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Evaluation of two Reverse Transcription–Polymerase Chain Reaction–Liquid based Hybridization (RT-PCR-LH) assays for early detection of dengue infection

Menaka D Hapugoda[1*], Maya B Gunasekera[2], Nilanthi R de Silva[1], Sunethra Gunasena[3], L.D.Prithimala[3], M Y D Dayanath[1] and W Abeyewickreme[1]

[1.] *Department of Parasitology, Faculty of Medicine, U' sity of Kelaniya, Ragama*

[2.] *No 19, Deal Place, Colombo 3*

[3.] *Department of Virology, Medical Research Institute, Colombo 8*

Dengue is an important flaviviral infection in Sri Lanka. In this study two RT-PCR-LH based assays were evaluated for definitive laboratory diagnosis of dengue infection by comparing with Heamagglutination Inhibition (HI), gold standard test for diagnosis of dengue infection. Acute and convalescent serum samples were collected from clinically suspected dengue patients warded at the North Colombo Teaching Hospital. Convalescent serum samples were collected 7-14 days after collecting the acute sample. All paired serum samples were tested by HI assay. Acute serum samples collected from serologically confirmed dengue patients by HI assay were used for evaluation of two RT-PCR-LH assays. This included serum samples from patients with fever 1-5 days (Group 1; n=38) and with fever more than five days (Group 2; n=42). Eight serum samples similarly collected but confirmed as non dengue by HI assay were also tested by both RT-PCR-LH assays. The two RT-PCR-LH assays involved the separate amplification of two regions of the virus by PCR: NS3 gene (assay A) and a noncoding region (assay B). The RT-PCR products from assay A and B (≅470bp and ≅235bp) were then separately hybridized in liquid phase with dengue specific radiolabelled oligonucleotides. Hybridized products were separated by polyacrylamide gel electrophoresis and visualized by autoradiography.

RT-PCR-LH assay A and assay B detected dengue virus in 100% and 97% of Group 1 samples respectively, whereas the assays (A and B) detected 59% and 43% of Group 2 samples. There is no significant difference between the two RT-PCR-LH assays (P=0.20). Eight paired sera confirmed as non dengue by HI were also negative by both RT-PCR-LH assays. Results indicate the absence of false positives by both RT-PCR-LH assays.

Both RT-PCR-LH assays can be used for early and definitive laboratory diagnosis of dengue infection using a single serum specimen collected within 1-5 days of illness.

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*menuhapu@yahoo.com

Tel: 011 2953412