JOURNAL OF CLINICAL MICROBIOLOGY, June 1994, p. 1614–1618
0095-1137/94/$04.00+0

Vol. 32, No. 6

Comparison of Nested PCR for Detection of DNA in Plasma with pp65 Leukocytic Antigenemia Procedure for Diagnosis of Human Cytomegalovirus Infection

FRANCOIS FREYMUTH,[1]* EVELYNE GENNETAY,[1] JOELLE PETITJEAN,[1] GENEVIEVE EUGENE,[1] BRUNO HURAULT DE LIGNY,[2] JEAN-PHILIPPE RYCKELYNCK,[2] CHRISTIAN LEGOFF,[2] PASCAL HAZERA,[3] AND CLAUDE BAZIN[3]

Virus Laboratory[1] and Departments of Nephrology[2] and Infectious Diseases,[3] University Hospital Caen, Caen, France

Received 18 October 1993/Returned for modification 6 January 1994/Accepted 24 February 1994

A nested PCR was used for the detection of human cytomegalovirus (HCMV) DNA in plasma. The presence of HCMV DNA and its correlation to pp65 leukocytic antigenemia were investigated with 299 blood samples from 45 organ transplant recipients and 63 AIDS patients. Of the 53 samples positive by nested PCR, 52 (98%) were also positive for leukocytic antigenemia and 23 had high levels of antigenemia (>50 positive cells per 2 × 10^5 leukocytes). Of the 246 samples negative in PCR, only 3 (1.2%) had highly positive antigenemia. For 15 patients having a high antigenemia level in the course of their disease, consecutive blood samples were studied and also assessed for viremia in culture. The extent to which HCMV DNA, detected by PCR, was present in plasma correlated with increased levels of HCMV leukocytic antigenemia for six of the eight AIDS patients and for all the organ transplant recipients. Positivity for HCMV DNA in PCR and for viremia in cell culture was usually restricted to the highest antigenemia levels. From a total of 69 blood samples, PCR and culture gave positive results, respectively, for 17 of 32 samples (53%) and 14 of 32 samples (43%) from transplant recipients and for 15 of 37 samples (40%) and 9 of 37 samples (24%) from AIDS patients. Our findings have shown a strong correlation between high levels of leukocytic antigenemia and HCMV DNA in plasma. The detection of HCMV DNA in plasma by this nested PCR can prove HCMV dissemination in blood, but it lacks the rapidity and simplicity of the leukocytic pp65 antigenemia procedure.

Human cytomegalovirus (HCMV) viremia is a marker of disseminated infection in immunosuppressed patients for whom the diagnosis of HCMV infections is clinically difficult. As polymorphonuclear leukocytes appear to be the major carriers of virus in blood (3), the procedures for the detection of HCMV viremia include viral isolation, direct viral antigen staining, and nucleic acid detection. The shell vial assay, faster than routine culture, is commonly used for the diagnosis of HCMV viremia (8, 13). The rapid immunologic detection of HCMV pp65 antigen in leukocytes is an early indicator of active HCMV infection (15, 21), and high antigenemia levels are usually related to symptomatic infections (5, 20, 21). Detection of HCMV DNA in leukocytes by PCR has also been proved to be an early indicator of active infection, more sensitive than culture or the presence of antigenemia (1, 7, 10, 17, 24). More recently there have been reports on the detection of HCMV DNA in plasma and serum (2, 9, 18, 25).

