

Review comments to the manuscript “Rapid detection of Enterococcus and vancomycin resistance using recombinase polymerase amplification (#64039)”

The authors developed a rapid method of isothermal recombinase polymerase amplification (RPA) combined with a lateral-flow(LF) strip for detection of vancomycin-resistant enterococci, especially the targets are VanA- and VanB- type *E. faecium* strains from clinical samples. The RPA-LF method performed within 30 min (RPA reaction was 20 min at 37degree and LF strip detection within 5 min). The method exhibited 100% sensitivity in both blood-culture and stool/rectal-swab samples without cross-reaction (100% specificity). The lower detection limit of the RPA-LF was approximately 10 times better than that of the conventional PCR method.

Comments and suggestions:

The developed method is simple, rapid, and more sensitive than the conventional PCR method. The method presented in this study could be worth and very convenient.

1. Line 53, 76, 92, etc.; the font of the numeral character “10, 20, 2018, etc.” in the manuscript should be checked and unified.
2. Line 108; “Identification” is correct.
3. Line 108-; in this study, authors developed the method for detection of *E. faecium* by its specific *ddl* gene. I would like to suggest that the development of the detection of *E. faecalis* by its specific *ddl* gene could be much worth and convenient in the clinical setting where the VR-*E. faecalis* strains are often isolated because *E. faecalis* is the major cause of enterococcal infection.
4. Line 179-; data of the final numbers of bacterial strains (CFU/mL of VRE) in the spiked stool samples after overnight culture could be valuable and important to estimate the sensitivity of the methods. It is better that a part of the sample data could be shown in this study.
5. Line 196-; To optimize the reaction conditions (temperature and time), the RPA-AGE methodology should be performed first, and then, RPA-LF assay should be considered. The order is reversed and inconsequential. The data shown in Figure 1 might be moved to the supplementary data if possible.
6. Line 204- (Line 250-); I wonder that the sensitivity of the conventional PCR method was ten times lower than RPA-AGE. Please discuss this issue and add the appropriate explanation in the Discussion section.
7. Line 210-(Table 3); The data shown in Table 1 is enough, and Table 3 might be removed from the manuscript or moved to the supplementary data.

8. Line 219-; in Discussion section, the comparison with the conventional real-time PCR for the VRE strains should be combined. Also, the description about the costs issue comparing with the various conventional methodology could be appeared in this manuscript. Beside the assay time and equipment necessity, the cost for the developed RPA-LF method to detect each VRE strain could be very important.