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Detection of recent dengue infection in patients sera by a novel Reverse Transcription-polymerase Chain Reaction-Liquid Hybridization (RT-PCR-LH) based assay

Early diagnosis of dengue which is essential for appropriate patient management is difficult with the immunodiagnostic test in routine use at present. We describe here a

Reverse Transcription-Polymerase Chain Reaction based Liquid Hybridization (RT-PCR-LH) technique which enables rapid and early diagnosis of dengue infection.

RT-PCR products of the NS3 gene of dengue virus prototypes and of 10 dengue positive sera identified by culture, were allowed to hybridize in liquid phase with a mixture of dengue specific radiolabelled oilgonucleotides. The products were separated by PAGE and visualized by autoradiography. Two DNA bands (~ 470 dp and ~ 455 dp) specific to dengue virus, were observed. Seventy eight clinically suspected dengue sera were tested by RT-PCR-LH method (which takes only 24h), and by IgM-ELISA and HAI tests for comparison.

Of the 78 sera tested, 57.8% was positive by RT-PCR-LH, 39.7% was positive by IgM-ELISA, and 18.4% had a HAI titre f2560. Of 72 cases where duration of fever was know, infection was detected by RT-PCR-LH in 50% of cases with <5d fever, and by IgM-ELISA in 4.5%. In cases with 5-15 d fever, RT-PCR-LH and IgM-ELISHA/HAI titre f2560 detected infection in 62% and 56%, respectively. The 10 sera which were negative by RT-PCR-LH, but were positive by either IgM-ELISA or HAI titre f 2560, were all >5d fever cases.

RT-PCR-LH assay was significantly better ($x2 = 9.28$, $p < 0.01$) than the other diagnostic technique for early detection of dengue. RT-PCR-LH together with IgM- ELISA were capable of detecting dengue infection in 72% of suspected cases.