103/A

Comparison of different RNA extraction methods for Dengue Reverse Transcription –Polymerase Chain Reaction (RT-PCR)

D D Adihetty[1], C Wellawaththage[2], W Abeyewickreme[2], K Abeywickrema[1], M D Hapugoda[2*]

[1] *Faculty of Science, University of Kelaniya*
[2] *Faculty of Medicine, University of Kelaniya, Ragama*

Reverse Transcription Polymerase Chain Reaction (RT-PCR) is currently used on a routine basis for the early laboratory diagnosis of dengue infection. Dengue viral RNA for RT-PCR can be extracted using different RNA extraction methods. It is important to select an appropriate RNA extraction method that gives a consistent high yield of pure RNA at an affordable cost for the routine laboratory diagnosis of dengue. The objective of this study was to select an efficient and cost effective RNA extraction method for dengue RT-PCR. Five RNA extraction methods (Quagen kit, Tryzol, detergent lysis by Np-40, direct boiling and size-fractionated silica) were compared using 3 criteria: amount of pure RNA extracted; result of RT-PCR based on the intensity of DNA band in agarose gel; and cost per test. Considering the amount of RNA extracted (mean value for five samples), the five RNA extraction methods listed according to the descending order are as follows: direct method by boiling (57 µg), size fractionated silica (52 µg), Tryzol (43 µg), Quagen kit (36 µg) and detergent lysis using NP-40 (4 µg). All RNA extraction methods gave satisfactory products in RT-PCR. The tryzol method gave a product with high intensity in RT-PCR. Considering the cost per test, the five RNA extraction methods listed according to the descending order are as follows: Quagen kit (Rs.1000), size fractionated silica (Rs. 546), detergent lysis using NP-40 (Rs. 114), Tryzol (Rs. 98) and direct method by boiling (Rs. 23). The Tryzol method could be recommended for routine laboratory diagnosis based on intensity of the resultant on intensity of resulted DNA band in agarose gel, purity and amount of RNA extracted and consistency and cost of extraction.

menakaha@yahoo.com Tel: 2960483, 0718733814