Monitoring of CMV Infections Using Real-Time PCR

Human cytomegalovirus (CMV) belongs to the family of Herpesviridae which enter a lytic cycle or establish a latent stage leading to livelong persistence in the host. During latency herpesviruses exist as an extrachromosomal episome and the production of infectious particles is suppressed. Reactivation from latency is triggered by stress, immunosuppression, and other factors. The primary CMV infection in healthy adults is usually asymptomatic, severe clinical manifestations are found in immunocompromised patients.

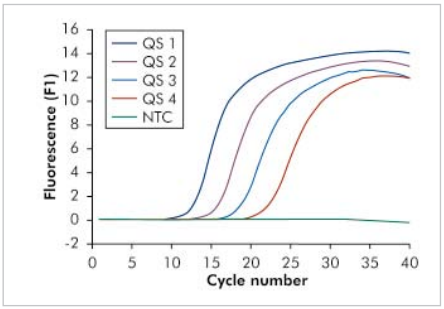

Fig. 1: Detection of the Quantitation Standards (CMV LC QS 1–4) in fluorimeter channel F1 of the LightCycler 1.1/ 1.2/1.5 Instrument. NTC: non-template control (negative control).

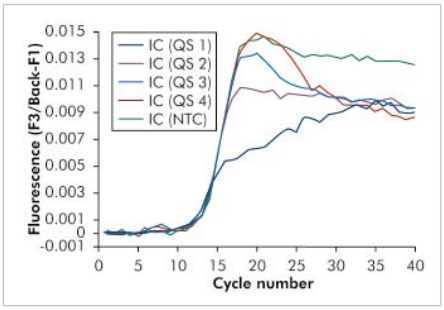

Fig. 2: Detection of the Internal Control (IC) in fluorimeter channel F3/Back-F1 of the LightCycler 1.1/1.2/1.5 Instrument with simultaneous amplification of Quantitation Standards (CMV LC QS 1–4). NTC: non-template control (negative control).

The Role of CMV in Immunocompromised Patients

CMV is a common pathogen which complicates treatment of immunosuppressed patients (transplantation, HIV patients). This leads to the development of clinical manifestations including retinitis, encephalitis, hepatitis, or pneumonitis. Development of a symptomatic CMV infection including organ involvement can lead to an invasive disseminated CMV Disease (CMVD) which may be fatal.

In organ transplant recipients CMV is a cause of considerable morbidity and mortality and is associated with a decreased survival rate. Over 50% of all transplant patients are actively infected by CMV by either reactivation of a latent infection or acquirement of primary infection via the transplanted organ. In addition to direct consequences, CMV acts as an immunomodulator increasing the risk for other opportunistic infections.

Beyond prophylactic and selective approaches. treatment of CMV infection after transplantation includes pre-emptive strategies with Ganciclovir based on the earliest sign of CMV infection. Pre-emptive strategies restrict treatment to patients with detectable CMV reactivation. This requires close monitoring of the patient and intensive viral surveillance. Monitoring of CMV strictly requires a sensitive and specific diagnostic tool with a high positive predictive value.

Diagnosis of CMV Infection

Among other technologies, real-time PCR became a widely used technique in molecular diagnostics. Qiagen employs this key technology to develop highly sensitive and specific assays for infectious disease diagnostics. These kits minimize contamination risk and ensure rapid turnaround time allowing fast diagnosis.

Qiagen's artus PCR Kits are based on real-time PCR and designed for the molecular detection of pathogens including diverse viruses and bacteria. All CMV PCR Kits are CE-marked diagnostic products which are specifically developed for rapid and quantitative detection of CMV from EDTA plasma.

In order to achieve the best analytical performance the kits are designed for the use on specific real-time PCR platforms. Kits are available for three different cyclers: ABI PRISM 7000, 7700, and 7900HT SDS, LightCycler 1.1/1.2/1.5/2.0 Instruments, and Rotor-Gene 3000.

Each kit includes a reaction mix containing all reagents and enzymes for specific amplification and detection (fig. 1). The ready-to-use format ensures convenient use and safe implementation into diagnostic workflows. Accurate quantitation is guaranteed by quantitation standards of defined concentration supplied with each kit. This facilitates monitoring of the viral load in infected patients. In addition, each Qiagen's artus CMV PCR Kit provides an Internal Control, a second heterologous amplification system to identify possible PCR inhibition and to monitor nucleic acid purification (fig. 2).

Detection of the Internal Control

To ensure highest sensitivity, Qiagen's artus CMV PCR Kits have been optimized to detect lowest numbers of CMV DNA (table 1).

The PCR Kits use harmonized amplification profiles for detection of herpes virus DNA allowing the parallel detection of the four herpes viruses CMV, EBV, HSV-1/2, and VZV on the LightCycler.

Conclusion

Qiagen's artus CMV PCR Kits offer reliable CMV diagnostics. Sensitive detection of CMV DNA from plasma guarantees the safe identification of an active viral replication. This is a prerequisite for appropriate treatment of immunosuppressed patients during the early phase of infection.

Table 1: Analytical sensitivities (95 % probability of detection) determined for the artus CMV PCR Kits on different real-time PCR platforms by Probit-analysis.

Real-time PCR cycler	Copies/PCR
ABI PRISM 7000 SDS	4.0
ABI PRISM 7700 SDS	4.0
ABI PRISM 7900HT SDS	3.4
LightCycler Instrument 1.1/1.2/1.5	4.9
LightCycler Instrument 2.0	6.5
Rotor-Gene 3000	4.8

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