developed for quantifying CMV DNA in immunocompromised patients

Results: The detection limit of TaqMan polymerase chain reaction assay for CMV-DNA was 10 genome per reaction and the linear measure interval was 1 to 107 copies per reaction ($r^2 = 0.999$). The reproducibility of the TaqMan assay was initially evaluated by interassay (between-runs) and intraassay (within-run). The interassay and intraassay coefficients of variation were 9.79% and 10.85%, respectively. The specificity of the assay was determined among herpesviridae subfamily. No positive signals were detected. Initial application of the quantitative real-time PCR to serum of infant indicated the effective of assay for CMV quantitation with 100% sensitivity ($n = 5$).

Conclusion: In-house TaqMan assays may potentially serve as a useful tool for rapid quantification of CMV infections in immunocompromised patients.

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71.008

Evaluation of Different Storage Conditions on Detection of Influenza A Virus Subtyping by Multiplex PCR Assay

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Isolation of influenza A virus by tissue culture is the traditional gold standard for laboratory detection and is important for tracking antigenic changes of the virus. However, viral isolation is not useful for rapid clinical diagnosis of infection and is not practical for large-scale epidemiological studies. The application of molecular diagnostics overcomes these limitations and increases the detection rate of influenza virus by 3 - 60%. Although optimal parameters for specimen storage to support subsequent virus isolation have been determined, these parameters have not been defined for nucleic acid testing. In order to define these parameters, we evaluated the ability to detect influenza A virus by molecular assays under different storage conditions, including temperature, storage medium, and time. We analyzed samples kept in different storage temperatures (4 °C, −20 °C, −70 °C), storage time (1, 7, 14 and 28 days) and different storage medium (viral transport medium or ethanol). These conditions were evaluated for both swabs and extracted RNA samples. The Luminex multiplex bead-based assay was used for molecular detection of influenza A virus matrix gene and hemagluttinin subytping. Although we were able to detect influenza A virus in all storage conditions, there was a 25—42% decrease in the mean fluorescent intensity (MFI) signal over time for both matrix and hemaglutinnin for swabs stored in VTM at 4 °C. Loss of MFI signal by swabs stored in VTM was minized by storage at either −20 °C or −70 °C. We did not observe a loss of MFI signal in swabs stored in ethanol at either 4 °C or −20 °C, although a slight decrease in MFI signal was observed in samples stored at −70 °C.(This really needs to be repeated, does not agree with other results). Results of our study suggest that ethanol storage of specimen may provide an optimal transport medium for samples collected at remote sites where maintenance cold-chain requirements may be difficult. Results from other respiratory viruses will also be presented.

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Virological and Immunological Monitoring of Human Cytomegalovirus Infection in Heart and Small Bowel/Multivisceral Transplantation

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Background: this study was carried out to assess the diagnostic and prognostic value of cytomegalovirus (CMV) determination in whole blood (heart and intestinal recipients) and in biopsy tissue samples (intestinal recipients) from transplant patients during virological surveillance and analyse the CMV T cell response after transplantation.

Methods: we monitored 30 heart transplant recipients (HTR) comprising three R-/D+ and 34 intestinal/multivisceral transplant (SBMTR) comprising three R-/D+ by CMV pp65 antigenemia and Real Time PCR . Immunological surveillance was done in 12 HTR and 9 SBMTR. T lymphocyte suspensions obtained using a Ficoll gradient were processed by ELISPOT, an immunoenzyme assay based on the search for T cells with viral specific antigens of one of the protein markers of cell activation (IFN-gamma). Around 2000 blood samples and 1200 intestinal biopsy specimens were processed for virological tests and 56 were analysed by ELISPOT

Results: 70% (21/30) and 44% (15/34) of HTR and SBMTR developed active CMV infection, respectively. Of the 21 HTR infected four had a mild-moderate symptomatic infection (leucopenia and fever). Of the 15 SBMTR infected patients two had a severe symptomatic CMV infection (pneumonia, enteritis and rejection) which led to the patient's death and three had a mild infection (fever).

Conclusion: quantitative determination of CMV in blood and organ biopsy by molecular tests is the elective assay for monitoring viral load, since it directly correlates with viral replication and clinical symptoms. The preliminary outcome of immunological monitoring shows i) a CMV T-cell immune response in the first month after transplantation was associated with a reduction in mean and peak CMV viral load and; ii) a good and early reconstitution or development of the CMV-specific T-cell response can shorten the duration of CMV infection and control the risk to incur repeated episodes linked to a recurrent infection.

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