We investigated, by a nested PCR, the presence of HCMV DNA in 299 plasma samples from 109 patients (46 organ transplant recipients and 63 AIDS patients) and its correlation to pp65 leukocyte antigenemia. For 15 patients who in the course of their disease had antigenemia related to a disseminated infection (≥40 positive cells per 2 × 10^5 leukocytes) consecutive blood samples were tested, and a comparison with levels of viremia in leukocyte culture was also made. HCMV antigen detection was done in our laboratory (5) with minor modifications to the reported procedures (15, 22). Leukocytes from 10 ml of a fresh heparinized blood sample were separated by sedimentation on a 6% dextran 70,000 solution for 30 min at 37°C and then centrifuged. The plasma was immediately frozen at −70°C. The contaminating erythrocytes were lysed in an NH_4Cl solution (8 g/liter) at 4°C. The leukocytes were washed and were suspended in minimal basal medium–2% fetal calf serum at 2 × 10^6 cells per ml. Cytocentrifuge preparations were made with spots of 2 × 10^5 cells (Cytospin-2; Shandon, Runcorn, United Kingdom). After fixation for 10 min in cold methanol-acetone (2:1), the spots were stained by indirect immunofluorescence with the anti-HCMV monoclonal antibodies C-10 and C-11 (Clonab; Biotest, Dreieich, Germany) and a 1:20 solution of fluorescein-conjugated goat anti-mouse immunoglobulin (Clonab Ig FITC; Biotest) as the second antibody. The number of positive cells (those having a fluorescent nucleus) in 2 × 10^5 leukocytes was counted. For culture, 400 μl of the suspension of leukocytes was inoculated onto a monolayer of human embryonic lung fibroblasts (MRC5 cells; bioMérieux, Lyon, France) in 25-cm^2 plastic flasks, and HCMV was identified by its characteristic cytopathic effect. Nucleic acids were extracted from plasma as described previously for extraction from serum (8). Frozen plasma samples were centrifuged for 10 min at 3,000 rpm and were filtered through a 0.45-μm-pore-size filter membrane (Millex-HA; Millipore, Molsheim, France). Then 100 μl of plasma was added to 100 μl of the lysing buffer, containing (at final concentrations) 100 mM KCl, 20 mM Tris-HCl (pH 8.3), 5 mM $MgCl_2$, 0.2 mg of gelatin per ml, and 0.9% Tween solution. Proteinase K was added to a final concentration of 60 μg/ml, and the mixture was incubated for 60 min at 56°C. Proteinase K was then inactivated by heating for 10 min at 95°C; this was followed by centrifugation for 10 min at 12,000 rpm. PCR amplification was performed within the conserved region of the immediate-early (IE) gene. The outer primer set

* Corresponding author.

TABLE 1. HCMV DNA findings by nested PCR for 299 plasma samples from 46 transplant recipients and 63 AIDS patients with different levels of HCMV pp65 antigenemia

No. of CMV antigen-positive cells[a]	No. of plasma samples	N-PCR result (no. [%] of samples)	
		Positive[b]	Negative[b]
<1	156	1 (0.6)	155
1–10	79	12 (15.1)	67
11–49	38	17 (44.7)	21
≥50	26	23 (88.7)	3
Total	299	53	246

[a] Per 2×10^5 leukocytes.
[b] For HCMV DNA, as detected by nested PCR (N-PCR) product bands on ethidium bromide-stained gel.

(6) consisted of MG1, positions 2333 to 2352 (5′-AGAGTCTG CTCTCCTAGTGT-3′), and MG2, positions 2602 to 2621 (5′-CTATCTCAGACACTGGCTCA-3′). The inner primer set (17) consisted of IE1, upstream primer 5′-CCACCCGT GGTGCCAGCTCC-3′, and IE2, downstream primer 5′-CCCGCTCCTCCTGAGCACCC-3′. The sizes of the PCR products of the outer primers and the nested primers are 289 and 162 bp, respectively. The DNA of the 10 μl of plasma was amplified in a 100-μl reaction volume containing 1.25 U of *Taq* polymerase (Hybaid), each of the four deoxynucleoside triphosphates at 0.2 mM each, 0.5 μM (each) primers MG1 and MG2, and the buffer system: 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 0.01% (wt/vol) gelatin. The reaction was performed in an automated thermal cycler (Hybaid). The tubes were preheated for 10 min at 94°C before 40 incubation cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C and, at the end, 8 min at 72°C. A 4-μl portion of the first amplification product diluted 80 times was amplified in a 50-μl new reaction mixture with *Taq* polymerase, the 4 deoxynucleoside triphosphates, the inner primers (IE1 and IE2) at 0.4 μM each, and the buffer system. Thirty incubation cycles of 1 min each at 94°C, 55°C, and 72°C were performed. The final PCR products were analyzed by electrophoresis on agarose gel stained with ethidium bromide on a UV light box. Contamination was rigidly controlled as recommended by performing plasma preparations and preparing PCR products in separate laboratory rooms (12). All plasma preparations and reactions were performed with known negative and positive controls. Each blood sample was tested in duplicate. When discrepancies were observed between the results of the duplicates, plasma samples were subsequently retested twice. To evaluate the presence of contaminating cellular DNA we amplified the β-globin gene in 25 plasma samples positive for antigenemia (16).

