

## Response letter

Reviewer: 1

This manuscript reports an evaluation of a new faecal test for *H. pylori* infection in adults.

Specific Comments:

1) The authors use the abbreviation/term THD from the start of their work without explanation.

THD is not an abbreviation, but it is the full name of the company that produced and provided the THD fecal test free of charge for all participants included in the study.

2) The Methods of the ABSTRACT refers to infection, without specifically indicating that they mean *H. pylori* infection.

According to the suggestion, we indicated "*Helicobacter pylori* infection" in Methods of the abstract of the revised manuscript.

3) The results of the ABSTRACT indicates that a certain number of participants performed endoscopy. One assumes that these participants were not all endoscopists! Suggest to change the word to "underwent".

The suggested correction has been included in both the results of the abstract and main text of the revised manuscript.

4) The results of the ABSTARCT talks about the 23 with histo and UBT, and then goes directly to discuss the utility of the faecal test: this suggests that this utility was in the 23 older patients. Suggest to make more clear that the utility referred to was in all subjects.

According to this reviewer's suggestion, the sentence was rephrased in the abstract of the revised manuscript in order to clarify that the THD fecal test diagnostic accuracy parameters were referred to the 290 participants who completed the study.

5) In line 1 of the INTRODUCTION, which population is being referred to?

In the revised manuscript, we clarified that the prevalence of *Helicobacter pylori* infection was referred to the general population worldwide.

6) The word "threatened" may be better replaced with the word "limited".

Thanks for the suggested change, which we have now done.

7) The methods study design section mentions that drugs may increase false negative testing, but this does not indicate which tests are being referred to.

As suggested, we detailed in the revised manuscript that the use of proton pump inhibitors, 2-histamine receptor antagonists, antibiotics and bismuth salts may increase false negative results of invasive and non-invasive current diagnostic tests for *Helicobacter pylori* infection.

8) The end of para 2 on page 8 is a bit repetitive.

According to this suggestion, we deleted the final sentences of the paragraphs "THD fecal test (index test)" and "<sup>13</sup>C-urea breath test (reference standard) and upper endoscopy".

9) In the RESULTS section (and elsewhere) the authors note that 294 were included and that 4 withdrew, yet they continue to present the data for 294 subjects. The data should be presented for 290 included subjects.

The baseline characteristics of the study population are referred to all participants enrolled in the study. However, as specified in the Results section of the main text, we reported the results of the “complete-case analysis” which included only the 290 participants who completed the study. In this section, we also mentioned that “in the best-worst case analysis, including all 294 participants enrolled in the study, there were small changes in THD fecal test diagnostic accuracy parameters”. The best-worst case approach was provided to avoid overestimation of diagnostic accuracy parameters.

10) Para 2 of the RESULTS includes basic details also presented in the Table. Suggest just the table to avoid repetition.

We performed the suggested correction in the revised manuscript by deleting from the text the basic details reported in the table.

11) Page 11 (and elsewhere) includes some incorrect word/sentence structure.

We thank the reviewer for their comments and have asked a native English speaker to review the manuscript again for word/sentence structure.

12) The authors refer to bacterium on page 11, without specifying which organism they are referring to.

We changed the term “bacterium” with “*H. pylori*”.

13) The value of the last sentence on page 11 is unclear. Is this reflecting the data of this work, or is conjecture arising from this work. Given that the current work was conducted in only one population (with one background disease prevalence), these comments should be in the DISCUSSION only.

We adjusted the sentence in order to clarify that the post-THD fecal test probability of *H. pylori* infection, for populations with different prevalence of this condition, was calculated using likelihood ratios estimated from our data. This type of statistical analysis allows generalizing the diagnostic test results obtained in a single population. Indeed, likelihood ratios, differently from the other diagnostic accuracy parameters, are not influenced by the variation of disease prevalence among different population.

14) The DISCUSSION could be enhanced with reorganisation to enhance flow and readability.

We thank the reviewer for their comment. In our revised discussion, we have now adopted the scheme advocated for by Richard Horton-‘The hidden research paper’ for writing discussion [JAMA. 2002; 287(21):2775-8]. The discussion is now structured as follows: i. Main findings; ii. Comparison with existing knowledge; iii. Strengths and limitations; iv. Implications for practice and research / Conclusions.

Reviewer: 2

THD should be corrected as "a new test" but mentioned in methods. A good paper to provoke treatment targeted to established resistance issues.

We thank the reviewer for the kind appreciation of our paper. According to the suggestion, we changed the term “THD fecal test” with “a new fecal test” in the title and introduction of both abstract and main text. Successively, we specified that “THD” is the full name of the company that produced and provided this new fecal test free of charge for all participants included in the study. Therefore, the company itself named the device “THD fecal test”.

Reviewer: 3

The manuscript is well-written, the study design correct, the method used are up-to-date and the statistics is robust. One remark: it would be better if the new test were compared with another stool antigen test, to see what brings new and better the THD test as compared to the older stool tests.

We thank the reviewer for the kind appreciation of our paper. Our choice of using urea breath test as the reference investigation was supported by two considerations: i. we standardized the test methodology in a multicentre Italian study [Aliment Pharmacol Ther 2003;17(10):1291-7]; ii. The European and Italian Guidelines require, for a correct interpretation of the result of current stool antigen tests, two pre-requisites (i.e. monoclonal antibody availability and validation "in loco") that we could not guarantee in our study population.