' (APPLIED SCIENCE) | 04 JULY 2017

A Research Protocol for the Development of a Multiplex Reverse Transcription Recombinase Polymerase Amplification Assay for the Simultaneous Detection of Dengue, Chikungunya, and Zika Viruses in *Aedes aegypti* Mosquitoes from Informal Settlements in Dar es Salaam

J, u, m, a, R, a, s, h, i, d, i, ,, G, r, a, c, e, M, w, a, m, b, e, n, e

DOI: https://doi.org/10.5281/zenodo.18570058

Abstract

Dengue, chikungunya, and Zika viruses are arthropod-borne pathogens of public health importance, transmitted primarily by Aedes aegypti mosquitoes. These viruses co-circulate in tropical regions, including Tanzania. Informal settlements in Dar es Salaam, with high population density and water storage practices, present suitable breeding sites for this vector. Surveillance often depends on singleplex molecular methods, which are inefficient for multi-pathogen screening, indicating a need for a rapid and field-adaptable multiplex tool. The purpose is to develop and validate a multiplex reverse transcription recombinase polymerase amplification (RT-RPA) assay for the simultaneous detection of dengue, chikungunya, and Zika virus RNA in Ae. aegypti. Specific objectives are: 1) to design and optimise primer and probe sets for a single-tube multiplex RT-RPA; 2) to determine the assay's analytical sensitivity and specificity against standard RT-qPCR; and 3) to screen field-collected Ae. aegypti from informal settlements in Dar es Salaam using the validated assay. The study comprises laboratory assay development and a cross-sectional field application. Ae. aegypti will be collected from informal settlements in Dar

es Salaam using BG-Sentinel traps and aspirators. Following morphological identification, pooled mosquitoes will be homogenised for nucleic acid extraction. The multiplex RT-RPA will target conserved genomic regions of the three viruses. Assay conditions, including temperature, time, and reagent concentrations, will be optimised. Analytical sensitivity will be determined using serial dilutions of synthetic RNA, and specificity will be assessed against related arboviruses. The validated assay will screen field-collected mosquito pools, with results confirmed by RT-qPCR. Findings will include the performance characteristics (sensitivity, specificity, limit of detection) of the novel multiplex RT-RPA assay. The results from screening field-collected mosquitoes will indicate the presence and distribution of the target viruses within the sampled Ae. aegypti populations in Dar es Salaam's informal settlements. This protocol will yield a validated multiplex RT-RPA assay. Its application is expected to provide evidence on the co-circulation of dengue, chikungunya, and Zika viruses in the study area, demonstrating the assay's utility for integrated arbovirus surveillance. The developed assay should be integrated into national vector-borne disease surveillance programmes to facilitate routine, simultaneous pathogen detection. Further work