Development of a new LAMP assay for diagnosing the main meningitis pathogens

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Introduction

Meningitis is a serious disease with significant burden in low- and middle-income countries. Current diagnostic tests are expensive and difficult to use in secondary health centres where resources are limited. LAMP assays could be an alternative method to bring molecular detection closer to patients. This study therefore aimed to develop standardized LAMP assays that could detect in parallel reactions the four main pathogens causing meningitis using improved gene targets.

Methods

Three LAMP reaction conditions were tested to determine the best method: the method of McKenna et al., 2011, the method of Tanner et al., 2014 and the method of Kim et al., 2012.

Optimisation tests were carried out for the LAMP condition, which did not work in the standard condition, by varying the temperature and by varying the concentration of DNA polymerase.

Following the tests, the LAMP condition described by Kim et al., 2012 was chosen as the best performing.

The samples were subjected to the LAMP test and the results were analysed. The performance of the test was evaluated using various formulae, including sensitivity, specificity, negative predictive value and positive predictive value.

Results

Of all the targets tested, only sodC, psaA, cfb and fucK showed signs of amplification using all three reaction conditions. However, the LAMP reactions using Thermopol buffer and the isotherm were not reproducible. These reaction conditions were therefore abandoned. The final reaction using 1X homemade buffer worked well, was reproducible and therefore was selected to perform the validation assays.

The LAMP test targeting the fucK gene showed a sensitivity, specificity, positive predictive value and negative predictive value of 100%. All Haemophilus influenzae samples tested positive, while the other bacteria in the panel tested negative (Table 2, figure 2).

The LAMP test targeting the cfb gene revealed that 6 out of 7 Streptococcus agalactiae were detected by the test, while the other bacteria in the panel were negative. Sensitivity was therefore 86%, specificity and positive predictive value 100% and negative predictive value 97% (Table 2, figure 3).

The LAMP test targeting the sodC gene showed a sensitivity, specificity, positive predictive value and negative predictive value of 100%. All Neisseria meningitidis samples tested positive, while the other bacteria in the panel tested negative (Table 2, figure 4).

The LAMP test targeting the psoA gene showed a sensitivity, specificity, positive predictive value and negative predictive value of 100%. All Streptococcus pneumoniae samples tested positive, while the other bacteria in the panel tested negative (Table 2, figure 5).

Conclusion

We have successfully developed rapid and sensitive LAMP assays for the detection of H. influenzae, S. agalactiae, N. meningitidis, and S. pneumoniae. They have been validated on DNA extracted from bacterial strains. Further validation with clinical samples is needed alongside further development to decrease the amount of sample handling required, making it more user-friendly, and ensuring and easier uptake in peripheral settings. Assays using methodologies like LAMP holds great promise for improving meningitis diagnosis and could contribute to more effective management and treatment of the disease in resource limited settings.

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