

# Comparison of real-time PCR and pp65 antigen assays for monitoring the development of Cytomegalovirus disease in recipients of solid organ and bone marrow transplants

Simona Marchetti, Rosaria Santangelo, Stefania Manzara, Sara D'onghia,  
Giovanni Fadda, Paola Cattani

*Institute of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy*

## SUMMARY

Cytomegalovirus (CMV) infection is a frequent complication in transplant recipients. This retrospective study compared real-time PCR (rt-PCR) and a pp65 antigen assay as tools for monitoring CMV infection in solid organ (SOT) and bone marrow (SCT) transplant patients. The study tested 2662 samples by rt-PCR, and 1284 specimens with a pp65 antigen assay. 24.3% of the rt-PCR samples and 4.1% of the pp65 antigen samples were positive. 793 specimens, from 230 patients, were tested with both assays. In 6.7% of samples, both tests were positive; in 72.7% both were negative; in the remaining 20.6% of cases, the results were discordant. CMV disease was diagnosed in 50 patients. Results from the two methods were poorly correlated ( $r=0.460$ ). The sensitivity of rt-PCR (94%) was higher than that of the pp65 antigen assay (27%). Both assays showed high specificity (92% and 99%, respectively). ROC curve analysis, performed separately for SOT and SCT patients, confirmed that rt-PCR outperformed the pp65 assay in the detection of CMV. These findings provide evidence that rt-PCR is a reliable diagnostic tool, and that it can be more effective than pp65 based assays in monitoring CMV infection progression and in guiding therapy in immunocompromised patients.

**KEY WORDS:** Cytomegalovirus, CMV, Real-time PCR, pp65 antigenemia, PPV, NPV

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## INTRODUCTION

Human cytomegalovirus (HCMV) is a ubiquitous member of the human herpes virus family. Up to 80% of healthy adults are seropositive, indicating previous exposure, established latency and a possible risk of viral reactivation. The reactivation mechanism appears to be strongly related to impaired control of the virus by the immune system. For this reason, CMV is one of the most important opportunistic pathogens in immunocompromised patients, including recipients of solid organ and bone marrow transplants and pa-

tients infected with human immunodeficiency virus (Mengelle *et al.*, 2003; Mori *et al.*, 2004; Schroeder *et al.*, 2005; Allice *et al.*, 2008; Fisher, 2009). Cytomegalovirus infections in these immunosuppressed patients can lead to serious diseases. They may also increase the incidence of other opportunistic infections and decrease patient survival. Active CMV infection occurs in 30%-75% of transplant recipients, with a mortality rate of 5% (Martin-Davila *et al.*, 2005; Gentile *et al.*, 2006; Madi *et al.*, 2007).

One approach to preventing CMV disease is the administration of antiviral agents after transplantation. The development and widespread use of sensitive, specific and reliable diagnostic assays for CMV thus plays an essential role in CMV prevention during the early post-transplant period, and can make a significant contribution to disease management (Razonable *et al.*, 2002a; Razonable *et al.*, 2002b; Kotton *et al.*, 2010).

*Corresponding author*

Paola Cattani

Institute of Microbiology

Università Cattolica del Sacro Cuore

Largo A. Gemelli, 8 - 00168 Rome, Italy

E-mail: pcattani@rm.unicatt.it

One tool, frequently and successfully used for this purpose has been the pp65 antigen assay for CMV, an immune-fluorescence technique based on the staining of peripheral blood leukocytes for lower matrix protein pp65 (Mazzulli *et al.*, 1993; Kusne *et al.*, 1999). However, the pp65 assay has low sensitivity, requires subjective interpretation and immediate sample processing and may be hard to perform in neutropenic patients (Gimeno *et al.*, 2008; Kotton *et al.*, 2010).

