Introduction

The human Cytomegalovirus (CMV) is a member of the family Herpesviridae and belongs to the subfamily betaherpesvirinae. It consists of icosahedral capsid with a linear double-stranded DNA genome of approximately 230 kbp, a surrounding tegument and an outer envelope. CMV has a worldwide distribution and infects humans of all ages, with no seasonal or epidemic patterns of transmission. The seroprevalence of CMV increases with age in all populations and ranges from 40 to 100%. Similar to infections with other herpesviruses, primary infection with CMV results in the establishment of a persistent or latent infection. Reactivation of the virus can occur in response to different stimuli, particularly immunosuppression. The vast majority of CMV infections are asymptomatic or subclinical, but congenital infections and infections in immunocompromised patients may be symptomatic and serious. In immunocompromised hosts, such as transplant recipients, HIV-infected or cancer patients, a CMV infection or reactivation may become a life-threatening disseminated disease.

Materials and Methods

Materials: One hundred eighty three, previously tested (urine, plasma and body fluids) clinical samples were selected and extracted using Abbott m2000sp protocol

Methods:
Method 1. Laboratory-developed test (LDT) CMV quantitation assay based on Qiagen ASR real time qPCR and Acrometrix quantification standards run on Abbott m2000rt instrument.
Method 2. RealStar® CMV PCR Kit 1.0 assay based on quantitative real-time PCR from Altona Diagnostics run on Roche LC480.

Analytical sensitivity and LOD were determined by run dilutions of the AcroMetrix® CMV Panel - Life Technologies and Altona standards provided in RealStar CMV kit.

Results

The RealStar® CMV PCR method demonstrated 100% agreement with the ASR CMV Qian assay; 114/114 positive samples were detected and 69/69 were not detected by each method. Of the 114 detectable samples, 5 were below the limit of quantification (LOQ) for each assay and thus correlated well. All detectable samples (N=114) were within tolerance +/- 0.5 log. Correlation regression was (R2=0.9941). Based on the Bland-Altman analysis, the mean difference (bias) between the two assays was -0.193, with 95% confidence intervals (-0.809 and 0.420). Analytical specificity was 100% when run against 47 different microbial and viral targets.

RealStar CMV assay had the following characteristics: LOQ = 150 IU/mL; linear range: 150-6,000,000 IU/mL; slope, -3.256; R 2 value=0.9987; PCR efficiency, 96%.

Conclusions

This study demonstrates that RealStar® CMV qPCR from Altona Diagnostics performs as well as Qiagen CMV ASR in-house assay. The performance characteristics are suitable for clinical diagnosis and monitoring of the transplant patient population.

References

6. CLSI MM17 – Verification and Validation of Multiplex Nucleic Acid Assays.
7. CLSI C24-A2 – Statistical Quality Control for Quantitative Measurements: Principles and Storage of Specimens for Molecular Methods