Reviewer's report

Title: Microarray of Surface-exposed Proteins of Rickettsia heilongjiangensis for Serodiagnosis of Far-eastern Spotted Fever

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Reviewer: Won-Jong Jang

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This paper by Qi and et al. demonstrates that some recombinant surface-exposed proteins (rSEPs) can be candidate antigens for serological diagnosis of Far-eastern spotted fever (FESF) caused by Rickettsia heilongjiangensis. Qi and et al. selected 11 SEPs which were identified in their previous study and fabricated them on a microarray using the rSEPs. They tested seventeen paired sera from patients suffered from FESF and 20 negative reference sera in the study. They suggested four of them are more likely candidate antigens for serological diagnosis of FESF.

Major revision:

1-ABSTRACT, page 2, lines 30-34; “Four rSEPs (rOmpA-2, rRplA, rRpsB, rSdhB) showed sensitivities of 71% to both acute- and convalescent-phase sera and the combination assay of these four rSEPs showed a higher sensitivity of 76% in recognizing the acute-phase sera or 88% in recognizing the convalescent-phase sera with a good specificity of 90%.” To diagnose infectious disease, detection of etiological agent and antibodies against the agent at the early stage of infection is the most important. Please, state about the usefulness of SEPs concerning about that.

2-INTRODUCTION, line 62-64 and DISCUSSION; It is interesting that authors tried to test the surface-exposed proteins as candidate and fabricated them on a microarray. Also, many studies have shown that membrane proteins of rickettsiae are suitable for diagnostic antigens of Rickettsial disease. Please, state previous data about serological assay using the membranes proteins as antigens comparing with SEPs.

3-Discussion, page 11, line 209-213; “Our results suggest that the remarkable variation in immune recognition patterns for FESF require multi-antigen combination to cover the different antibody responses and thus achieve the highest possible test sensitivity. Therefore, the four rSEPs may be considered as more likely candidate antigens for the serological diagnosis of FESF, especially rSdhB, with its sensitivity of 82% to the acute-phase sera and 76% to the convalescent-phase sera with specificity of 90%. Furthermore, refinement of the production of fusion molecules comprised of these SEPs and the reaction conditions of microarray assay described herein may lead to improve the sensitivity and specificity for the serodiagnosis of FESF.” In this study, a single
antigen showed low specificity. Multiple-antigen combination could be induced more severe cross-reactivities with other antigens. Please state the possibility and strategy of overcome it.

4-Conclusion, page 13, line 266-; “In conclusion, the eleven SEPs were serologically characterized with paired sera from FESF patients, and four rSEPs (rOmpA-2, rRplA, rRpsB, and rSdhB) are more likely candidate antigens for the serological diagnosis of FESF. In addition, an optimized microarray composed with the four rSEPs may give an acceptable sensitivity for serological diagnosis of FESF during both the 269 acute and convalescent phase.” Adding the data of optimized microarray composed with four antigens would provide more reliable evidence of the antigens are suitable for diagnosis and improve this paper.