A more recent technique is quantification of CMV-DNA by rt-PCR. The method seems to be accurate, rapid, highly sensitive and highly specific. Especially in recipients of bone marrow transplants, it appears to be more effective than the pp65 assay as a support for decisions on the initiation of antiviral treatment (Mori *et al.*, 2004; Gimeno *et al.*, 2008). Yet, although rt-PCR usually has high sensitivity, it has a lower specificity than the pp65 assay. This means it is necessary to determine clinically appropriate threshold values for a positive diagnosis of CMV infection. Studies published to date have involved a broad range of different methods, different kinds of specimen (plasma, whole blood, peripheral blood leukocytes, peripheral blood mononuclear cells), and different categories of patient (recipients of bone marrow transplants; recipients of solid organ transplants). In these conditions, it has been difficult to achieve a consensus concerning the ideal cutoff point for the diagnosis of active CMV infection (Razonable *et al.*, 2002a; Razonable *et al.*, 2002b; Schroeder *et al.*, 2005; Gentile *et al.*, 2006; Kotton *et al.*, 2010). Future research will need to focus on the implementation of automated instrumentation making it possible to achieve standardized, reproducible results. As a contribution to this goal, we present a retrospective study comparing the sensitivity and specificity, and the positive and negative predictive values of quantitative rt-PCR and a pp65 antigen assay in the monitoring of CMV reactivation in a large sample of immune-compromised patients (recipients of solid organ and bone marrow transplants).

## MATERIALS AND METHODS

### Patients and clinical specimens

The study included 2909 whole-blood (WB) specimens submitted for CMV detection to the

Institute of Microbiology at the Università Cattolica del Sacro Cuore, Rome, Italy, in the period from October 2004 to August 2007. The samples were collected from 478 solid organ and bone marrow transplant recipients. 1284 samples were tested with the pp65 antigen assay; 2662 samples were tested with rt-PCR, 793 samples from 230 patients were tested with both methods. Of this latter group of patients 45.2% (104/230) had undergone solid organ transplantation. The group included 92 recipients of kidney transplants (median age: 50 years, range 18-77), 11 liver transplant patients (median age: 52 years, range 23-51), and 1 heart transplant patient (38 years). The remaining 54.8% of patients (126/230) were recipients of blood marrow transplants (median age 51 years, range 16-81). Immunosuppression protocols and prophylaxis regimens differed according to the specific transplant programs. The median number of samples was 3 (range, 2-46) per patient. All blood specimens were collected in EDTA-tubes and divided in two aliquots for the two assays. Aliquots were stored appropriately for further processing.

### CMV infection and disease definitions

Symptomatic CMV infections, (CMV syndrome and CMV disease) were defined according to standard, previously published criteria (Ljungman *et al.*, 2002).

### CMV antigen assay

The CMV pp65 antigen assay was carried out within 4 hours of specimen collection, using the Light Diagnostic™ Cytomegalovirus (CMV) pp65 Antigenemia Immunofluorescence Assay (IFA) (Chemicon International, Temecula, USA) according to the manufacturer's instructions. The result of the assay was expressed as the number of CMV antigen-positive cells per  $2 \times 10^5$  leukocytes in the slide.

### DNA extraction

Total nucleic acids were extracted from 350  $\mu$ l of EDTA-anticoagulant whole blood and eluted in 200  $\mu$ l of elution buffer using the automatic Bio Robot Ez1 extractor (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol.

### Quantitative real-time PCR

CMV DNA was quantified using a commercially

available real-time PCR test: TaqMan Q-CMV Rt Complete KIT (Cepheid by Nanogen Advanced Diagnostic S.r.l., Torino, Italy) according to the manufacturer's protocol.

For each sample, we amplified the exon 4 region of the CMV MIEA (major immediate early antigen). To control for the presence of factors inhibiting DNA amplification, we also amplified the 5' UTR region of the human beta-globin gene. The CMV probe for CMV was labeled with the FAM fluorophore and quenched by the MGB-NFQ group; the internal inhibition control was labeled with the VIC fluorophore and quenched by the MGB-NFQ group. PCR reactions were performed using an ABI PRISM 7000 Sequence Detection System (PE Biosystems, Foster City, CA, USA). Copy numbers for CMV DNA were obtained by linear extrapolation of the cycle threshold (CT) values from the standard curve (range: from  $10^2$  to  $10^5$  copies). The analytical sensitivity of the assay, as assessed by the manufacture, allows the quantification of approximately 20 to  $1 \times 10^6$  molecules of target DNA/reaction.

