

ANSWERING REVIEWERS



Dear Editor,

Thank you for your careful review of our manuscript originally entitled “Diagnosis before and after treatment of *Helicobacter pylori* infection: New approaches and treatment of the infection” We have considered the reviewer comments and respond as follows:

Title: A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication

Author: Elvira Garza-González, Guillermo Ignacio Perez-Perez, Garza Héctor Jesús Maldonado, Francisco Javier Bosques-Padilla

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5916

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 reviewer

Reviewer 1.

Non-invasive methods to diagnose the infection, such as urea breath test and stool antigen test, are treated only in the section dedicated to methods to verify the eradication, following the antibiotic treatment (for a choice of the authors). This gives the incorrect impression that infection can be detected only by invasive methods. I, therefore, would suggest to move the treatment of the above reported methods to the first part of the manuscript as “non-invasive methods to establish an infection”, comprehending urea breath test, stool antigen test and serology, and to retreat briefly the suitability of these techniques to verify the success of treatment.

Response:

Before serology, the title “non-invasive methods to detect an infection” was added.

Now “Test used to detect eradication of infection” is a subtitle for methods such as urea breath test and stool antigen test

Additionally, the title: “Invasive methods to detect infection by *H. pylori*” was added before histology

First line of abstract: *H. pylori* is not an infection, it causes an infection. Change “is” with “causes”.

Response: The phrase was changed

Second line of paragraph of Diagnosis of infection: change “each have” with “each one has”.

Response: The change was made.

Ibidem, IV line: add “there” to “.... Are histological”

Response:

The correction was made

First paragraph of Histology, IV line: add neoplasia to “....and dysplasia....”

Response: The correction was made

First paragraph of Culture, V line: change “overproduction” with “overpresence” or similar.

Response: The correction was made

Ibidem, last sentence: add “...preferably in broth (any kind) with 15%-20% of glycerol.” after “stored at -80 °C.

Response: The sentence was added.

7. Same pg., last but one line. Change “media” with “medium” (it is Latin, singular name; plural is “media”).

Response: The word was changed.

Second paragraph of PolymeraseResistance to clarithromycin is mostly attributable to mutations in the 23S rRNA gene; just mention the efflux pumps and their role in resistance to antibiotics.

Response: A brief comment in relation to efflux pumps was added.

Ibidem, Ref. 30 deals with microdoses of labeled urea, not minimally invasive procedures; perhaps ref 22 fits better.

Response: The reference was changed

First paragraph of Urea breath test, IV line: change “expired” with “exhaled”.

Response: The word was changed

Concomitant therapy, 3rd line: modify the sentence “... This therapy involves the administration of three antibiotics together ” in “This therapy involves the simultaneous administration of three antibiotics”

Response: The sentence was changed

Hybridizing therapy, IV line: add for how long the treatment should be administered.

Response: This information was included.

Ibidem, ref 41 is not correct; its title is “Current options for the treatment of Helicobacter pylori”. Perhaps a different publication should be quoted.

Response: The reference was corrected.

Finally, the correct name of one of the authors, Perez-Perez GI, is Pérez-Pérez GI. Similarly, Megraud should be Mégraud. I do not know whether the Journal is careful about these things.

Response: The last names were corrected.

Reviewer 2.

The Minireview of the authors describes the diagnosis before and after treatment of *H. pylori* infection. They divided the manuscript in three main chapters. The first chapter talks about *H. pylori* diagnosis before treatment. Here they explain the invasive diagnostic methods like histology, culture, PCR, and rapid urease test. Also serology is presented in this chapter, but only few studies are cited.

Response: We revised the text and included more studies.

There are plenty of reviews trying to facilitate this complex problem of *H. pylori* diagnosis and treatment. This manuscript gives a good overview of the current status of diagnosis and treatment. However, serology could be explained more precisely, with its possibility of discrimination the infecting strain by looking at antibody responses to different virulence factors. Also, in areas where technical equipment for UBT is not available, a good serologic test is still the most efficient and affordable test for *H. pylori* prior to endoscopy.

Response: We included information related to the use of serology for discrimination of the infecting strain by looking at antibody responses to different virulence factors. We also included the valuable comment of the reviewer in relation to the fact that the serologic tests are a good alternative in populations that lack UBT testing.

In general, the authors should be more critical, especially when it comes to deviations from the current guidelines in daily routine. This is the case regarding histology, staining procedures, indication for treatment, and assessment of antibiotic resistance.

Response: We added some critical comments

Major points:

The introduction doesn't talk about the treatment of *H. pylori* infection. Importantly, the authors should introduce if and which diagnostic measures can be of help for deciding upon the need for treatment (i.e. histology and multiplex serology).

Response: We added information about treatment in the introduction section. The need of treatment is decided by the clinician according to the clinical condition of the patient.

In the section describing Histology it is stated that the updated Sydney Classification is set as “gold standard” *but this approach is scarce in daily practice because of the large number of biopsies suggested.* The authors should add a conclusion/recommendation (i.e that less biopsies can lead to underestimation and sampling error, and that HE stain alone also can lead to false negatives.)

Response: We added a conclusion.

Fluorescent in situ hybridization: The method is only described but not judged. Again, in a review the authors should be more critical and support the reader with appraisements of the methods mentioned. In my eyes, FISH is barely used - if at all – in daily routine since it is still too technically tedious and expensive.

Response: A critical commentary was introduced

At the end of section describing culture, you state that it is less recommended to freeze gastric biopsies. Could you give a reference here?

Response: We cannot provide a reference. We deleted the sentence.

Differences in sensitivity are mentioned but not explicitly stated. Please provide parameters.

Response: More information about sensitivity and specificity is mentioned in the manuscript.

