OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Hepatitis Monthly
Article DOI: 10.5812/hepatmon.106273
Published in: Hepatitis Monthly: 20(9); e106273

Peer Review Report for "Evaluation of a Novel Enzyme-Linked Immuno Assay Model to Detect E2 Antigen and Antibodies Against Core, NS3, NS4, and NS5 Antigens of Hepatitis C Virus"

Author(s): Ali Kargar Kheirabad, Rakhshandeh Nategh, Fazel Shokri, Kobra Razavi Pashabeyg, Mehdi Norouzi

Review Timeline:

Submit Date: 9 Jun 2020
Revised Date: 8 Sep 2020
Accepted Date: 10 Sep 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Mohammad Heiat: Reviewer | **Revision** (0) — 4 Jul 2020

Dear AE
The reviewing process has been finalized.
- However, the title of the article is quite interesting, but the authors have not been successful in writing and presenting scientific data and justification despite many efforts. Therefore, the article needs a thorough major revision.
- In this article, the workload is high and the method presented is quite confusing and misleads the reader. It is strongly recommended that authors avoid addressing the surrounding issues and articulate the original text in a more concise manner and using clearer illustrations.
All comments are provided in the attached word file.
Sincerely

REFEREE: Reviewer | **Revision** (0) — 28 Jul 2020

Dear AE,

The authors of this original article designed six ELISA strategies for testing HCV in all samples. Experiments have been done properly with appropriate controls and validations. The data is presented well and interpreted with accuracy. The study is in the scope of the journal, and therefore I can recommend this work for publication. Some minor points can make it better than this; including:

- Please indicate in the Abstract what is the “recommended test” means?
- Please summarize section “1.3.1 Demographic Characteristics” in a Table.
- Please draw tables base on journal guidelines.
- Authors should make more discussion about their data. There is no discussion about the comparison or their results with previous similar studies.

Thanks.

Heidar Sharafi: Associate Editor | **Revision** (0) 11 Aug 2020

Dear EIC,

The manuscript was reviewed by two expert reviewers. While the topic and findings are interesting, major concerns have been raised. I would like to ask the authors to address all the concerns and comments. Here, I add my comments to those of the reviewers:

1. In the Introduction, "According to the report that conducted in Pakistan in 2016, the incidence of HCV ...". I recommend presenting the data from Iran.
2. The English expression and syntax of the manuscript are weak and need to be edited by a native academic English editor.
3. In line 67, "... they have insufficient credit for positive results (25%) in low... ". It is vague what are authors addressing as insufficient credit.
4. The aim of the study is not clear. What diagnostic tool is referring to? The aim is included in two different paragraphs while they can be included as a single paragraph to clearly express what is going to be achieved in this study.
5. In many parts, it has been included as "positive sample" and "negative sample" however, it is not clear what is positive or negative in the samples.
6. There is great concern with different sampling methods for the two included populations. Sampling among blood donors seems to have biased to include donors with HCV infection or seromarker while among patients on hemodialysis it seems there is no bias. These different sampling methods and pooling the data of both groups can greatly influence the diagnostic characteristics of tests. These should be clarified through the manuscript and discussed if needed as a limitation.
7. In lines 105-111, the authors stated that they collected data of HBV, HIV, ALT, and AST and history of addiction, transfusion and etc. I don't believe these are needed for this study and also authors have not included these data in the manuscript.
8. Did they obtain the consent from blood donors?
9. The condition of blood donors described as HCV positive is unclear and should be described clearly if they have current or past HCV infection. In addition, the different strategies for diagnosis among these two populations can influence the results as well (all hemodialysis patients underwent HCV RNA and HCV Ab detection while the same is not true for donors).
10. The results of groups D and E by the ELISA tests are not included.
11. Line 202, group B, 104+3=107?

12. In Table 1, 95%CI for all diagnostic characteristics can be added as well. positive or true positive? negative or true negative?
13. Table 2 can be reformed to express the data more clearly.
14. Line 246, "Based on this survey, ..." which survey?
15. References 3 and 6 are the same.
16. Reference 14 is not related to the 4th generation ELISA methods.
17. References 25 and 26 are completely unrelated to their corresponding statement in the manuscript body.
Regards

Seyed Moayed Alavian: EIC | **Revision** (0) 17 Aug 2020

Dear Author,
The reviewers' comments are mentioned. Please correct the details as mentioned above carefully according to reviewers' request.
Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Answer to Associate Editor's Comments:

Dear AE;
The following is your helpful comments and the action taken

1. In Introduction, "According to the report that conducted in Pakistan in 2016, the incidence of HCV ...". I recommend to present the data from Iran.
it was edited

2. The English expression and syntax of manuscript is weak and needs to be edited by a native academic English editor.
many parts of manuscript were edited

3. In line 67, "... they have insufficient credit for positive results (25%) in low... ". It is vague what are authors addressing as insufficient credit.
it was edited to "and due to their high false positive records, they have low positive predictive value (25%) in low risk people (16, 17)."

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This part was deleted

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No Sir! it was edited

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Some unnecessary parts were removed.