To prepare HCMV DNA from plasma we used the technique described by Ishigaki et al., which is simple, rapid, and without any procedure of phenol-chloroform extraction (9). A light amplification of β-globin DNA was observed in 18 of the 25 plasma specimens; 9 of them were HCMV DNA positive by PCR, and 9 were negative. Thus, this technique cannot eliminate all debris from lysed cells in plasma. Of the 299 blood samples tested by this technique, 143 had HCMV antigenemia. Thus this technique appeared to be more sensitive than PCR for HCMV DNA detection, which gave positive results for 53 of 299 plasma samples (Table 1). Of the 53 samples positive by PCR, 52 (98%) had corresponding antigenemia. The HCMV DNA in plasma correlated with the numbers of HCMV antigen-positive leukocytes, as seen in a linear progression between four groups of antigenemia levels (<1 cell, 1 to 10 cells, 11 to 49 cells, and >50 cells positive per 2×10^5 polymorphonuclear leukocytes) and the percentages of HCMV DNA-positive plasma, respectively: 0.6%, 1; 15.1%, 12; 44.7%, 17; and 88%, 23. These findings showed a strong correlation between high levels of antigenemia in leukocytes and HCMV DNA in plasma. Of the 246 samples negative by PCR, 3 (1.2%) had highly positive leukocytic antigenemia. In these cases, either HCMV DNA was not present or the PCR result was a false negative. For the 53 positive PCR products, a 289-bp specific fragment was already apparent after the first amplification in 61% of the specimens. This could indicate a distinctive amount of HCMV DNA in plasma samples.

During early HCMV infection few antigen-positive cells are present in blood. The number rapidly increases in patients developing HCMV disease and then decreases after recovery (5, 20, 21). Eight of the 63 AIDS patients and 7 out of the 46 organ transplant recipients had high levels of antigenemia, ≥40 positive cells per 2×10^5 leukocytes. In the AIDS

TABLE 2. Comparison of nested PCR, leukocytic pp65 antigenemia procedure, and culture for eight AIDS patients with high antigenemia levels

Patient no.	Disease	Wk	No. of HCMV antigen-positive cells[a]	Culture result	DNA N-PCR result[b]
01		0	0	0	0
		8	2	0	0
	Interstitial pneumonia[c]	19	63	+	+
		20	10	0	+
		21	6	0	+
02		0	12	0	+
		4	1	0	0
	Retinitis	12	19	0	+
		13	50	+	+
		14	5	0	0
		18	55	+	0
		22	7	+	0
03	Retinitis	0	>50	+	+
		4	2	0	0
		11	15	0	0
	Relapse[d]	20	200	0	+
		21	67	0	0
		22	8	0	0
04		0	2	0	0
		4	4	+	0
	Pneumonitis, hepatitis[d]	8	52	0	+
		11	200	0	+
		12	20	0	+
05		0	1	0	0
		2	0	0	+
	Polynephritis[e]	3	4	0	+
		4	>50	0	+
		6	41	+	0
		8	0	0	0
06		0	0	0	0
		8	2	0	0
	Esophagitis	12	42	+	0
		16	1	0	0
07		0	0	0	0
	Myocarditis[e]	12	43	+	+
08		0	14	0	0
	Encephalitis[e]	5	>50	0	0

[a] Per 2×10^5 leukocytes.
[b] For HCMV DNA in plasma by nested PCR (N-PCR).
[c] *Pneumocystis carinii*.
[d] Relapse under ganciclovir treatment.
[e] Association with HCMV not determined.

TABLE 3. Comparison of nested PCR, leukocytic pp65 antigenemia procedure, and culture for seven organ transplant recipients with high antigenemia levels

Patient no.	Transplanted organ	Clinical status, disease, or symptom	Wk	No. of HCMV antigen-positive cells[a]	Culture result	DNA N-PCR result[b]
10	Kidney	Asymptomatic	0	4	0	0
			2	11	0	0
			3	48	+	+
			5	9	0	0
11	Liver	Hepatitis[c]	0	500	0	+
			1	115	0	+
			2	50	0	0
			3	2	0	0
12	Liver		0	0	0	0
		Hepatitis	2	20	+	+
			3	12	0	+
			5	16	0	0
		Relapse	6	220	+	+
			7	58	+	+
			8	22	0	+
			9	7	0	0
			13	1	0	0
13	Kidney		0	10	+	+
		Fever	1	72	+	+
			2	9	0	+
14	Kidney		0	0	0	0
			4	16	0	0
		Thrombocytopenia	5	28	+	+
			7	159	+	+
			8	1	0	0
15	Kidney	Asymptomatic	0	0	+	0
			2	10	+	0
			3	6	+	+
			4	98	+	+
16	Kidney	Asymptomatic	0	400	+	+
			1	400	+	+
			2	ND[d]	0	0

