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DNA-based detection of Herpes Simplex Virus 1 and 2 in clinical samples and its usefulness as a screening test for viral encephalitis

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Both Herpes Simplex Virus (HSV) types 1 and 2 are contagious, and may be transmitted by asymptomatic carriers. HSV has been associated with encephalitis and meningitis where 70% of infections occur in individuals with a previous history of HSV.

For acute infections, virus detection is the method of choice. Viral culture takes 24 hours, and allows detection of asymptomatic shedding. PCR is the most practical method available for the early detection of HSV in Sri Lanka. It can detect the virus in primary infections, asymptomatic shedding, and latency.

The objective of this study was to optimise and establish a low-cost in-house PCR-based molecular diagnostic assay to detect HSV 1 and 2 in clinical specimens, and determine its usefulness as a screening test for viral encephalitis. Samples suspected of HSV infection (n=358) were tested between June 2003 and April 2006. Of the samples received for testing 307 (85.8%) were cerebrospinal fluid specimens, 36 (10%) were blood, and 15 (4%) were samples from vaginal swabs, blister fluids and eye swabs. DNA was extracted from these clinical specimens using an in-house guanidium thiocyanate method followed by nested PCR and agarose gel electrophoresis, using

primers specific for the glycoprotein D gene of both HSV 1 and 2 viruses. The assay was controlled for sensitivity, specificity and contamination by the use of known HSV specimens and water blanks. No cross-reactivity was seen with *Varicella zoster*, *Haemophilus influenzae* and *Neisseria gonorrhoeae.* Validation of the PCR was done using a reference strain of HSV 1 and 2. Estimation of the sensitivity of the PCR was done using dilutions of the culture, for which the viral titre had been specified. Sensitivity was set at one hundred virus particles in 0.1 ml of specimen.

Of the samples suspected of HSV, 26 were found to be positive for HSV Type 1 or 2 (7.26%). Most patients tested belonged to the age group 16-45 years (52.3%). 15.4% belonged to the 0-15 years age group, while the rest were over 46 years old. Of the suspected CSF samples, 6.2% were positive; of the blood samples, 8.3% were positive. Of the other mentioned samples, 28.6% were positive for HSV 1 or 2. The chemical cost for offering this test using a commercially available kit would range from Rs.1,400 (Cinnagen, Iran) to around Rs.18,000 (Roche, USA). The chemical cost of performing our in-house assay is Rs.750. We have optimised and established a rapid PCR-based test for HSV, which is useful as a screening test in viral encephalitis.

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