Author's response to reviews

Title: A novel method for detection of HBVcccDNA in hepatocytes using rolling circle amplification combined with in situ PCR

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Author’s response to reviews: see over
Dear Miss Sheryl Ramos,

On behalf of my co-authors, we thank you for your letter and for the reviewers’ comments and suggestion concerning our manuscript entitled “A novel method for detection of HBVcccDNA in hepatocytes using rolling circle amplification combined with in situ PCR” (ID: 7819834501178802). We appreciate you and reviewers very much for the positive and constructive comments and suggestions on our manuscript. Those comments are very valuable and very helpful for revising and improving our paper. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the point-by-point response to the reviewer’s comments as flowing.

Responds to the reviewer’s comments:

**Reviewer #1:**

1. **English writing.**

**Response:** Many thanks for the reviewer’s suggestion. According to the review’s suggestion, we have corrected the English grammar, typographical and punctuation errors in the revised manuscript.

2. **Table 1 does contain some HBV DNA load data, so details of which assay was used should be described in Materials and Methods.**

**Response:** According to the review’s suggestion, “serological markers
and quantification of HBV DNA and cccDNA” was added in the part of materials and methods.

3. The authors claim that the sensitivity of their test is two copies/cell but no evidence is provided as to how this was deduced.

Response: Considering the reviewer’s suggestion, we have supplemented “quantitation of intrahepatic HBV cccDNA levels were normalized by the amount of human genomic (hg)-beta-actin in the samples. Cell numbers were calculated based on an estimation of 6.667 pg/hgDNA per cell” in the revised version accordingly.

4. The sub-heading RCA treatment would be better described as RCA assay.

Response: RCA assay was described in the part of discussion.

5. Repeatability

Response: “Intra-assay and inter-assay variability experiments” have been further described according to the reviewer’s suggestion.

6. Electron microscope or a conventional light microscope.

Response: We are very sorry for our writing mistake in the original manuscript. We used a conventional light microscope. We have made correction in the revised manuscript.

7. Figure headings.

Response: We have made correction according to the Reviewer’s
8. The positive HBV ccc DNA staining is difficult to recognize at the x200 magnification and this will be further exacerbated when the figures are reduced on publication. They authors may wish to consider only including the x400 magnification which ably illustrates their results.

Response: We think the combination of x200 magnification and x400 magnification in the manuscript may be has a better effect than a single x400 magnification on publication.

Reviewer #2:

1. Some of the descriptions in materials and methods are not acceptable, such as “after deparaffinizing by normal method”, more details should be added. Especially, as a key part of this work, the descriptions regarding RCA should be clearer.

Response: Many thanks for the reviewer’s suggestion. We have supplemented the more details regarding RCA and deparaffinizing according to the review’s suggestion in the revised manuscript.

2. The purposes of some sample preparation steps should be explained in materials and methods part or results part, for example why AP should be removed after PSAD treatment, why RCA should be divided into two steps?

Response: We have made correction according to the reviewer’s
suggestion in the revised manuscript.

3. **In this manuscript, the authors totally investigated 26 HBV infected patient samples, but only few results are shown. If the rest could be added, it would be more convincing.** Of course, the authors could summarize a table by semi quantitative analysis of all staining results instead of adding all of them directly.

**Response:** We have made correction according to the reviewer’s suggestion in the revised manuscript.

4. **It seems that the data shown in Table 1 are average values which do not reflect the truth of every single one. It would be helpful to the readers if data of each patient could be listed in the table mentioned above.**

**Response:** We have made correction according to the reviewer’s suggestion in the revised manuscript.

5. **How did the authors design the primers for RCA and IS-PCR? More sequence information should be included, such as GenBank accession number and corresponding position of each primer.**

**Response:** We have made correction according to the reviewer’s suggestion in the revised manuscript.

On behalf of my co-authors, we thank you for your time again. If you have other questions or problems regarding our manuscript, please don’t hesitate to contact me.
Looking forward to hearing from you.

Best Regards

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