### Statistical analysis

The correlation between quantitative values from the real-time PCR and the pp65 assay was measured using the Spearman correlation coefficient. Differences between results from the two assays were assessed using the Mann-Whitney U test and Fisher's exact test. Receiver Operating Characteristic (ROC) curves were computed and used to determine optimal cut off values for the two assays. Statistical analysis was performed using EpiInfo software version 3.5 and MedCalc software version 8.0. *P* values less than 0.05 were considered statistically significant.

## RESULTS

The pp65 assay detected pp65 antigen in 52/1284 samples (4.1%) (median: 3 positive cells on  $2 \times 10^5$  leukocytes - range, 1-300). 948 samples (73.8%) tested negative. 284 (22.1%) specimens were considered unsuitable for the pp65 assay, due to very low levels of leukocytes.

Rt-PCR detected CMV DNA in 646/2662 samples (24.3%). The remaining 75.7% ( $n=2016$ ) tested negative. The assay found a median of  $2.2 \times 10^3$  copies/ml (range,  $43-1.8 \times 10^7$ ). All specimens were

positive for beta-globin DNA, confirming the quality of the DNA preparations.

The presence of a subset of samples tested with both assays made it possible to compare the performance of the two tests. 53/793 (6.7%) samples tested positive on both assays; 577 (72.7%) tested negative on both; in the remaining 163 samples (20.6%) the two methods gave discordant results. In these samples, real-time PCR detected viral DNA in 162/163 cases (99.4%) (median:  $2.3 \times 10^3$  copies/ml - range,  $4.3 \times 10^1-2.4 \times 10^6$ ). In each of these cases, the pp65 assay gave a negative result. Only 1 sample (0.6%) tested negative by real-time PCR and positive by the pp65 assay (5 positive cells on  $2 \times 10^5$  leukocytes). This sample came from a patient with CMV disease.

Samples that tested positive on the pp65 assay showed significantly higher CMV-DNA levels (median:  $2 \times 10^4$  copies/ml; range,  $0-10^7$ ) than those for samples that had tested negative (median: 0 copies/ml; range,  $0-2.4 \times 10^6$ ) ( $p < 0.0001$ ).

For the purposes of the analysis, we classified each sample according to the result of the pp65 assay (Table 1). Group 1 included all samples that tested negative on the assay ( $n=740$ ). Group 2 included samples ( $n=25$ ) with a low value (1 to 3 positive cells) for the antigen. Groups 3 ( $n=22$ ), 4 ( $n=3$ ), and 5 ( $n=3$ ) included samples respectively with moderate (4 to 20 positive cells), high (21 to 100 positive cells), and very high ( $>100$  positive cells) antigen values. Table 1 shows the load of CMV DNA load values for each group.

The Spearman coefficient for the correlation between DNA load and numbers of CMV pp65-positive leukocytes was relatively weak ( $r=0.460$ ,  $p < 0.0001$ ). We observed, however, that all groups with high antigen values had higher median DNA load than groups with lower antigen values, the only exception being Group 5.

Comparing data from CMV pp65 antigenemia with the data from CMV DNA assay (ranging from  $10^2$  copies/ml to  $10^6$  copies/ml), we found that the sensitivity increased from 24.4% to 83% and its specificity decreased from 99.8% to 93.9%. The pp65 assay was less sensitive than rt-PCR both for low viral loads and for the higher loads that characterize active CMV infection (Table 2). Among the samples tested with both assays, 178 came from 50 patients (50/230, 21.7%) with CMV disease (Table 3). We used this diagnosis to estimate the sensitivity, specificity, positive predic-

TABLE 1 - Comparison of CMV DNA load in whole blood specimens according to the result of the pp65 assay. CMV pp65 specimens were classified into 5 groups. The table shows mean viral load and standard deviation (Std Dev), median load, minimum (Min) and maximum (Max) values for each group.

Antigenemia groups	Samples N° (%)	CMV DNA (copies/ml)				
		Mean x 10 <sup>4</sup>	Std Dev x 10 <sup>4</sup>	Median x 10 <sup>4</sup>	Min x 10 <sup>6</sup>	Max x 10 <sup>6</sup>
Group I	740 (93.3%)	0.97	10.2	0.00	0.00	2.40
Group II	25 (3.2%)	5.91	23.84	0.30	0.12	12.00
Group III	22 (2.8%)	31.97	38.38	27.00	2.5	55.00
Group IV	3 (0.4%)	184.32	169.27	220.00	0.06	3.33
Group V	3 (0.4%)	333.54	577.17	0.50	0.12	10.00
Total	793 (100%)					

TABLE 2 - Sensitivity and specificity of CMV pp65 antigen assay using quantitative real-time PCR as a reference.

