Answering reviewers' comments

January 31, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7501-review.doc).

Title: Diagnosis of *H.pylori*: What should be the gold standard?

Author: Saurabh Kumar Patel; Chandra Bhan Pratap; Ashok Kumar Jain; Anil Kumar Gulati; Gopal Nath

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7501

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers.

Comment 1: Page 23 of the article needs to be reviewed. Is PCR the best method under all conditions? This test costs much higher than other tests and it could not be conducted in many regions due to the costs and shortage of equipment. Coincidentally, these regions are susceptible to this infection. Is the proposed method the best for children? Would it not be better to pick the best method based on age and conditions? I propose that the best diagnostic method be defined based on different conditions in the conclusion.

Clarifications/Modifications: Although, PCR may be used as gold standard, provided that the chances of contamination are taken care off, the method to be used should be proposed on the basis of level of available diagnostic facilities. If endoscopic facility is not available in periphery or underdeveloped regions, diagnosis by SAT using monoclonal antibody based kits may be applied on stool specimens. In situations where UBT system is available but endoscopic facility is not available then this test should be considered the best option. Although, serology is often misleading but it may be the best in children where sanitary conditions are satisfactory and prevalence of the infection is low especially

in pediatric age group. Therefore, utility of each of the invasive and non invasive tests are almost equally important depending upon the given clinical situation.

Comment 2a: First of all, this review was based on very old papers. Review paper should be included more recent papers published in 2010-2013. For example, the authors used the term "more recent" in the section of RUT. However, this paper was published in 1994.

Clarifications/Modifications: The newer references have been consulted and referenced. In RUT section the term "more recent" has been removed according to reference.

Comment 2b: Some sentence did not include a reference. For example, in the section of culture, the authors stated that the recommendation is not to consume these drugs 2 weeks prior to endoscopy. However, we cannot see a reference for this sentence. In page 16, the authors stated that these include a number of surface or secretory antigens such as CagA, various subunits of urease, heat shock proteins, catalase and LPS to mention a few. In page 17, the specificity of serology is satisfactory (90%). In page 19, antibody levels can persist in the blood of individuals cured of *H. pylori* infection for long periods of time. However, we cannot see references for these sentences.

Clarifications/Modifications: In support of the statements references have been added.

"To avoid false negative results, Megraud and Lehours (2007) recommended that not to consume these drugs 2 weeks prior to endoscopy [36] as is done for rapid urease test [37-38]."

"These include a number of surface or secretory antigens such as *Cag*A, various subunits of urease, heat shock proteins, catalase and LPS to mention a few [149-153]."

"The sensitivity and specificity of serology is satisfactory i.e. 80- 90% [166]."

"Antibody levels can persist in the blood of individuals cured of *H. pylori* infection for long periods of time [179]."

Comment 2c: The authors concluded that PCR is best test for the detection of *H. pylori*. They showed the limitation of UBT or other tests because they can be affected by PPI. However, they did not mention the influence of PPI for PCR.

Clarifications/Modifications: An advantage of PCR as summarized by Dus *et al*. (2013) in their review article both spiral or coccoid forms could be detected and PPI will not influence the efficacy of PCR based detection.

Comment 2d: In the section for UBT, the authors cited a paper from Gisbert and Pajares. However, it seems to be for a stool antigen test.

Clarifications/Modifications: Reference has been updated: **Gisbert JP,** Pajares JM. Review article: ^{13}C-urea breath test in the diagnosis of *Helicobacter pylori* infection - a critical review. *Aliment Pharmacol Ther* 2004; 20(10):1001-17. DOI: 10.1111/j.1365-2036.2004.02203.x; PMID: 15569102**.**

Comment 2e: The authors cited Maastricht III. However, we can see Maastricht IV now.

Clarifications/Modifications: Recommendations of Maastricht IV has been reviewed and important and relevant recommendations are mentioned in review article e.g. However, EHSG has recommended in its publication in 2012 that by using monoclonal antibody SAT may be used as it gives equivalent diagnosis accuracy to UBT.

Comment 3a: There are some language mistakes (e.g page 5 "It has been established that approximately 80% and 95% *H.pylori* infection is cause of the gastric ulcer and (GU) duodenal ulcer (DU) [9]." May be written as "It has been established that approximately 80% and 95% of gastric ulcer (GU) and duodenal ulcer (DU) are caused by H.pylori, respectively).

Clarifications/Modifications: The sentence has been change as per suggestion of reviewer.

Comment 3b: In the abstract RUT should be explained before the abbreviation as was done in the text.

Clarifications/Modifications: In the abstract also RUT has been spelled fully.

Comment 3c: In page 9, second sentence "The recommendation is not to consume these drugs 2 weeks prior to endoscopy." The authors should provide an updated an valid reference for this sentence. This also is true for the sentence in page 9 "The recommendation is, therefore, to take two biopsy specimens from the antrum as well as two specimens each from the anterior and posterior corpus."

Clarifications/Modifications:As per suggestions references have been added.

"Although PPI has no antibacterial activity at the recommended dosage in gastric mucosa [35] but they indirectly interfere with *H. pylori* distribution in the stomach by altering the pH of its bacterial habitat, causing its disappearance in the antrum tissue. To avoid false negative results, Megraud and Lehours (2007) recommended that not to consume these drugs 2 weeks prior to endoscopy [36] as is done for rapid urease test [37-38].

The recommendation, by Megraud and Lehours (2007) is, therefore, to take two biopsy specimens from the antrum as well as two specimens each from the anterior and posterior corpus [36]."

Comment 3d: In page 14, first sentence "Use of UBT is often considered as the gold standard test in the diagnosis of *H. pylori* infection [114]." Here, the reference is very old (1987). I also think the gold standard is the histology for the diagnosis of *H. Pylori*. The UBT may have comparable results with histology, but the noninvasiveness of the UBT make it more cheaper, appliable and favorable.