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References 3 ***was edited***

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it was edited

Dear AE;

In addition to your comments, worshipful reviewers have some comments. The following is action taken to these respectable comments:

All comments of Reviewer *1* ***are provided in the attached word file and all of them edited according to the attached file. Edited parts are highlighted in the text.***

Reviewer 2 ask to indicate in the Abstract what is the “recommended test” means?

it was edited to “this Antigen and antigen/antibody detection tests”

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Heidar Sharafi: Associate Editor | **Revision** (1) 29 Aug 2020

Dear EIC,
The responses to reviewer 1's comments have not been received. Reviewer 1's comments are included in a word file of their manuscript in the previous peer-review round. I invite authors to address those comments as well.
Regards

Seyed Moayed Alavian: EIC | **Revision** (1) 5 Sep 2020

Dear Author,
The reviewers' comments are mentioned. Please correct the details as mentioned above carefully according to reviewers' request.
Thanks.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

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Dear AE;

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All comments of Reviewer *1* ***are provided in the attached word file and all of them edited according to the attached file. Edited parts are highlighted in the text.***

The comments and action taken to them:

1. *In background of abstract, there is comment “No need” for Hemodialysis (HD) subjects are considered as a high-risk group for hepatitis C virus infection.*

Background of abstract was edited to “The serological measurement of anti-hepatitis C virus antibody is widely used for the first line diagnosis of HCV infection and increasing performance criteria of these tests is very important for screening of the infection.”

2. *For objectives of abstract, there is comment "It is not perfect"*

Objectives of abstract was edited to "In this study, we have optimized a novel Enzyme Immuno Assay model to detect E2 antigen with or without sample pretreatment in combination with antibodies against core, NS3, NS4, and NS5 antigens of hepatitis C virus and compared the performances of these assays with Indirect Antigen (Ag), Biotin/ HRP labeled Antigen Sandwich, methods of enzyme-linked immunosorbent assay (ELISA) for their ability in detection of HCV."

3. *For methods of abstract, there is comment "How can we come up with the method through this words?"*

Methods of abstract was edited to "107 positive and 415 negative controls from volunteer whole blood donors in Blood Transfusion Organization and 204 blood samples from patients under hemodialysis treatment in Tehran and Bandar Abbas hemodialysis centers were included in the study, and 6 different methods of ELISA test were used for detection of Anti-HCV antibodies and/or HCV Antigens in serum samples."

4. *For results of abstract, there is comment "So superficial"*

Results of abstract was edited to "Our results show that E2 Antigen detection alone or combination with Antibody detection in comparison to other methods for detection of antibodies, have the highest accuracy value (99% and 98% respectively) with regard to sensitivity, specificity and accuracy. Besides, the combined Ag / Ab ELISA test output was closer to the Real-time PCR results."

5. *For conclusions of abstract, there is comment "Where is Novelty"*

Conclusions of abstract was edited to "Our result demonstrate that this new approach to Antigen and antigen/antibody detection tests increased performance criteria of serologic detection of HCV specially in HD patients who might experience longer window period."

6. *For introduction of manuscript, there are two comments "Without relation to topic" and "The miscellaneous notes which can be wrapped up in one short paragraph"*

In the first part of the introduction, the hepatitis C virus, proteins and the importance of its infection are discussed to determine the importance of diagnosing of this infection. However, according to the comment received from Associate Editor, the sentence "According to the report that conducted in Pakistan in 2016, the incidence of HCV seroconversion among chronic HD patients is about 53.37%" was changed to "According to the our previous report that conducted in Iran in 2016, the incidence of HCV seroconversion among chronic HD patients is about 3.36% (8)." and its reference was corrected. In addition, sentence "In addition, third generation ELISAs, mostly use detection kits, do not provide infection diagnosis before Seroconversion step, and due to their high false positive records, they have insufficient credit for positive results (25%) in low risk people" edited to "In addition, third generation ELISAs, mostly use detection kits, do not provide infection diagnosis before Seroconversion step, and due to their high false positive records, they have low positive predictive value (25%) in low risk people"

The second part, which the comment "The miscellaneous notes which can be wrapped up in one short paragraph" is about it, was divided into 3 separate paragraphs to provide reviewer opinion.

7. *In the first part 1.2.1 subjects and Serums, there is comment "The report style is confusing"*

This part edited to "Serum specimens were collected from volunteer whole blood donors in Iran Blood Transfusion Organization (IBTO), and from patients under hemodialysis treatment in Tehran and Bandar Abbas hemodialysis centers.

IBTO specimens included 107 positive and 415 non-reactive for antibody test samples. Because of low prevalence of infection in blood donors, random sampling was limited and confirmed positive and non-reactive samples were collected. Positive samples were confirmed by NAT. Among hemodialysis patient samples, there were 202 negative and 2 positive samples for HCV NAT test."

