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Development of a multiplex real-time PCR assay for the simultaneous detection of *Leptospira* spp. and dengue viruses

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Introduction and Objective(s): Dengue is a hyper-endemic viral infection and leptospirosis a major public health threat as an emerging infectious disease in Sri Lanka. Both have similar early clinical manifestations that vary from mild febrile illness to severe disease and are notifiable diseases in Sri Lanka. The present study aimed to develop a real-time PCR assay to detect acute leptospirosis and dengue virus (DENV) infection simultaneously.

Methods: A 2-step multiplex real-time reverse transcription PCR assay was developed to simultaneously detect RNA of DENV and *Leptospira*, using the QuantiTect Probe RT-PCR Kit (Quiagen, Germany). Primers and probes were designed to match the 16s rRNA of pathogenic *Leptospira* and a consensus sequence shared by the 4 DENV serotypes. Double-quenched probes with Iowa Black FQ, and an internal ZEN Quencher were used for each primer. Different 5′ fluorophores FAM and HEX were used for Leptospira and DENV respectively. Specificity of the assay was determined with a panel of leptospiral samples having pathogenic serovars and all 4 DENV serotypes. Additionally, RNA from other bacteria and viruses were also subjected to the multiplex assay. RNA from liquid cultures of all viruses and bacteria were extracted using column-based method with PureLink™ RNA Mini Kit (Invitrogen, USA).

Results: All 6 tested pathogenic *Leptospira* species and 4 DENV serotypes were positive for the assay. Twelve other bacteria and 9 viruses gave negative results for this multiplex assay, showing a high specificity for testing the indented microbes.

Conclusion: We established a multiplex real time PCR approach, which is cost and time saving for simultaneous detection of 2 pathogens of public health concern in Sri Lanka. Given that leptospirosis and dengue fever have similar initial clinical presentations, the ability of this approach to test for both infections at the same time is of great significance, particularly during epidemics. The high specificity of the assay, as demonstrated, may be attributed to the use of Double-Quenched Probes. This test will be validated using patient samples to develop strict protocols to confirm infection using human blood samples. If successful, this approach could be used in the local laboratory.

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Keywords: Real-time PCR, leptospirosis, dengue, simultaneous detection

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