[a] Per 2×10^5 leukocytes.
[b] For HCMV DNA in plasma by nested PCR (N-PCR).
[c] Ganciclovir treatment.
[d] Not done.

patients, the correlation between increasing levels of antigenemia and a specific HCMV organ disease was confirmed in only four cases (Table 2). Among the seven transplant recipients, the association was found in only three (Table 3). However, two kidney recipients were monitored for only 2 weeks, and later development of disease cannot be excluded. If a high antigenemia level reflects an intensive HCMV infection, one should observe an HCMV load in the systemic circulation. The presence of increased HCMV DNA in plasma correlated with increased levels of HCMV leukocytic antigenemia in six of the eight AIDS patients and in all the organ recipients. In most cases the detection of HCMV DNA in plasma was restricted to the highest antigenemia levels. However, for some patients (no. 2, 5, and 12 to 15) HCMV DNA in plasma was detected 1 or 2 weeks before the peak of antigenemia. For four patients (no. 1, 4, 12, and 13) the PCR result remained positive with a low antigenemia level. On the other hand, two AIDS patients (no. 6 and 8) had a negative PCR result in spite of high antigenemia levels: 42 and >50 positive cells, respectively. Thus, nested PCR has the capability to detect HCMV DNA in plasma of blood samples from patients with high levels of HCMV pp65 leukocytic antigenemia. For organ transplant recipients, two studies reported a correlation between an active HCMV infection, the presence of HCMV DNA in serum or plasma samples, and a positive virus isolation from leukocytes (2, 23). HCMV DNA was detected at an early stage of HCMV infection, was confirmed after a median interval of 1 week, and remained positive longer than did samples isolated in culture. It was also recently reported for kidney transplant patients that PCR of HCMV from plasma samples had higher sensitivity (68%) than detection of CMV-pp65 leukocytic antigenemia (55%) but did not discriminate as well between clinical HCMV infection and asymptomatic infection (25).

For the seven organ transplant recipients, positive viremia in culture was associated with the presence of HCMV DNA and with high antigenemia levels, except for with one patient (no. 11) who had ganciclovir treatment. For AIDS patients there are more discordant results between culture, HCMV DNA detection, and antigenemia. Samples from two treated patients (no. 3 and 4) were negative for HCMV viremia. The viremia preceded the detection of HCMV DNA for one patient (no. 4) and followed it for two others (no. 2 and 5). For a total of 69 blood samples from these two groups of patients, PCR for HCMV detection in plasma appeared more sensitive than culture for detection of viremia: PCR and culture gave positive results, respectively, for 17 of 32 samples (53%) and 14 of 32 samples (43%) from transplant recipients and for 15 of 37 samples (40%) and 9 of 37 samples (24%) from AIDS patients. However, the conventional culture used in this study was reported to be less sensitive than the shell vial assay (5). A

recent study has shown that infectious HCMV was present in two of eleven 1-ml samples of filtered plasma positive by PCR for HCMV DNA (18). Low or nonexistent titers of infectious HCMV in some plasma samples can indicate that cell-free viruses are infectious in the plasma for a short time because they are labile or neutralized by specific antibodies, or that plasma might contain more defective interfering particles than infectious virions. It has also been reported that defective virions contain incomplete, defective HCMV DNA (19) and that PCR is capable of detecting the DNA of these defective virions (4).

