27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P091 Clinical epidemiology of viral infections

Category: 1c. Influenza and respiratory viruses

25 April 2017, 12:30 - 13:30
P1901

Evaluation of a LAMP (loop-mediated isothermal amplification) assay as a screening tool to detect CMV in critically ill patients

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Background:
Cytomegalovirus (CMV) is a DNA virus in the *Herpesvirus* family. Seroprevalence of human CMV is very high worldwide. Following primary infection, the virus remains latent, being able to cause recurrences.
CMV is an important pathogen in immunocompromised individuals, but its role in immunocompetent patients is unclear and controversial. Several studies have shown that CMV infection is common in immunocompetent critically ill patients and is associated with a poor clinical outcome.
Traditionally, CMV has been studied by cell culture, but it presents two main limitations: low sensitivity and long time required for the virus to grow. Molecular techniques represent an important advance in both sensitivity and response time, however they remain expensive.
The objective of this study was to develop a LAMP (Loop-mediated isothermal amplification) assay to detect CMV in bronchoalveolar lavage (BAL) from patients admitted to the Intensive Care Unit (ICU) and compare this method with PCR and culture.

Materials/methods:
BAL samples from 52 patients admitted to our ICUs were analyzed. BAL was cultured in human fibroblast cells and monitored for up to 3 weeks for sign of infection. A BAL was considered positive for CMV when cytopathic effect was observed on cell cultures and then confirmed by inmunofluoresce detection of the antigen.

Samples were initially detected using a real-time quantitative PCR (ELITechGroup, Italy). Total DNA from BAL samples was extracted using a QIAamp DNA Mini kit (Qiagen GmbH, Germany), according to the manufacturer's instructions.

In 42 samples, extraction was performed and evaluated using a LAMP assay. In 22 samples, LAMP was performed directly on the sample and after a boil for 10 minutes. For LAMP amplification we used the Isothermal Master Mix from Optigene and the primers previously published. Positive samples for other *Herpesvirus* (herpes simplex virus 1 and 2, Varizella-Zoster virus, Epstein-Barr virus, human herpesvirus 6) were tested to prove the specificity of these LAMP primers.

Results:
The gold standard was the real-time PCR. When comparing sensitivity, it was much higher with LAMP (95.8% after extraction, 58.3% on BAL directly, 66.7% after boil) than with culture (23.1%). As for the specificity, with both methods it was 100% (Table 1).
The mean response time for the cell culture was 19 days, compared to the LAMP technique which was one hour.
There were no cross-reactions with other virus belonging to the herpesvirus family.

Conclusion:
LAMP amplification method could be used as a screening method to detect CMV in ICU patients from BAL samples. It is a simple, cheap, sensitive, specific and rapid amplification assay.

Table 1. Sensitivity and specificity of culture and the different variants of LAMP compared to real-time PCR.

	Sensitivity	**Specificity**
Culture	**(6/26)23.1%**	**(28/28)100%**
LAMP/extracted DNA	**(23/24)95.8%**	**(18/18)100%**
LAMP/BAL directly	**(7/12)58.3%**	**(11/11)100%**
LAMP/boiled BAL	**(8/12)66.7%**	**(11/11)100%**