CRITICAL CARE

LETTER

Need to evaluate the performance of real-time PCR assays for the quantitation of cytomegalovirus DNA load in lower respiratory tract specimens

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There is an increasing appreciation for the potential clinical value of the quantitation of cytomegalovirus (CMV) DNA in the lower respiratory tract in critically ill patients lacking canonical immunosuppression, in view of the possible pathogenic role of CMV in these patients [1]. No data have been published on the analytical performance of real-time PCR assays for this purpose.

We present our data on the performance of the COBAS® AmpliPrep/COBAS® TaqMan CMV PCR Assay (Roche Diagnostics, Mannheim, Germany) for the quantitation of CMV DNA in tracheal aspirates (TA). This CMV PCR assay has been approved recently by the US Food and Drug Administration for use with plasma specimens [2]. We chose TA for the analyses because of the simplicity of their collection in critically ill patients undergoing mechanical ventilation.

We pooled TA obtained from five ICU patients with undetectable CMV DNA levels in TA and in plasma specimens [3]. The pool was fluidized by treatment with *N*-acetyl cysteine (1 % in PBS) at a 1:1 volume ratio (vortexed for 30 seconds, incubated for 10 minutes at room temperature and centrifuged at 1,600 rpm for 10 minutes). The pellet was then resuspended with distilled water to obtain the initial volume. Four aliquot portions were spiked with different quantities (2.69, 3.69, 4.69, and 5.69 $\log_{10}$ IU/ml) of the First World Health Organization International Standard for CMV (National Institute for Biological Standards and Control, Hertfordshire, UK) [4]. The testing pools were assayed in heptuplicate on three consecutive days.

The fitted regression line between copies/ml and IU/ml for tracheal aspirates was $y = 1.0013x - 0.0517$ ($R^2 = 0.999$). According to our calculations, 1 copy/ml was equated to 0.90 IU/ml (similar to that for plasma specimens [5]). The overall intra-assay and inter-assay coefficients of variation were 8.2 % (95 % confidence interval (CI), −0.6 to 17.0 %) and 10.77 % (95 % CI, 1.69 to 19.8 %), which are slightly higher than those for plasma specimens [5]. The analytical performance of the assay was analyzed with clinical samples containing different copy numbers of CMV DNA. The data are shown in Table 1. The overall intra-assay and inter-assay coefficients of variation were 14.0 % (95 % CI, 9.8 to 18.1 %) and 15.4 % (95 % CI, 12.1 to 18.8 %), respectively.

Our data indicate that this CMV PCR assay performs well with fluidized TA containing low to intermediate CMV DNA loads (between 500 and 50,000 copies/ml), and may therefore be used for monitoring CMV replication in the lower respiratory tract in critically ill patients undergoing mechanical ventilation.

Abbreviations

CI: Confidence interval; CMV: Cytomegalovirus; PBS: Phosphate-buffered saline; PCR: Polymerase chain reaction; TA: Tracheal aspirates.

Competing interests

DN has received honoraria from Roche Diagnostics for participating in several conferences. The remaining authors declare that they have no competing interests.

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Table 1 Performance of the cytomegalovirus PCR assay for quantitation of cytomegalovirus DNA load in tracheal aspirates

Group (number of specimens)[a]	Range of CMV DNA loads (copies/ml)[b]	% Coefficient of variation (95 % confidence interval)	
		Intra-assay	Inter-assay
A (10)	150 to 851	14.0 (9.8 to 18.7)	15.4 (12.1 to 18.8)
B (10)	1,234 to 8,781	10.6 (7.8 to 14.0)	16.2 (11.2 to 21.5)
C (10)	11,013 to 56,000	11.7 (4.9 to 18.5)	12.8 (7.3 to 18.3)
A + B + C (30)	150 to 56,000	14.0 (9.8 to 18.1)	15.4 (12.1 to 18.8)

CMV PCR assay, COBAS® AmpliPrep/COBAS® TaqMan Cytomegalovirus PCR Assay (Roche Diagnostics, Mannheim, Germany). [a]Tracheal aspirates were obtained from 20 patients with septic shock of abdominal origin admitted to the ICU. On the basis of their cytomegalovirus (CMV) DNA content, the specimens were grouped into three categories: A, between 150 and 1,000 copies/ml; B, between 1,000 and 10,000 copies/ml; and C, >10,000 copies/ml. The specimens were divided into the above referred groups based on two criteria: the precision of the Roche PCR assay for plasma specimens was shown to vary depending upon the CMV DNA content – in this sense, the coefficient of variation was maximum for specimens containing <1,000 copies/ml, minimum for those containing >10,000 copies/ml, and intermediate for specimens containing CMV DNA loads between 1,000 and 10,000 copies/ml [5]; and the majority of tracheal aspirates from critically ill patients displaying an episode of active CMV infection contain CMV DNA loads <1,000 copies/ml, so the knowledge of the performance of the PCR assay with specimens containing CMV DNA loads within this range (150 to 1,000 copies/ml) is of particular clinical interest. Only occasionally, tracheal aspirates contain >10,000 copies/ml. [b]As determined by the CMV PCR assay. This PCR assay targets the CMV UL54 gene (DNA polymerase) and displays a limit of detection of 100 copies/ml (91 IU/ml), a limit of quantification of 150 copies/ml (164 IU/ml), and a linear quantification range from 150 to 10,000,000 (2.18 to 7.0 $\log_{10}$) copies/ml for plasma specimens.

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