The specificity of the PCR used in this study is achieved by the use of a specific set of internal primers, which reacts with a large number of HCMV strains and does not cross-react with the DNA of other herpesviruses or with human cellular DNA (17), and by direct identification, by their size, of the first and second amplified products after ethidium bromide-stained agarose gel electrophoresis (11). It has been reported that nested PCR successfully eliminates the high background levels detected by oligomeric hybridization and overcomes false-positive reactions caused by fragmented DNA (14). This technique has been chosen for its simplicity and rapidity over a single PCR with detection by a labeled probe, which should provide a similar level of sensitivity. As reported previously, the detection sensitivity of these primers used in a single PCR reached 10 virions (17), or 10 fg of HCMV DNA (9). The nested PCR used in this study has been shown to be more sensitive: five copies (1 copy per 30,000 cells) (6). In a high proportion of our plasma samples (18 of 25) we observed the presence of cellular DNA by amplification of the β-globin gene, which proved that HCMV DNA detected in plasma could be released from lysed cells. But Spector et al., using another technique, failed to detect DNA of the HLA-DQα gene in filtered (pore size, 0.2 μm) plasma specimens and suggested that HCMV DNA in plasma was supported by cell-free virus (18). Since many of the progeny HCMV virions are attached to cell fragments, this point is difficult to resolve; more extended studies are necessary for an accurate understanding.

In summary, the findings presented here showed a strong relation between high pp65 antigenemia levels in leukocytes and HCMV DNA in plasma, suggesting that in most disseminated infections with or without HCMV disease in a specific organ(s), virus particles are released from the infected cells into the plasma. Further studies with immunosuppressed patients are necessary to evaluate the precise sensitivities and specificities of these two techniques and to obtain comparative correlations between HCMV infection and disease. The detection of HCMV DNA in plasma by nested PCR is reliable for proving intensive HCMV dissemination in blood, but nested PCR lacks the rapidity and simplicity of the leukocytic pp65 antigenemia procedure.

This work was supported in part by a grant from the Agence Nationale de Recherche sur le SIDA, Action Coordonnée No. 11.

REFERENCES

1. **Boland, G. J., R. A. de Wegler, M. G. J. Tilanus, C. Ververs, K. Bosboom-Kalsbeek, and G. C. de Gast.** 1992. Detection of cytomegalovirus (CMV) in granulocytes by polymerase chain reaction compared with the CMV antigen test. J. Clin. Microbiol. **30:**1763–1767.
2. **Brytting, M., W. Xu, B. Wahren, and V. A. Sundqvist.** 1992. Cytomegalovirus DNA detection in sera from patients with active cytomegalovirus infections. J. Clin. Microbiol. **30:**1937–1941.
3. **Dankner, W. N., J. A. McCutchan, D. D. Richman, K. Hirata, and S. A. Spector.** 1990. Localization of human cytomegalovirus in peripheral blood leukocytes by in situ hybridization. J. Infect. Dis. **161:**31–36.
4. **DeRossi, A., A. Amadori, L. Chieco-Binachi, C. Giacquinto, F. Zacchello, A. Buchbinder, F. Wong-Staal, R. C. Gallo, and C. S. Peckham.** 1988. Polymerase chain reaction for early diagnosis of pediatric HIV infection. Lancet **ii:**278.
5. **Freymuth, F., J. Petitjean, B. Hurault de Ligny, X. Troussard, J. P. Ryckelynck, M. Leporrier, J. J. Ballet, and F. Galateau.** 1992. Antigène cytomegalique leucocytaire. Nouvelle méthode diagnostique de l'infection à cytomegalovirus après transplantation. Presse Med. **21:**1710–1713.
6. **Georgescu, M. M., P. Morand, F. Sarrot-Reynauld, P. Innocenti, and J. M. Seigneurin.** 1992. Une nouvelle technique de diagnostic des infections actives à cytomegalovirus: la "nested-polymerase-chain-reaction." Rev. Med. Intern. **13:**S304.
7. **Gerna, G., D. Zipeto, M. Parea, M. G. Revello, E. Silini, E. Percivalle, M. Zvattoni, P. Grossi, and G. Milanesi.** 1991. Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant recipients by determination of viremia, antigenemia, and DNAemia. J. Infect. Dis. **164:**488–498.
8. **Gleaves, C. A., T. F. Smith, E. A. Shuster, and G. R. Pearson.** 1984. Rapid detection of cytomegalovirus in MRC-5 cells inoculated with urine specimens by using low-speed centrifugation and monoclonal antibody to an early antigen. J. Clin. Microbiol. **19:**917–919.
9. **Ishigaki, S., M. Takeda, T. Kura, N. Ban, T. Saitoh, S. Sakamaki, N. Watanabe, Y. Kohgo, and Y. Niitsu.** 1991. Cytomegalovirus DNA in the sera of patients with cytomegalovirus pneumonia. Br. J. Haematol. **79:**198–204.
10. **Jiwa, N. M., G. W. van Gemert, A. K. Raap, F. M. van de Rijke, A. Mulder, P. F. Lens, M. M. M. Salimans, F. E. Zwaan, W. van Dorp, and M. van der Ploeg.** 1989. Rapid detection of human cytomegalovirus DNA in peripheral blood leucocytes of viremic transplant patients by the polymerase chain reaction. Transplantation **48:**72–76.
11. **Kaneko, S., S. M. Feinstone, and R. H. Miller.** 1989. Rapid and sensitive method for the detection of serum hepatitis B virus DNA using the polymerase chain reaction technique. J. Clin. Microbiol. **27:**1930–1933.
12. **Kwok, S. Q., and R. Higushi.** 1989. Avoiding false positives with PCR. Nature (London) **239:**237–238.
13. **Mazeron, M. C., T. Berbar, M. C. Guillemin, R. Colimon, A. Roseto, and Y. Perol.** 1983. Production d'anticorps monoclonaux contre le cytomegalovirus humain. C. R. Acad. Sci. Paris Sér. III **297:**305–308.
14. **Porter-Jordan, K., E. I. Rosenberg, J. F. Keiser, J. D. Gross, A. M. Ross, S. Nasim, and C. T. Garrett.** 1990. Nested polymerase chain reaction for the detection of cytomegalovirus overcomes false positives caused by contamination with fragmented DNA. J. Med. Virol. **30:**85–91.
15. **Revello, M. G., E. Percivalle, M. Zavattoni, M. Parea, P. Grossi, and G. Gerna.** 1989. Detection of human cytomegalovirus immediate early antigen in leukocytes as a marker of viremia in immunocompromised patients. J. Med. Virol. **29:**88–93.
16. **Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim.** 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230:**1350–1354.
17. **Shibata, D., W. J. Martin, M. D. Appleman, D. M. Causey, J. M. Leedom, and N. Arnheim.** 1988. Detection of cytomegalovirus DNA in peripheral blood of patients infected with human immunodeficiency virus. J. Infect. Dis. **158:**1185–1192.
18. **Spector, S. A., R. Merrill, D. Wolf, and W. M. Dankner.** 1992. Detection of human cytomegalovirus in plasma of AIDS patients during acute visceral disease by DNA amplification. J. Clin. Microbiol. **30:**2359–2365.
19. **Stinski, M. F., E. S. Mocarski, and D. R. Thomsen.** 1977. DNA of human cytomegalovirus: size heterogeneity and defectiveness resulting from serial undiluted passages. J. Virol. **31:**231–239.
20. **The, T. H., M. van der Ploeg, A. P. van der Berg, A. M. Vlieger, M. van der Giessen, and W. J. van Son.** 1992. Direct detection of cytomegalovirus in peripheral blood leucocytes. A review of the