Antigen assay	N° of samples Threshold levels (x) of CMV DNA (copies/ml)									
	<10 <sup>2</sup>	≥10 <sup>2</sup>	<10 <sup>3</sup>	≥10 <sup>3</sup>	<10 <sup>4</sup>	≥10 <sup>4</sup>	<10 <sup>5</sup>	≥10 <sup>5</sup>	<10 <sup>6</sup>	≥10 <sup>6</sup>
Positive	1	52	6	47	24	29	37	16	48	5
Negative	579	161	629	111	703	37	731	9	739	1
Sensitivity (%)	24.4%		29.7%		43.9%		64%		83%	
Specificity (%)	99.8%		99.0%		96.6%		95.2%		93.9%	

TABLE 3 - CMV pp65 antigen assay as a predictor for CMV disease (a) and rt-PCR as a predictor for CMV disease (b).

(a)

Methods	Patients with or without CMV disease				
	Patients without CMV disease	Patients with CMV disease	Total	Sensitivity %	Specificity %
Negative	611	129	740	27%	99%
Positive	4	49	53		
Total	666	178	793		

(b)

Methods	Patients with or without CMV disease				
	Patients without CMV disease	Patients with CMV disease	Total	Sensitivity %	Specificity %
Negative	568	9	577	94%	92%
Positive	47	169	216		
Total	615	178	793		

TABLE 4 - ROC analysis: sensitivity, specificity, Negative Predictive Value (NPV), Positive Predictive Value (PPV), calculated for solid organ (SOT) and bone marrow (SCT) transplant recipients. Rt-PCR vs. pp65 antigen assay.

ROC analysis	CMV real-time PCR		CMV pp65 antigen	
	SOT	SCT	SOT	SCT
Sensitivity %	97.0	93.8	22.4	30.6
Specificity%	98.2	98.5	98.6	99.7
NPV	99.1	98.2	80.7	83.7
PPV	94.2	94.6	83.3	97.1

tive value (PPV), and negative predictive value (NPV) of the two assays. Analysis showed that rt-PCR was more sensitive but less specific than the pp65 assay (sensitivity: 94% vs 27%, specificity 92% vs 99%). As shown in Table 3, the PPV and the NPV of the two assays were 92% and 93% respectively for rt-PCR, and 96% and 57% for the pp65 assay (these values were calculated using 2x2 table).

Among the blood samples that tested positive for CMV DNA, 169 came from patients with a diagnosis of CMV disease. DNA viral loads in these samples were significantly higher (median,  $4.8 \times 10^3$  copies/ml; range,  $2.8 \times 10^2$ -  $1.0 \times 10^7$ ) than in

samples (n=47) from patients without CMV disease (median,  $5 \times 10^2$  copies/ml; range, 43- $3.8 \times 10^4$ ;  $P < 0.0001$ ). The majority of pp65-positive samples (49/53; median 4.0; range, 1-300) came from patients with CMV disease; only 4 samples came from patients without CMV disease (median 4.0; range, 1-10;  $P = 0.6564$ ) (Table 3).

To compare the diagnostic performance of the two essays, we computed ROC curves. As regards the pp65 assay, analysis showed that the number of pp65-positive cells in samples of  $2 \times 10^5$  leukocytes was not significantly different between patients with and without CMV disease and that the presence of a single positive cell should be taken as an indication of CMV infection (Gimeno *et al.*, 2008) both in solid organ and bone marrow transplant recipients. On this basis, we computed the sensitivity, specificity, NPV, and PPV for recipients of solid organ transplants and for recipients of bone marrow transplants (Table 4).

For the rt-PCR assay analysis of the ROC curve suggested that the trigger points for intervention were of 3000 copies/ml for recipients of solid organ and 1000 copies/ml for recipients of bone marrow transplants. On this basis, the sensitivity, specificity, NPV and PPV of the assay for recipients of solid organ transplants and of bone marrow transplants are shown in Table 4.

