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

Andrea Vergara[1], Izaskun Alejo[1], Ana Cervilla[1], Mireia Navarro[2], Cristina Rodríguez[1], Jordi Vila[1,2], Mª Ángeles Marcos[1,2].

[1]Department of Clinical Microbiology-CDB, Hospital Clínic - Universitat de Barcelona, Spain

[2]Institut de Salut Global (ISGlobal-Hospital Clínic), Barcelona, Spain

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Objectives

Cytomegalovirus (CMV) is a DNA virus in the *Herpesvirus* family. The seroprevalence of human CMV is very high worldwide. Following primary infection, the virus remains latent, being able to cause recurrences. CMV is recognized as 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 that this 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 have represented an important advance in both sensitivity and response time, however they remain expensive techniques.

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.

Methods

BAL samples from 54 patients admitted to our ICUs were used in the present study. 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 a cytopathic effect was observed on conventional cell cultures and then confirmed by inmunofluorescence detection of the antigen. Cytomegalovirus was initially detected in positive samples by a real-time quantitative PCR (ELITechGroup, Italy). Total DNA from BAL samples was extracted using a QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions.

In 42 samples, extraction was performed and then evaluated using a LAMP assay. In 23 samples, the 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 designed elsewhere (1). 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 the clinical 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 2).

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. Primers used for cytomegalovirus LAMP assay.

Primer name	Sequence
F3	TTCGCGCATGATCTCTTCG
B3	GAGGAATGTCAGCTTCCCAG
FIP	ATCGACCCGCTGGAAAATACCGTTTTGTTGCTGGAACGCAGCTCTT
BIP	ACTGCTGAGGTCAATCATGCGTTTTTATCTTCATCGCCGGGAACTC
LPF	GGTACTGGAACTTTACTCGCAGA
LPB	TTGAAGAGGTAGTCCACGTACTC

Table 1. Sensitivity and specificity of culture and the different variants of LAMP performances compared to the gold standard (real-time PCR).

	Sensitivity	Specificity
Culture (n=54)	(6/26) 23.1%	(28/28) 100%
LAMP on extracted DNA (n=42)	(23/24) 95.8%	(18/18) 100%
LAMP on BAL directly (n=23)	(7/12) 58.3%	(11/11) 100%
LAMP on boiled BAL (n=23)	(8/12) 66.7%	(11/11) 100%

References

1. Development and Evaluation of Loop-Mediated Isothermal Amplification Assay for Rapid and Inexpensive Detection of Cytomegalovirus DNA in Vitreous Specimens from Suspected Cases of Viral Retinitis. Reddy *et al*. J Clin Microbiol. 2010;48(6):2050-2.

Contact: **Andrea Vergara Gómez**
Hospital Clínic, Barcelona
E-mail: **VERGARA@clinic.ub.es**