Development of a colorimetric RT-LAMP assay with novel primers for detection of Dengue virus in *Aedes aegypti* mosquitoes

M. D. S. Janaki[1,2], I. Sanchez-Vargas[3], O. C. Ratnayake[3], S. Elmegerhi[3], O. V. D. S. J. Weerasena[1], V. S. Aryaprema[4], N. Fernando[1], S. M. Handunnetti[1], P. P. S. L. Pathirana[1], H. A. Tissera[5], J. Rovnak[3], R. Perera[3]

[1]*Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka*
[2]*National Dengue Control Unit, Ministry of Health, Public Health Complex, Colombo 05, Sri Lanka*
[3]*Center for Vector-borne Infectious Diseases, Colorado State University, USA*
[4]*Anastasia Mosquito Control District, Florida, USA*
[5]*Epidemiology Unit, Ministry of Health, De Saram Place, Colombo 10, Sri Lanka*

Dengue poses a threat to public health both globally and locally. Rapid detection of the Dengue virus (DENV) in mosquitoes is crucial for prompt and proactive control measures and interventions. Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) provides a rapid, sensitive, and affordable solution for this purpose. RT-LAMP for detection of all four DNV serotypes using 24 primers (6 primers per each serotype) is currently available. The objective of this study was to find out the possibility of developing a new RT-LAMP assay by reducing the number of primers required. A comprehensive bioinformatic analysis was carried out using Geneious software to identify a conserved region in all four serotypes (DENV 1-4). However, consistently conserved regions for all serotypes was not found to develop a simple assay to detect all four serotypes. Therefore, based on previous studies which targeted 3' untranslated region (3'UTR) of DENV 2, two separate sets of primers (5 primers per each set) were designed; Primer set 1 using Eiken's Primer Explorer V4 and primer set 2 using NEB LAMP Primer Design Tool. Both primer sets were assessed using RNA from cultures of all four dengue serotypes and WarmStart Colorimetric LAMP master mix (NEB, USA) including UDG and Syto 9 green, fluorescent dye. Primer set 1 showed positive colour changes and peak amplifications specificity only for DENV 2 RNA within 40 min. Primer set 2 showed positive reactions with DENV 2 first, followed by DENV 3 and 4, within 40 min. Based on the results primer set 2 (5 primers) was selected and as a further development, DNV 1 detection primer set (6 primers) from above currently available LAMP assay was added for the detection of DNV 1. However, when the assay was conducted with extracts (homogenates) from Aedes aegypti mosquitoes infected with each of the four serotypes, the assay was positive only with extracts of mosquitoes infected with DENV 1 or 2, indicating that mosquito extracts resulted in weaker reactions compared to purified viral RNA, emphasizing the need for further optimization of the assay with the novel primers.

Keywords: *Dengue, Dengue virus serotypes, RT-LAMP, Aedes aegypti, Primer optimization*

Acknowledgements: This work was supported by the NRC (19-065) & Center for Vector-borne Infectious Diseases, Colorado State University, USA and constitutes a part of the PhD studies of author MDSJ.