A comparison between the ROC curves for the two assays, including all patients, showed that the area under the ROC curve for the DNA-based

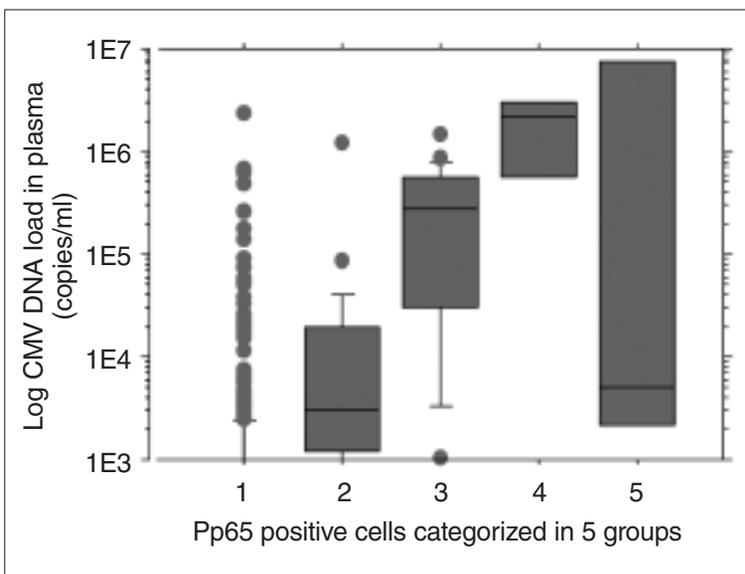


FIGURE 1 - Comparison of CMV DNA loads and levels of pp65 antigen in whole blood specimens from solid organ transplant patients and from bone marrow transplant recipients. Specimens are classified in five groups according to their recorded antigen levels (1-5).

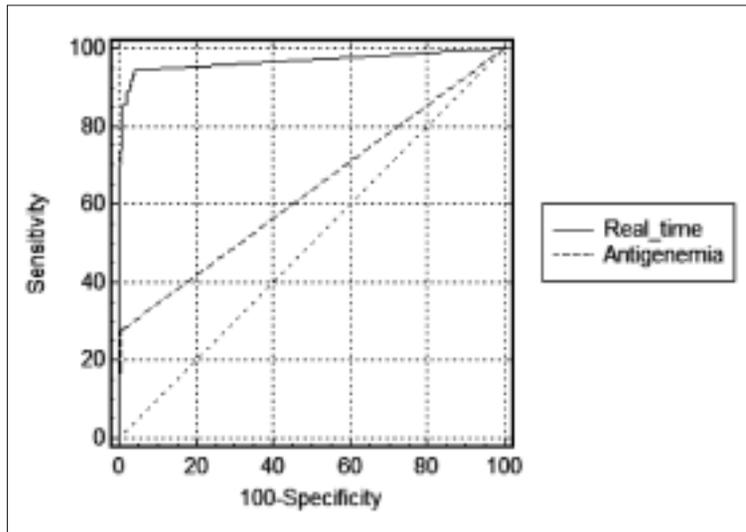


FIGURE 2 - Comparison of ROC curves for rt-PCR and pp65 antigen assays.

assay (AUC:  $0.968 \pm 0.009$ ; 95% CI: 0.953-0.979) was significantly larger than the equivalent area for the pp65 assay (AUC:  $0.635 \pm 0.025$ ; 95% CI: 0.600-0.668) ( $p < 0.0001$ ) (Figure 2).

We went on to compare series of samples taken after transplantation considering the first sample to test positive with either method. In 18 (19.1%) of CMV positive patients ( $n=94$ ) the same sample tested positive on both assays. In series yielding discordant results, samples from 75 patients (79.8%) tested positive by rt-PCR before they tested positive on the pp65 assay. Only in 1 case (1.1%), did the pp65 assay give a positive result before the DNA assay ( $p < 0.0001$ ). In all patients with positive results on both assays the pp65 assay returned negative before the rt-PCR assay.

