Optimization of Reverse Transcriptase Polymerase Chain Reaction based method for the detection of the Dengue Virus

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The major public health problem in the tropical countries including Sri Lanka is the Dengue (DEN). Diagnosis of the disease as early as possible would improve the patient management, vector controlling, and lower the fatality rate. Thus, early diagnosis of DEN has become a major requirement in clinical setup. Therefore, the aim of this study was to optimize a RT-PCR based method for the detection of the DENV and serotypes.

Different extraction methods namely; Trizol, Silica and commercial kit based methods were used to determine the suitable extraction method for the DENV from clinical samples (NS1 positive). The optimization of RT-PCR including synthesis of cDNA was performed. In addition, the optimization of nested PCR was carried out from cDNA to differentiate DENV serotypes.

A commercially available RNA extraction kit (CEYGEN) showed successful results for isolating RNA from viral samples. Complementary DNA (cDNA) was synthesized using DC-2C primer, incubating at 80°C for 4 min. 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 performed at 72°C for 10 min using DC-1S and DC-2C primers. The nested PCR was successfully optimized with proper amplification conditions and different annealing temperatures 50°C, 42°C, 50°C and 50°C for different serotypes D1, D2, D3 and D4 respectively.

Altogether, three samples were confirmed as positive for DENV diagnostics and were identified as D1, D4 and D2 DEN serotypes with the band sizes at 490 bp, 398 bp and 230 bp respectively. Therefore, in this study, RT-PCR and nested PCR methods were optimized to diagnose DENV as well as serotypes of DENV. Analysis of large number of clinical samples is needed for the evaluation of the method.

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