

**Optimization and evaluation of a reverse transcriptase loop amplification test for
O'nyong'nyong virus**

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ABSTRACT

O'nyong'nyong fever, caused by infection with a mosquito-borne East African alphavirus, is an acute, non-fatal illness characterized by fever and polyarthralgia. Alpha viruses such as Chikungunya and O'nyong'nyong are endemic in East Africa and have caused extensive epidemics and therefore their rapid diagnosis is a priority. In this study, a novel, cheap and rapid method of gene amplification known as Reverse Transcriptase Loop Amplification Test was optimized for ONNV detection. Other methods such as; RT-PCR, Cell culture, plaque assay were also performed for purposes of comparison with RT-LAMP. The objective of this work was to optimize and evaluate an RT-LAMP assay for detection of O'nyong'nyong virus. One hundred samples were prepared. 40 of the samples were spiked with a high concentration of ONNV antigen (1:1000) while 40 these samples were spiked with low concentration of ONNV antigen (1:10000) and 20 few of the samples were not spiked with the ONNV antigen. All the samples were blinded and coded to avoid bias. These samples were analyzed using plaque assay, RT-PCR, and RT-LAMP. Plaque assay was used as the gold standard in this study. Out of the 100 samples tested by plaque assay 40 (11-30 pfu/ml) tested positive, 30 (0 pfu/ml) negative and 30 (1-10 pfu/ml) indeterminate. Using conventional RT-PCR 40 samples were positive, 30 negative and 30 indeterminate. By RT-LAMP 40 samples were positive, 23 negative and 37 indeterminate. RT-LAMP detected 7 samples as indeterminate that conventional PCR and plaque assay did not detect. The sensitivity and specificity of RT-PCR and RT-LAMP was calculated using plaque assay as the gold standard. The sensitivity of RT-LAMP was 100% whereas that of RT-PCR was 85%. The specificity of RT-LAMP was 77% whereas that of RT-PCR was 100%. RT-LAMP is the best screening test for sensitivity whereas RT-PCR is the best screening test for specificity. Reliability testing was done by calculating the positive (PPV) and negative (NPV)

predictive values. PPV of RT-LAMP was 85% whereas that of RT-PCR was 100%. NPV of RT-LAMP was 100% and that of RT-PCR was 77%. In terms of cost and more detailed analysis RT-LAMP emerged as the best test amongst the three assays carried out in this study. The research findings reported in this work will contribute towards the diagnosis of viral infections especially ONNV and other emerging arboviruses particularly in outbreaks. Further optimization and evaluation should be done to establish RT-LAMP as a tool for surveillance of not only O'nyong'nyong virus but also other emerging viruses.