## DISCUSSION

This study compared the effectiveness of rt-PCR and a pp65 antigen assay in the detection of CMV infection in recipients of solid organ and bone marrow transplants. Our results provide evidence that rt-PCR can be extremely useful in detecting the reactivation of CMV during the early post-transplant period.

We note, furthermore, that rt-PCR may be the only effective assay for patients with severe neutropenia or leukopenia. 22% of our specimens proved unsuitable for testing with the pp65 assay because of an insufficient number of leuko-

cytes, a common condition in these patients. By contrast, the rt-PCR assay was successful for all the specimens to which it was applied. rt-PCR also has a number of additional advantages. The procedure is faster than the pp65 assay, is easier to handle and provides standardized processing for large numbers of specimens (Yakushiji *et al.*, 2002; Bordilis *et al.*, 2005; Onishi *et al.*, 2006).

The fact that tests on consecutive samples tested positive with rt-PCR before testing positive on the pp65 assay is additional evidence that rt-PCR can provide a sensitive tool for monitoring patients at high risk of CMV disease (Nitsche *et al.*, 2003; Hernando *et al.*, 2005; Sanghavi *et al.*, 2008; Kotton *et al.* 2010).

As mentioned earlier, our work showed a relatively weak correlation between DNA load and antigen values, confirming earlier findings by Nitsche (2003), but contrasting with results from other studies (Li *et al.*, 2003; Garrigue *et al.*, 2006). As observed by others (Kalpoe *et al.*, 2004), we detected a wide range of values of CMV DNA load within distinct Groups classified according to the result of the pp65 assay. Although earlier studies showed that copy numbers increase in direct proportion to antigen levels, for all levels of antigen (Gault *et al.*, 2001; Li *et al.*, 2003), our findings are partially contrasting. The discrepancy could be due to the very small number of samples ( $n=3$ ) in our Group 5, or to differences between the two analytical approaches (molecular amplification versus IFA).

As expected, the ROC curve analysis for real-time

PCR showed that this test was more sensitive than antigenemia assay, with similar specificity. Possible explanations include the sensitivity of the pp65 assay to delays in sample processing and the difficulty of detecting the antigen in neutropenic patients.

We also observe that rt-PCR test gave positive results in 21.7% of asymptomatic patients, though recording viral loads that were significantly lower than in patients with CMV disease ( $500$  vs  $4.8 \times 10^3$ ). Though low viral loads are not necessarily a sign of disease, the ability to detect of such loads may provide useful indications for follow-up. Trends in viral loads over time may be more important in predicting disease than any absolute viral load value (Li *et al.*, 2003, Kotton *et al.*, 2010).

Our observations thus support suggestions by other authors (Nitsche *et al.*, 2003; Hernando *et al.*, 2005) that rt-PCR is more effective than the pp65 assay in monitoring CMV disease progression and in guiding therapy.

We observe, finally, that rt-PCR has a high sensitivity and that there is currently no clear agreement on the ideal cut-off for the diagnosis of active CMV infection. Treatment decisions in the clinic should therefore be taken with caution, considering trends in viral load and the sensitivity of the methods used in individual laboratories and not just absolute values recorded in a single test.

