Reviewer’s report

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

Version: 3 Date: 27 April 2014

Reviewer: DAWEI CAI

Reviewer’s report:

In this manuscript, Zhong et al tried to combine rolling circle amplification with in situ PCR for HBV cccDNA detection. The authors investigated biopsy liver tissue samples from 26 HBV infected patients, and compared different treatment methods among IS-PCR alone, PSAD treatment + IS-PCR, and PSAD treatment + RCA + IS-PCR. The evidences presented in this manuscript demonstrated that incorporation of PSAD treatment, RCA and IS-PCR is better than IS-PCR alone. However, as a methodology article, descriptions of the materials and methods part are not clear, what’s more, the results part is oversimplified. Some specific concerns are listed below:

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.

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?

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. If authors could show the quantification data of each sample calculated by authors’ previous method (ref. 14), it would be great.

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.

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.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.