Development and Evaluation of a SYBR Green-based Multiplex Reverse Transcriptase-polymerase Chain Reaction for Rapid Diagnosis and Serotyping of Dengue and Chikungunya Viruses

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Background/Objective
Dengue virus (DENV) and Chikungunya virus (CHIKV) have emerged as the two most important arthropod-borne viruses responsible for large and geographical epidemics. Accurate diagnosis of these two viruses remains challenging due to their similar clinical manifestation, common transmission vectors, geographical distribution and seasonal correlation.

Method
In the present study, we developed and evaluated a rapid, cost-effective molecular assay to simultaneously detect, quantify and differentiate DENV-1, 2, 3, 4 and CHIKV using SYBR Green I-based one-step multiplex real-time RT-PCR. DENV is serotyped based on melting temperature analysis of PCR amplicon that is specifically corresponding to only each of DENV-1, 2, 3, 4 and CHIKV.

Result
The detection limit of the assay was determined to be 20 RNA copies/reaction for DEN-1, 10 RNA copies/reaction for DEN-2, 50 RNA copies/reaction for DEN-3, 5 RNA copies/reaction for DEN-4 and 10 RNA copies/reaction for CHIKV. Furthermore, our assay is not cross-reacting with the panel of RNA viruses validated in this study. In addition, the present assay was evaluated using clinical serum samples and the sensitivity for DENV-1, 2, 3, 4 and CHIKV was found to be 78.58%, 92.86%, 92.86% 92.86%, and 96.7%, respectively, and the specificity of the assay is 100%.

Conclusion
Our assay has the potential as a point-of-care clinical molecular diagnostic platform for DENV and CHIKV in acute-phase patient serum samples.