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#### REFERENCES

- ALLICE T., CERUTTI F., PITTALUGA F., VARETTO S., FRANCHELLO A., SALIZZONI M., GHISETTI V. (2008). Evaluation of a novel real-time PCR system for Cytomegalovirus DNA quantitation on whole blood and correlation with pp65-antigen test in guiding pre-emptive antiviral treatment. *J. Virol. Methods*. **148**, 9-16.
- BORDILS A., PLUMED J.S., RAMOS D., BENEYTO I., MASCAROS V., MOLINA J.M., CORDOBA J., GARCIA J., CRUZ J.M. (2005). Comparison of quantitative PCR and antigenemia in Cytomegalovirus infection in renal transplant recipients. *Transplant. Proc.* **37**, 3756-3759.
- FISHER R.A. (2009). Cytomegalovirus infection and disease in the new era of immunosuppression following solid organ transplantation. *Transpl. Infect. Dis.* **11**, 1398-2273.
- GARRIGUE I., BOUCHER S., COUNZI L., CAUMONT A., DROMER C., NEAU-CRANSAC M., TABRIZI R., SCHRIVE M.H., FLEURY H., LAFON M.E. (2006). Whole blood real-time quantitative PCR for Cytomegalovirus infection follow-up in transplant recipients. *J. Clin. Virol.* **36**, 72-75.
- GAULT E., MICHEL Y., DEHEE A., BELABANI C., NICOLAS J.C., GARBARG-CHENON A. (2001). Quantification of human Cytomegalovirus DNA by real-time PCR. *J. Clin. Microbiol.* **39**, 772-775.
- GENTILE G., PICARDI A., CAPOBIANCHI A., SPAGNOLI A., CUDILLO L., DENTAMARO T., TENDAS A., CUPELLI L., CIOTTI M., VOLPI A., AMADORI S., MARTINO P., DE FABRITIIS P. (2006). A prospective study comparing quantitative Cytomegalovirus (CMV) polymerase chain reaction in plasma and pp65 antigenemia assay in monitoring patients after allogeneic stem cell transplantation. *BMC Infect. Dis.* **6**, 167.
- GIMENO C., SOLANO C., LATORRE J.C., HERNANDEZ-BOLUDA J.C., CLARI M.A., REMIGIA M.J., FURIO S., CALABUIG M., TORMO N., NAVARRO D. (2008). Quantification of DNA in plasma by an automated real-time PCR assay (Cytomegalovirus PCR KIT) for surveillance of active Cytomegalovirus infection and guidance of preemptive therapy for allogeneic hematopoietic stem cell transplant recipients. *J. Clin. Microbiol.* **46**, 3311-3318.
- HERNANDO S., FOLGUEIRA L., LUMBRERAS C., SAN JUAN R., MALDONADO S., PRIETO C., BABIANO M.J., DELGADO J., ANDRES A., MORENO E., AGUADO J.M., OTERO J.R. (2005). Comparison of Cytomegalovirus viral load measure by real-time PCR with pp65 antigenemia for the diagnosis of Cytomegalovirus disease in solid organ transplant patients. *Transplant. Proc.* **37**, 4094-4096.
- KALPOE J.S., KROES A.C.M., DE JONG M.D., SCHINKEL J., DE BROUWER C.S., BEERSMA M.F.C., E.C.J. CLAAS. (2004). Validation of clinical application of Cytomegalovirus plasma DNA load measurement and definition of treatment criteria by analysis of correlation to antigen detection. *J. Clin. Microbiol.* **42**, 1498-1504.
- KOTTON C.N., KUMAR D., CALIENDO A.M., ÅSBERG A., CHOU S., SNYDMAN D.R., ALLEN U., HUMAR A., on behalf of The Transplantation Society International CMV Consensus Group. (2010). International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation. *Transplantation*. **89**, 779-795.
- KUSNE S, GROSSI P, IRISH W., ST GEORGE K., RINALDO C., RAKELA J., FUNG J. (1999). Cytomegalovirus pp65 antigenemia monitoring as a guide for preemptive

