DEVELOPMENT OF SINGLE STEP REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) ASSAY TO DETECT CHIKUNGUNYA VIRUS IN CLINICAL SAMPLES

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DEVELOPMENT OF SINGLE STEP REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) ASSAY TO DETECT CHIKUNGUNYA VIRUS IN CLINICAL SAMPLES

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Chikungunya is an arborviral disease caused by the Chikungunya virus (CHIKV). The virus is transmitted by *Aedes* mosquitoes and mainly prevalent in tropical and subtropical countries. At present there is no specific therapy or vaccine available against the infection. Therefore early detection of the virus is crucial in patient management. The main objective of this study was to optimize and establish an inexpensive single step reverse transcriptase polymerase chain reaction (RT-PCR) assay for rapid, specific and sensitive detection of Chikungunya virus in clinical samples and to compare the sensitivity of the assay with the currently used two step RT-PCR/ nested assay.

Chikungunya virus infected blood samples were used to develop the single-step RT-PCR assay. Two protocols were tested by using a virus specific primer set targeting the E1 region of the viral genome. The RT-PCR/nested assay was performed in parallel as a control, to compare the specificity, sensitivity and speed of the assay. For the RT-PCR/nested assay, the E2 region was targeted for amplification. The protocol which showed 83.3% sensitivity towards Chikungunya virus was selected as the suitable protocol for the single-step RT-PCR assay. When compared with the RT-PCR/nested assay, the single-step RT-PCR assay showed comparable detection ability in detecting the virus in clinical samples. However, in dilution studies a lower sensitivity was seen compared to the RT-PCR/nested method. Importantly, this assay has reduced the turn over time and the post-PCR contamination which is possible in a RT-PCR/nested assay and has the capability to detect low volumes of the viral load in minute quantities of blood. The findings of this study demonstrate the potential clinical application of single-step RT-PCR as a molecular diagnostic tool for rapid, sensitive, specific and cost effective detection of Chikungunya virus in clinical samples.