antigenemia and polymerase chain reaction. Transplantation **54:** 193–198.

21. **van den Berg, A., W. van der Bij, W. J. van Son, J. Anema, M. van der Giessen, J. Schirm, A. M. Tegzess, and T. H. The.** 1989. Cytomegalovirus antigenemia as a useful marker of symptomatic cytomegalovirus infection after renal transplantation: a report of 130 consecutive patients. Transplantation **48:**991–995.
22. **Van der Bij, W., J. Schirm, R. Torensma, W. J. van Son, A. M. Tegzess, and T. H. The.** 1988. Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. J. Clin. Microbiol. **26:**2531–2535.
23. **Wolf, D. G., and S. A. Spector.** 1993. Early diagnosis of human cytomegalovirus disease in transplant recipients by DNA amplification in plasma. Transplantation **56:**330–334.
24. **Zipeto, D., M. G. Revello, E. Silini, M. Parea, E. Percivalle, M. Zavattoni, G. Milanesi, and G. Gerna.** 1992. Development and clinical significance of a diagnostic assay based on the polymerase chain reaction for detection of human cytomegalovirus DNA in blood samples from immunocompromised patients. J. Clin. Microbiol. **30:**527–530.
25. **Zweygberg Wirgart, B., L. Claesson, B. M. Eriksson, G. Tufvesson, T. Tottermen, and L. Grillner.** 1993. CMV DNA amplification from plasma and CMV pp65 antigen detection in leucocytes for early diagnosis of CMV infection in kidney transplanted patients. Presented at the IXth International Congress of Virology, Glasgow, Scotland, 8 to 13 August 1993.