- therapy: a cost effective strategy for prevention of cytomegalovirus disease in adult liver transplant recipients. *Transplantation*. **68**, 1125-1131.
- LI H., DUMMER J.S., ESTES W.R., MENG S., WRIGHT P.F., TANG Y.W. (2003). Measurement of human Cytomegalovirus loads by quantitative real-time PCR for monitoring clinical intervention in transplant recipients. *J. Clin. Microbiol.* **41**, 187-191.
- Ljungman P., Griffiths P., Paya C. (2002). Definitions of Cytomegalovirus infection and disease in transplant recipients. *Clin. Infect. Dis.* **34**, 1094-1097.
- MADI N., AL-NAKIB W., MUSTAFA A.S., SAEED T., PACSA A., NAMPOORY M.R.N. (2007). Detection and monitoring of Cytomegalovirus infection in renal transplant patients by quantitative real-time PCR. *Med. Princ. Pract.* **16**, 268-273.
- MARTIN-DAVILA P., FORTUN J., GUTIERREZ C., MARTI-BELDA P., CANDELAS A., HONRUBIA A., BARCENA R., MARTINEZ A., PUENTE A., DE VICENTE E., MORENO S. (2005). Analysis of a quantitative PCR assay for CMV infection in liver transplant recipients: an intent to find the optimal cut-off value. *J. Clin. Virol.* **33**, 138-144.
- MAZZULLI T., RUBIN R.H., FERRARO M.J., D'AQUILA R.T., DOVEIKIS S.A., SMITH B.R., THE T.H., HIRSCH M.S. (1993). Cytomegalovirus antigenemia: clinical correlations in transplant recipients and in persons with AIDS. *J. Clin. Microbiol.* **31**, 2824-2827.
- MENGELLE C., SANDRES- SAUNÉ K., PASQUIER C., ROSTAING L., MANSUY J.-M., MARTY M., DA SILVA I., ATTAL M., MASSIP P., IZOPET J. (2003). Automated extraction and quantification of human Cytomegalovirus DNA in whole blood by real-time PCR assay. *J. Clin. Microbiol.* **41**, 3840-3845.
- MORI T., MORI S., KANDA Y., YAKUSHIJI K., MINEISHI S., TAKAUE Y., GONDO H., HARADA M., SAKAMAKI H., YAJIMA T., IWAO Y., HIBI T., OKAMOTO S. (2004). Clinical significance of cytomegalovirus (CMV) antigenemia in the prediction and diagnosis of CMV gastrointestinal disease after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* **33**, 431-434.
- NITSCHKE A., OSWALD O., STEUER N., SCHETELIG J., RADONIC A., THULKE S., SIEGERT W. (2003). Quantitative real-time PCR compared with pp65 antigen detection for Cytomegalovirus (CMV) in 1122 blood specimens from 77 patients after allogeneic stem cell transplantation: which test better predicts CMV disease development? *Clin. Chem.* **49**, 1683-1685.
- ONISHI Y., MORI S.I., HIGUCHI A., KIM S.W., FUKUDA T., HEIKE Y., TANOSAKI R., MINEMATSU T., TAKAUE Y., SASAKI T., FURUTA K. (2006). Early detection of plasma Cytomegalovirus DNA by real-time PCR after allogeneic hematopoietic stem cell transplantation. *Tohoku J. Exp. Med.* **210**, 125-135.
- RAZONABLE R.R., PAYA C.V., SMITH T.F. (2002a). Role of the laboratory in diagnosis and management of Cytomegalovirus infection in hematopoietic stem cell and solid-organ transplant recipients. *J. Clin. Microbiol.* **40**, 746-752.
- RAZONABLE R.R., BROWN R.A., WILSON J., GROETTUM C., KREMERS W., ESPY M., SMITH T.F., PAYA C.V. (2002b). The clinical use of various blood compartments for Cytomegalovirus (CMV) DNA quantitation in transplant recipients with CMV disease. *Transplantation*. **73**, 968-973.
- SANGHAVI S.K., ABU-ELMAGD K., KEIGHTLEY M.C., ST GEORGE K., LEWANDOWSKI K., BOES S.S., BULLOTTA A., DARE R., LASSAK M., HUSAIN S., KWAK E.J., PATERSON D.L., RINALDO C.R. (2008). Relationship of Cytomegalovirus load assessed by real-time PCR to pp65 antigenemia in organ transplant recipients. *J. Clin. Virol.* **42**, 335-342.
- SCHROEDER R., MICHELON T., FAGUNDES I., BORTOLOTTA A., LAMMERHIRT E., OLIVEIRA J., SANTOS A., BITAR A., KEITEL E., GARCIA V., NEUMANN J., SAITOVITCH D. (2005). Antigenemia for Cytomegalovirus in renal transplantation: choosing a cutoff for the diagnosis criteria in Cytomegalovirus disease. *Transplant. Proc.* **37**, 2781-2783.
- YAKUSHIJI K., GONDO H., KAMEZAKI K., SHIGEMATSU K., HAYASHI S., KUROIWA M., TANIGUCHI S., OHNO Y., TAKASE K., NUMATA A., AOKI K., KATO K., NAGAFUJI K., SHIMODA K., OKAMURA T., KINUKAWA N., KASUGA N., SATA M., HARADA M. (2002). Monitoring of Cytomegalovirus reactivation after allogeneic stem cell transplantation: comparison of an antigenemia assay and quantitative real-time polymerase chain reaction. *Bone Marrow Transplant.* **29**, 599-606.