The authors mention the role of culture to assess antibiotic resistance, which is *mainly used to confirm the antibiotic sensitivity of the bacteria after two treatment failures.* Given the dramatically rising resistance rates in many parts of the world, the authors should critically discuss this. Can we really wait with resistance assessment until two treatments have failed? Also, this emerging situation may lead to a much broader need of culture in the future.

Response: We added a specific comment in the new manuscript. “Given the increase in antibiotic resistance rates, especially to clarithromycin and metronidazole, it would be desirable that more laboratories be able to perform the culture and susceptibility testing, to avoid having to wait for two treatment failures”

Serology: The first sentence (*In general, tests containing complex antigen mixtures...*) is rather a conclusion and should be placed at the end of the first paragraph.

Response: The sentence was changed.

I do not agree with the last sentence (*its positive predictive value is poor when used*

in populations with a low prevalence of infection by H. pylori [27].).

Response: The sentence was deleted

While the authors are correct that the PPV depends on the prevalence, low PPV is only observed for tests with poor specificity. This has been overcome by tests employing multiple antigens. The authors should cite such test system, for example Formichella et al., Clin Vaccine Immunol. 2013 Sep 4. [Epub ahead of print] A novel immuno-line assay based on recombinant virulence factors enables highly specific and sensitive serological diagnosis of H. pylori infection.

Response: We agree with the reviewer. We included the valuable information mentioned in the reference and the reference.

Urea breath test: It should be mentioned that corpus-predominant gastritis can produce false-negative 13C-urea breath test results, which could lead to a major mis-assessment of such patients which are in high need for endoscopy and/or eradication. This is a major drawback especially in high risk populations. Further, recent data indicate that the sensitivity and NPV of UBT is better after three months compared to six weeks.

Response: We added this valuable comment to the new manuscript

Stool antigen test: sensitivity and specificity are nowhere named in the paragraph. These are highly variable. Please give numbers and examples. It should be mentioned that SAT is only recommended for diagnosis in children.

Response: We added diagnostic accuracy data for the stool antigen test. We found data regarding the use of the stool antigen test in children. We added this information.

Treatment: Resistance to amoxicillin and metronidazole has remained relatively stable. I don't agree. There is no resistance to Amoxicillin, and resistance to Metronidazole has risen to >50% in many geographic areas (southern and eastern Europe, Asia, ...).

Response: We agree with the reviewer. We changed the information and added some references.

such as clarithromycin for respiratory infections. The authors should add ..or metronidazole for gastrointestinal infections.

Response: The phrase was changed

Test and treat: This aspect should be discussed more critically: When patients with persistent dyspepsia are treated for H. pylori, only 10% benefit in the long term. The

other mentioned indications require diagnosis by endoscopy, so diagnosis of *H. pylori* infection can be done by histology. Thus, it is unclear why UBT or SAT should be used.

Response: We agree with the reviewer. Test and treat is discussed more critically. The sentence related to UBT and SAT was deleted.

Treatment regimens: The authors should give the eradication rates of the studies they cite (i.e. 41, 46, 48 etc.).

Response: References were updated therefore some references were deleted. Now eradication rates and relevant data of most of the citations are given.

Hybridizing therapy: The authors cite a review but not the study itself. This should be avoided. Also, I was not able to find any study with this term. Please cite correctly.

Response: The cite was corrected

Minor points:

Page 5 second paragraph “inability to obtain specimens from different areas of the stomach”. Inability seems the wrong word here. It is possible and recommended to obtain specimens from different areas of the stomach, but often not performed in daily routine. This sentence should be rephrased to underscore the need of several biopsies *due to a patchy distribution of H. pylori*. Also, it should be added that the sensitivity can significantly be increased by increasing the number of biopsies and employing specific stains. Sensitivities of 53% should not occur anymore.

Response: We agree with the reviewer. The paragraph was corrected.

Page 5: *H. pylori* is also observed within gastric glands.

Response: The phrase was added

Rapid urease test section: Line 3, change pH incubator to pH indicator

Response: The word was changed

Third line treatment section: last paragraph, line 4, change “should avoid if possible” to “should be avoided”

Response: The phrase was changed

Page 10, line 1: pH incubator should be pH indicator.

Response: The word was changed

Rapid urease test: it should be mentioned that the test specificity decrease over time during incubation, since the test often becomes false positive after longer incubation.

Response: The information was added in the revised manuscript.

Please revise (p20): This therapy is designed as sequential therapy in areas where ~~there is a~~ resistance to clarithromycin **is greater than 20% and **where** a quadruple therapy based on bismuth is not available.**

Response: The phrase was corrected

The manuscript contains some typos and linguistic mistakes that should be corrected. Page numbers and line numbers would facilitate proofreading.

Response: Page numbers were added

Reviewer 3.

Few data are available in children compared with adults. Studies specifically conducted in children should be an important aspect of this revision. They should be added.

Response: Some important data in children were added

I suggest include data about therapy in women who are pregnant or breastfeeding if the clinical condition of the woman requires treatment.

Response: Available information in Pubmed related to pregnant women was added.

The revision of literature is exhaustive; however several references could be updated because there are a plentiful number of references on this subject in the last years.

Response: References were updated

Modalities of therapy such as sequential, concomitant and hybridizing therapy should be more exhaustive commented.

Response: Sequential, concomitant and hybridizing therapy are more exhaustively commented

In the same line, some comments about therapy with probiotics could be informative.

Response: Information related to the use of probiotics was added.

We thank the editor and reviewer for their comments and hope that the revisions will help to clarify the manuscript. We believe that the manuscript has been strengthened as a result of this process.

Thank you in advance for considering this work.

Sincerely,

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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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