8. *In the next part 1.2.1 subjects and Serums, there is comment "Out of study scope" for "For each subject, demographic features and history of the associated risk factors including age, sex and hemodialysis duration were recorded." sentences. So, this sentence was deleted.*
9. *In the next part 1.2.1 subjects and Serums, there is comment "What are their relation to study scope" for "levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) were measured enzymatically (Pars Azmoon co.) using Auto Analyzer HITACHI 902." sentences. So, because of this result not showed in this manuscript, this sentence was deleted.*
10. *Finally, In the next part 1.2.1 subjects and Serums, there is comment "Even samples from IBTO??" for "Before enrollment, all members, who had agreed to participate in the research, were given a secure informed consent." Sentences*

It was edited to "Before enrollment, all hemodialysis patients, who had agreed to participate in the research, were given a secure informed consent. In addition, each hemodialysis was questioned about addiction, blood transfusion, jaundice or liver disease."

11. *For the 1.2.4 Tests Set up Procedure part of manuscript there are two comments "Was immobilized on…." And "*As before"

Those are edited according to the comments.

12. *For the* 1.2.*5 Statistical analysis part of manuscript there is a comment "For comparison of two pair qualitative variables. Why here???"*

Because of the change in the results calculations according to the AE reviewer, this part was edited to "Variables were compared in a pair-wise fashion using McNemar's, westgard QC recommendation and Medcalc Software Ltd,tests." And the reference of these methods was added to the manuscript.

13. *For the 1.3.2 Comparisons among different ELISA methods in HCV diagnosis there are two comments about "Summarize in a sample" and table number2*

These comments have very overlaps with the comments of AE. So, some parts of the presentation of the results were edited and the paragraph "The total of 726 samples were categorized into 5 groups: Group A contained 415 negative blood donor samples and group B contained 106 positive blood donor samples, which both were

confirmed by IBTO with Real-time PCR and antibody detection. Group C included 202 hemodialysis patients' samples with negative HCV results, and group D consisted of one HCV positive hemodialysis patient that is positive in both third-generation ELISA test and RT-PCR. Finally, group E consisted of a hemodialysis patient who was positive only by Real-time PCR.
Antigen sandwich (HRP) ELISA test presented: In group A; 414 negative samples and 1 positive sample, in group B; 104 positive samples and 3 negative samples, in group C; 201 negative samples and 1 positive sample. Screening samples with Antigen sandwich (Biotin) ELISA demonstrated: group A; 414 negative specimens and 1 positive sample, group B; 1 negative sample and 106 positive samples, group C; 201 negative samples and 1 positive sample.
Analyzing samples with combined Antigen & Antibody ELISA indicated: group A; 1 positive sample and 414 negative samples, group B; totally positive samples, group C; 201 negative samples and 1 positive sample.
In E2 Antigen Sandwich ELISA, both treated and Non-treated results were the same. In group A; all specimens were negative, group B; 104 positive samples and 3 negative samples, group C; all samples were negative." was changed to "The total of 726 samples were categorized into 5 groups: Group A contained 415 negative blood donor samples and group B contained 107 positive blood donor samples confirmed by NAT method. Group C included 202 hemodialysis patients' samples with negative HCV results in both third-generation ELISA test and RT-PCR, and group D consisted of one HCV positive hemodialysis patient that is positive in both third-generation ELISA test and RT-PCR. Finally, group E consisted of a hemodialysis patient who was positive only by Real-time PCR.
In all assays, if there was a discrepancy among results, including false positive or false negative, the test results were reevaluated by retesting.
Antigen sandwich (HRP) ELISA test presented: In group A; 414 negative samples and 1 positive sample, in group B; 104 positive samples and 3 negative samples, in group C; 201 negative samples and 1 positive sample. sample of group D was positive and sample of group E was negative.
Screening samples with Antigen sandwich (Biotin) ELISA demonstrated: group A; 414 negative specimens and 1 positive sample, group B; 1 negative sample and 106 positive samples, group C; 201 negative samples and 1 positive sample. sample of group D was positive and sample of group E was negative.

Analyzing samples with combined Antigen & Antibody ELISA indicated: group A; 1 positive sample and 414 negative samples, group B; totally positive samples, group C; 201 negative samples and 1 positive sample. All samples of group D and E were positive.

In E2 Antigen Sandwich ELISA, both treated and Non-treated results were the same. In group A; all specimens were negative, group B; 104 positive samples and 3 negative samples, group C; all samples were negative. All samples of group D and E were positive."
In addition, the results of the both tables were also edited.
14. For the Discussion paragraph 1 there is a comment "Low priority to mention"
Some sentences of this section edited according to the comments of reviewer 1 and AE.
15. For the Discussion paragraph 1 there is a comment for "E2 proteins" should be "E2 protein
It was edited

16. *For the final paragraph of Discussion there is a comment “This part can not be considered as conclusion It is might be suitable for introduction or discussion.”*

This section deals with the limitations of conducting research. In addition to the fact that this section has been edited as a comment of AE, its mandatory presence at the end of the discussion is Unavoidable.

Reviewer 2 ask to indicate in the Abstract what is the “recommended test” means?

it was edited to “this Antigen and antigen/antibody detection tests”

OPEN PEER REVIEW

Revision (2)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Seyed Moayed Alavian: EIC | **Revision** (2) 10 Sep 2020

Dear Author,
The document is reviewed carefully by the reviewers. According to their comments and your revisions as requested, this manuscript is accepted.
Thanks.