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Real-time *Bst* DNA polymerase-based assay for rapid diagnosis of acute leptospirosis

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Introduction and Objectives: Laboratory diagnosis is crucial for leptospirosis, especially during the acute stage, due to the variable nature of the disease. Among several laboratory tests, *flaB*-nested PCR is one of the methods used to diagnose acute leptospirosis by detecting the DNA of the pathogenic leptospires. However, it takes nearly 4.5 hours to obtain the results. We developed a rapid real-time *Bst* DNA polymerase-based assay which gives rapid results in less than 20 minutes. This study evaluated the *Bst* DNA polymerase-based assay using the 16S rRNA gene against nested PCR using the *flaB* gene to diagnose acute leptospirosis.

Methods: Confirmed *flaB*-positive and negative samples were obtained from the Department of Microbiology, Faculty of Medicine, University of Peradeniya. Fifty known positive and 50 negative DNA samples were screened using the newly developed *Bst* DNA polymerase-based assay. WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix (New England Biolabs, UK) and a set of six primers (doi:10.1128/JCM.00481-12) targeting the 16S rRNA gene of *Leptospira* spp. were used in the assay. The assay was performed at 65 °C for 30 minutes (60 cycles, 30 seconds per cycle) in a real-time PCR machine (BioRad® CFX96, USA). The results were then read by real-time fluorescence detection via the SYBER channel of the real time machine and compared with the *flaB*-nested PCR results.

Results: The assay's turnaround time has been reduced to 15-20 minutes. Of the 50 positive samples, 46 samples tested positive with a sensitivity of 92.0% (95% CI: 80.8% to 97.8%). Of the 50 leptospirosis negative samples, 44 tested negative, with a specificity of 88.0% (95% CI: 75.7% to 95.5%). The positive predictive value was 57.5% (95% CI: 38.87% to 74.22%), negative predictive value was 98.4% (95% CI:96.03% to 99.38%) and test accuracy was 88.6% (95% CI: 80.7% to 94.1%). The area under the receiver operated characteristic (ROC) curve was 0.890 at $p< 0.0001$ (95% CI: 0.812 to 0.944).

Conclusions: The newly designed real-time *Bst* DNA polymerase-based assay appears to be a rapid method for detecting *Leptospira* DNA and can be used as an alternative for *flaB*-nested PCR after validating the test using many samples for comparison.

Keywords*: Leptospirosis diagnosis, flaB-nested PCR, rapid real-time Bst DNA polymerase-based assay, LAMP*

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