Abstract No: STEM 29 Science, Technology, Engineering and Mathematics

# Application of rolling circle amplification (RCA) to detect direct amplification of dengue virus in patient serum samples.

**Manilgama T.[1], Seneviratne K. N.[1] Jayathilaka N.[1*]**

Rolling Circle Amplification (RCA) is an isothermal amplification process that can be utilized for rapid amplification of target nucleic acids. In contrast to PCR, which uses thermocycling to mediate denaturation, annealing, and subsequent extension, RCA can be performed at a single reaction temperature making RCA an attractive solution for disease diagnosis based on amplification of pathogen nucleic acids at resource limited settings. In addition, PCR-based detection of pathogenic RNA involves the additional steps required to make complementary DNA copies of the target for amplification. Dengue is a mosquito vector borne viral RNA infection that largely affects urban and semi-urban, sub-tropical and tropical areas. While majority of dengue fever patients recover with careful hospital monitoring some patients may develop severe complications that result in mortality. Therefore, early diagnosis is critical for screening the patients that require hospital management and to prevent exceeding hospital capacity during a dengue outbreak. We developed a direct RCA of dengue virus RNA in serum samples from dengue virus positive patients using Phi 29 DNA Polymerase for the disease diagnosis at resource limited settings. Serum samples were collected from patients suffering from dengue virus infection based on a positive NS 1 antigen test within four days from fever onset with informed consent (n=3). Serum samples collected from healthy individuals were used as the controls (n=3). Multiple Displacement Amplification (MDA) was used to increase the amplification efficiency. Positive control reactions were carried out using a circularized 66 bp linear 5´phosphorylated probe that contained a complementary sequence to all four dengue serotypes and a forward primer against the conserved target region on the probe at 30 $_{o}$C overnight. The product formation was confirmed by gel electrophoresis following restriction enzyme digestion of the RCA/MDA products with EcoRI. The RCA/MDA products were quantified using a ssDNA dye. Direct isothermal amplification of dengue virus from serum samples collected from dengue infected subjects confirmed that RCA/MDA reaction specifically amplifies dengue virus in patients while no amplification was detected for the serum samples collected from healthy volunteers. Since RCA/MDA can be used for direct gene expression analysis of mRNA and micro RNA in resource limited settings, this novel method can be used for simultaneous disease diagnosis and early prognosis of severe dengue based on differential expression in resource limited settings.

**Keywords:** Severe Dengue, Phi29 enzyme, Rolling Circle Amplification, Multiple Displacement Amplification and Serum.

[1] Department of Chemistry, Faculty of Science, University of Kelaniya, Sri Lanka
∗ njayathi@kln.ac.lk