Clarifications/Modifications: The recent references has been added(Please see refernces [118-120]).

Although, PCR may be used as gold standard, provided that the chances of contamination are taken care off, the method to be used should be proposed on the basis of level of available diagnostic facilities. If endoscopic facility is not available in periphery or underdeveloped regions, diagnosis by SAT using monoclonal antibody based kits may be applied on stool specimens. In situation where UBT system is available but endoscopic facility is not available then this test should be considered the best option. Although, serology is often misleading but it may be the best in children where sanitary conditions are satisfactory and prevalence of the infection is low especially in pediatric age group. Therefore, utility of each of the invasive and non invasive tests are almost equally important depending upon the given clinical situation.

Comment 3e: The reference 116, which was mentioned as comprehensive review for UBT test, is about stool antigen test, not for UBT test

Clarifications/Modifications: Reference has been updated: **Gisbert JP,** Pajares JM. Review article: ^{13}C-urea breath test in the diagnosis of *Helicobacter pylori* infection - a critical review. *Aliment Pharmacol Ther* 2004; 20(10):1001-17. DOI: 10.1111/j.1365-2036.2004.02203.x; PMID: 15569102**.**

Comment 3f: The important and main references are old (e.g; authors referenced the Maastrich III as 158; but the new Maastrich consensus Maastrich IV/Florence Consensus Report was published in 2012).

Clarifications/Modifications: Recommendations of Maastricht IV has been reviewed and important and relevant recommendations are mentioned in review article e.g. However, EHSG has recommended in its publication in 2012 that by using monoclonal antibody SAT may be used as it gives equivalent diagnosis accuracy to UBT.

3 References and typesetting were corrected

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

Sincerely yours,

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Answering reviewers' comments

June 20, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7501-review.doc).

Title: Diagnosis of *H. pylori*: What should be the gold standard?

Author: Saurabh Kumar Patel; Chandra Bhan Pratap; Ashok Kumar Jain; Anil Kumar Gulati; Gopal Nath

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7501

We have tried to improve the manuscript as per suggestions of the reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer (**Reviewer code:** 02536325).

The authors propose a very comprehensive review of diagnostic methods for *H. pylori* infection, questioning about which method should be considered the gold standard. Although some parts of the manuscript are extensive, I found the text very informative. I have the following general comments:

Comment 1.1: The authors would benefit if they did the review of the English language.

Clarifications/Modifications: As per reviewer's suggestion, Authors have carefully checked for all possible grammatical, language and typographical errors.

Comment 2.2: The authors refer to Kozak et al. (1997) on page 21, but this reference was not found at the end of the text (the informed reference is the number 203, but it refers to El-Nasr et al (2003).

Clarifications/Modifications: The sentence has deleted from the revised manuscript.

Comment 2.3: I suggest the following abbreviation*:* *Helicobacter* (*H.*) *pylori* in the first time they appear in the text and after the authors may use *H. pylori* (with space between words).

Clarifications/Modifications: As per reviewer's suggestion, Authors have carefully checked for abbreviation of *Helicobacter pylori* (*H. pylori*) and used as *H. pylori* in revised manuscript.

Comment 2.4: The authors have added some specific information in the text, but they did not report the related references.

Clarifications/Modifications: As per reviewer's suggestion, Authors have revised the manuscript and reported the related references.

Comment 2.5: In the item 3.2.1 (antibody detection) I believe the text is very extensive, and then I suggest that authors should decrease the text if it is possible.

Clarifications/Modifications: Authors have revised and rewriting of the the serology section has been done.

Comment 2.6: in the reference 90, I suggest reviewing the name of the authors..

Clarifications/Modifications: As per reviewers suggestion authors have reviewed the name of authors(Rocha *et al.*, 2005)

Comment 2.1: Abstract: page 3 - I am not sure if I understand: ... "In invasive tests ..." 9th line.

Clarifications/Modifications: The sentence has been change as per suggestion of reviewer.

Comment 3.2b Introduction: page 5 - 4th line: Do the authors want to give the reference for the statement: ... "and its prevalence ranges between 20-80%"?.

Clarifications/Modifications: As per reviewer's suggestion authors have cited the refrence for the above statment.

Comment 2.3: Page 5 - last line: I am not sure if I understand: ..."to pin point/S...".

Clarifications/Modifications: The sentence has been change as per suggestion of reviewer.

Comment 2.4: Page 6 - item 2.1.1 (staining methods): in this topic I suggest that authors may consider the possibility of detecting bacteria using H&E, although this staining is commonly used in combination with Giemsa for the histological diagnostic. .

Clarifications/Modifications:Authours have revised the histology as per suggestion of reviewer.

Comment 2.5: Page 6 - item 2.1.2 (histological picture): I suggest that authors may want to mention about the possibility to find coccoid forms of *H. pylori* in the gastric mucosa,

considering that some factors as prior antibiotic treatment can affect the bacterial morphology.

Clarifications/Modifications: As per suggestion of reviewer, authors have repored in histology section about the possibility of finding coccoid form of *H. pylori* in the gastric mucosa, considering that some factors as prior antibiotic and/treatment can affect the bacterial morphology.

Comment 2.6: Page 6 - item 2.1.3 (limitations): Do the authors want to inform the reference related to the percentage of the prevalence of H. heilmannii in the gastric mucosa?

Clarifications/Modifications: The sentence has deleted during in revised manuscript.

Comment 2.7: Page 8 - 2nd line: ...about the quantity of bacteria which is required for a positive result in the test, do the authors want to give the reference for this statement?

Clarifications/Modifications: Reference was cited for above statment.

3 References and typesetting were corrected

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

Sincerely yours,

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