Reviewer's report

Title: Rapid, Simple and Sensitive Detection of Spotted Fever Group Rickettsias by Loop-Mediated Isothermal Amplification of the ompB Gene

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Reviewer: Rong Fang

Reviewer's report:

The manuscript entitled as "Rapid, Simple and Sensitive Detection of Spotted Fever Group Rickettsias by Loop-Mediated Isothermal Amplification of the ompB gene" established a novel, specific, economical and sensitive diagnosis approach for rickettsial diseases. Compared to the conventional diagnosis approaches currently used for rickettsial diseases, this method is particularly useful for timely diagnosis and in rural areas. The authors determined the specificity of the approach using 44 strains of rickettsiae, and other clinical pathogenic bacteria which may cause symptoms similar to the manifestations of rickettsial infections in human. LAMP ompB assay showed a greatly enhanced sensitivity compared to regular PCR as evidence by amplifying as little as 5 copies of target gene in reference plasmid each reaction. Therefore, this report is important for early diagnosis of rickettsial disease in the field. However, there some comments need to be addressed:

Major Compulsory Revisions

1, Table 4, How will the authors explain about “case 9”? Why the culture was positive, but all PCR and LAMP was negative? Usually, PCR is more sensitive than cell culture.

2, Table 4, could the authors explain why some cases were diagnosed via PCR while others diagnosed through IFA? If IFA is the golden standard method, why would you skip it? Is it due to the unavailability of the patient serum?

3, Page 8, line 131, since LAMP assay is a novel approach, the authors may want to describe what the positive and negative results are, respectively. Or how to evaluate the results? How many bands should be expected? Are the results judged only by vision? Could you convert it to optical density or concentration?

4, Page 8, line 149, what is the parameter determine the Cvi and CVo? How did you calculate it? Did you calculate it based on the concentration or raw optical densities? If there any reference cited?

Minor Essential Revisions

5, Please be advised that “SFGR” is better spelled out as “spotted fever group rickettsiae”.

6, Page 2, line 38, it is suggested to correct as “emerging and re-emerging infectious diseases”
7. Page 2, line 41, it is suggested to correct as “only five species of spotted fever group rickettsiae were confirmed as human pathogens”

8. Table 4, case 7, the diagnosis type was repeated two times as “gltA gene amplified and gltA gene amplified”, please delete the redundant one.

9. Page 11, line 179, it should be “table 3”, instead of “table 1”

10. Page 12, line 193, the author mentioned “the 11 samples analyzed by IFA were also subject to ......” according to the previous description, among 11 samples, there is one sample was confirmed as positive through pathogen isolation or cell culture.

11. Page 4, Line 4, it mentioned as “44 strains of the order rickettsiales”, How many strains of rickettsiae were listed in Table 1?

Discretionary Revisions

12. Page 11, Fig 3, the symbols need to be optimized in order to better present the data.

13. Page 14, line 221 describe the sensitivity as “5 copies per reaction”. However, the same page line 223 described as “100 copies/µl”, are they comparable?

14. Page 2 line 42, you might want to give a reference to show the currently recognized ten pathogenic rickettsial species.

15. Page 3, line 62, it is suggested to correct as “in humans in the rural area of China”.

16. Page 8, Line 143, this paragraph mentioned the specificity of the LAMP assay. In addition, the specificity was also described on page 4. The authors may combine these two parts into one.

17. Page 9, Line 157, why were only seven out of 27 rickettsial species positive by LAMP assay? I noticed that there are more than seven strains of spotted fever group rickettsiae listed in Table 1. Are the strains of spotted fever group rickettsiae theoretically positive if the primers were designed according to the conserved region of ompB?

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests