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A COST EFFECTIVE FEASIBLE METHOD TO TRANSPORT DENGUE VIRAL NUCLEIC ACID IN AMBIENT TEMPERATURE FOR NEXT GENERATION SEQUENCING

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Although various diagnostic methods are available for the confirmation of dengue viral infections (DENV) in Sri Lanka, advanced genomic analysis techniques such as next generation sequencing (NGS) method still remains scarce. Since the DENV is a RNA virus, there is a need to transport either the DENV infected patient's samples or the extracted RNA samples to laboratories abroad for downstream evaluations such as NGS. RNA is labile and thus RNA samples should be preserved at -80°C to ensure the sample integrity. Shipping RNA samples is costly as the samples must be maintained in dry ice during shipment. The present study describes a cost effective and a feasible method to transport DENV nucleic acid for NGS. Though it is a generally used method for transporting DNA and cDNA, this method has not been used for the transport of DENV nucleic acid for downstream applications like NGS so far.

Total RNA was extracted (Qiagen, Cat No 5206) and converted to cDNA using random hexamers and M-MLV reverse transcriptase (Promega). Quantity and quality of cDNA was evaluated using Optizen Pop Bio Nanohandler before blotting. Known concentrations of cDNA samples were blotted onto a sterile Whatman filter paper and dried using the speed vac concentrator. The procedure was repeated at least three to four times to concentrate 40µl of cDNA on the filter paper. After drying, the filter papers containing cDNA were packed using sterile polythene, sealed and shipped in an envelope to the Virology Laboratory at the University of Calgary, Canada. After 5 days of shipment cDNA was eluted from the filter paper in 150µl of sterile water and the quantity and quality were tested using the Nano drop. One of the eluted cDNA samples was submitted for NGS resulting high quality sequences of DENV-1. This study demonstrates that this blotting method is an efficient and cost effective means of transporting DENV cDNA in ambient temperature for further analysis.

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