

Real-Time Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP) Assay as a Rapid Diagnostic Tool for Emerging Viruses

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Loop Mediated Isothermal Amplification (LAMP), a novel method of gene amplification is emerging as a valuable rapid diagnostic tool for rapid detection of infectious diseases. We have developed and evaluated one-step single tube real-time accelerated quantitative reverse transcription loop mediated isothermal amplification (RT-LAMP) assays for rapid detection of some of the recently emerged human viral pathogens viz; West Nile, SARS and Dengue viruses. The whole procedure is very simple and rapid wherein the amplification can be obtained in 20-30 min by incubating all the reagents in a single tube with reverse transcriptase and Bst DNA polymerase at 63°C. The RT-LAMP assay was found to be 10 to 100 fold more sensitive as compared to conventional RT-PCR, with a detection limit of 0.01 to 10 PFU of virus. The specificity of the RT-LAMP assay systems for respective viruses was established by cross-reaction studies with other closely related viruses. The evaluation of SARS RT-LAMP assay with 49 suspected and 10 healthy throat wash specimens revealed 100% and 87% sensitivity and specificity respectively with regard to RT-PCR. The evaluation of West Nile RT-LAMP assay with 8 of spiked and 6 negative human serum samples revealed 100% concordance with RT-PCR in terms of sensitivity and specificity. The applicability of RT-LAMP assay for clinical diagnosis of dengue virus infection was validated by examining limited number of DF and DHF patient serum samples of each serotypes. The RT-LAMP assay could correctly identified the serotypes with a 100% concordance with one step serotype specific direct RT-PCR. The field application of RT-LAMP assay made easy by employing the SYBR Green-I dye mediated naked eye visualisation protocol following incubation in a water bath. Thus the RT-LAMP assay reported here has the advantages of rapid amplification, simple operation and easy detection with potential usefulness for clinical diagnosis and surveillance of viral diseases in developing countries without requiring any sophisticated equipments or skilled personnel.

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