli, Daniele Calistri

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5840

The manuscript has been improved according to reviewers' suggestions:

1. The formatting has been checked.
2. The text has been revised according to the suggestions of the reviewers and all changes in the manuscript have been indicated in red.

Reviewer (1):

The topic is actual. However the paper present several limitation: 1) a long historical introduction and a commentary on the origin of circulating RNA and DNA that can be safely shortened 2) a very limited discussion of the clinical implications of plasma determination 3) an imbalance in references: several papers before 2008, few recent papers. For instance is quite incredible that they do not quote the paper of Misale (Nature 2012, 486: 532-6) and the several commentaries that have underscored how the quantification of circulating DNA can be applied during treatment of CRC cancer.

- 1) This part of the manuscript has been shortened according to the reviewer's suggestion.
- 2) We have extended our discussion of the clinical implications of plasma on pages 16-17 of the Conclusions.
- 3) More recent papers have been inserted into the text, including that by Misale et al. (ref. 76). However, in our opinion the most important papers on this topic were already included in the original version.

Reviewer (2):

SUGGESTIONS 1- Although the review refers to "nucleic acids", the authors have barely addressed the use of RNA as a diagnostic tool. While there are others, I would suggest at least addressing the work of Kanaoka's lab (Kanaoka S. et al., Gastroenterology 2004; Hamaya Y. et al., Br J Canc. 2010) who clearly demonstrated the usefulness of detecting specific transcripts in the stools for diagnosing colorectal cancer. See also Koga Y. et al., Cancer Sci 2008 and Yu Y.J. et al., Cancer Epidemiol Biomarkers Prev 2008 for additional studies by other groups. The potential of RNA analysis should also be addressed in the conclusions. 2- For more clarity, I would suggest referring to "circulating and stool nucleic acids" in the title and elsewhere in the text. 3- On page 9 (EARLY DIAGNOSIS OF CRC), line 5, please verify that CRC is the 2nd (? , not the 4th?) cause of cancer deaths in women. 4- On page 13, end of 2nd paragraph, please update the knowledge of SEPT9 since this marker has already been commercialized as a CRC diagnostic tool based on studies such as those published by Gruetzmann R. et al., PLOS ONE 2008 and Ahlquist D.A. et al., Clin Gastroenterol Hepatol 2011. 5- On page 16, 2nd paragraph, lines 10-12: for more clarity, please provide the reference for the study by Imperiale et al., at the end of the sentence. It may also be worth considering a recent meta-analysis of 20 studies on the diagnostic value of stool DNA testing for CRC screening (Yang H. et al., Can J Gastroenterol, 2013).

- 1) The potential of RNA analysis is now discussed in the Conclusions (pages 16-17) and References (refs. 104, 105, 106 and 107) pertaining to the use of RNA to detect specific transcripts in stool samples for CRC diagnosis have been added (pages 14-15).
- 2) We have modified the original title, as suggested.
- 3) We confirm that CRC in women is the 2nd cause of death (ref. 42 – Jemal et al. *CA Cancer J Clin* 2011; **61**: 69-90).
- 4) As requested we have updated the information on SEPT9 (pages 10-11) and added some references (refs. 72, 73, 74 and 75).
- 5- The reference (94) for Imperiale's study has been added on page 13 and reference to the recent meta-analysis on the diagnostic value of stool DNA testing for CRC screening has also been inserted (ref. 94).

Reviewer (3):

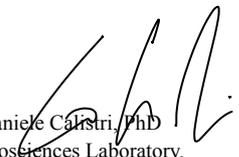
It is an interesting, well-written review article containing a lot of information in molecular basis that evaluates the role of circulating free DNA in blood and stools as a biomarker for early diagnosis and follow-up of colorectal cancer. There were no points to address.

- 3) References and typesetting were corrected.

The language content of the text has been thoroughly revised by a native English speaker.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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