

**003/A**

**Clinical utility of PCR and Real Time PCR assays for Cytomegalovirus, Hepatitis B and Hepatitis C infections**

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Reactivation of cytomegalovirus (CMV), Hepatitis B (HBV) and C (HCV) viruses from the status of latency is seen in immunocompromised individuals and such reactivation is often associated with morbidity and mortality in such individuals. The prevalence of these viral infections in a selected population of patients referred to the Molecular Diagnostic Laboratory at the Durdan's Hospital, Colombo, during the period from August 2007 to May 2008 were studied using qualitative PCR assays. All specimens from patients with suspected clinical diagnoses of either CMV or HBV or HCV infections were analyzed. Of 176 samples analyzed for CMV 78 were positive (37 males, 29 females) and majority of them are patients from a nephrology unit. Out of 40 and 10 samples analyzed from males and females, respectively, 22 and 4 were positive for HBV. Twenty six samples were analyzed for HCV and only 6 were found to be infected with viruses and all of them were from males. Although PCR detection of these viral DNA/RNA is a sensitive method to detect infection, it lacks specificity for the detection of active viral disease and for monitoring the efficacy of antiviral therapy. Therefore, Real-time PCR (RT-PCR) assays for the detection and quantification of CMV-DNA, HBV-DNA and HCV-RNA were developed using SYBRgreen1 chemistry. The assays developed are capable of detecting viral particles in blood samples and quantifying viral DNA accurately over a broad range of input target copies ( $10^2 - 10^8$  copies/ml) and therefore, can be used to predict the reactivation of viruses by comparing with published kinetic criteria in clinical guidelines. Post PCR analyses of Real-time PCR products by agarose gel electrophoresis revealed bands having the same intensity for a wide range of target copies ( $10^3 - 10^8$  copies/ml). In contrast, RT-PCR elicited higher cycle threshold for the descending order of concentration of target copies. Therefore, based on these results, it is evident that the intensity of conventional PCR bands should not be used for the assessment of viral reactivation or for monitoring therapeutic intervention and for this purpose RT-PCR is the method of choice

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