904/A/Poster

Optimization of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) based method for the detection of the dengue virus and identification of dengue virus serotypes using serotype specific polymerase chain reaction

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Dengue (DEN) is considered as the most significant mosquito borne viral disease in Sri Lanka. It is caused by the dengue virus (DENV) and is transmitted to human beings by biting of an infected female mosquito of *Aedes aegypti*. Early diagnosis of DEN has become a major requirement in clinical diagnosis in order to reduce the mortality rate and improve patient management. Therefore, the aim of this study was to optimize a low cost reverse transcriptase polymerase chain reaction (RT-PCR) based method for detection of DENV by selecting a low cost RNA extraction method and also to optimize a serotype specific PCR to differentiate serotypes of DENV.

Serum samples (n = 20) were collected from DEN confirmed cases from Government Hospitals, checked for the presence of NS1 protein and stored at -80°C until extraction. RNA was isolated using the different RNA extraction methods from all DEN confirmed serum samples, which included Trizol, Silica and CEYGEN Virospin R^{TM}. The best RNA isolation method was identified for the extraction of DENV. Complementary DNA (cDNA) synthesis was optimized using the most successful RNA extraction method. Further, the RT-PCR and nested PCR were optimized using cDNA.

The RNA isolation showed successful results with commercially available low cost RNA extraction kit (CEYGEN). The RT-PCR amplification conditions were optimized as denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 15 sec, 55°C for 15 sec, 72°C for 30 sec, and the final extension was carried out at 72°C for 10 min. The serotype specific PCR was successfully optimized with proper amplification conditions. Different annealing temperatures were used such as 50°C, 42°C and 50°C for different DENV serotypes D1, D2, D3 and D4, respectively. Overall, three samples were confirmed as positive for DENV. The D1, D2 and D4 DENV serotypes were identified with band sizes of 490, 230 and 398 base pairs respectively. Therefore, in this study, RT-PCR and serotype specific PCR methods were successfully optimized to diagnose DENV as well as serotypes of DENV and with further evaluations this method could be used in diagnosis of DENV.

Keywords: RNA extraction, cDNA synthesis, Reverse Transcriptase Polymerase Chain Reaction; nested Polymerase Chain Reaction.

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