JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1996, p. 2959–2962 Vol. 34, No. 12
0095-1137/96/$04.00+0

Evaluation of the Digene Hybrid Capture System for Detection and Quantitation of Human Cytomegalovirus Viremia in Human Immunodeficiency Virus-Infected Patients

TONY MAZZULLI,[1,2,3]* SERENA WOOD,[3] ROBERT CHUA,[3] AND SHARON WALMSLEY[2,4]

Departments of Microbiology[1] and Medicine,[2] University of Toronto, Mount Sinai and Princess Margaret Hospitals,[3] and The Toronto Hospital,[4] Toronto, Ontario, Canada

Received 23 July 1996/Returned for modification 14 August 1996/Accepted 3 September 1996

The Digene Hybrid Capture System (DHCS) is a solution hybridization antibody capture assay for the chemiluminescent detection and quantitation of cytomegalovirus (CMV) DNA in leukocytes. This assay was compared with the CMV antigenemia assay and shell vial and tube cultures for the detection of CMV in 234 blood specimens from 72 patients with human immunodeficiency virus. Intra- and interrun precision of the DHCS assay gave coefficients of variation of 17.8 and 16.3%, respectively. The correlation coefficient for the quantitative results obtained by the DHCS assay and the antigenemia assay was 0.911 (95% confidence interval, 0.885 to 0.930). Agreement between the DHCS assay and the other three assays ranged from 83 to 86%. The DHCS assay detected 71, 87, and 84% of specimens that were positive by antigenemia, shell vial cultures, and tube culture, respectively. A total of 92% of specimens that were positive by the DHCS assay were also positive by at least one of the other assays. Evaluation of the usefulness of quantitation of CMV DNA by using the DHCS assay and its correlation with clinical disease demonstrated that, with some exceptions, patients with clinical CMV disease tended to have high levels of DNA whereas asymptomatic patients tended to have low or undetectable levels. Overall, the DHCS assay provided a rapid, quantitative, and objective measure of CMV activity in leukocytes, but results did not always correlate with clinical disease.

Infection with cytomegalovirus (CMV) is common. In healthy, nonimmunocompromised individuals, CMV infection tends to be mild or asymptomatic. However, in patients with suppression of their cell-mediated immunity, such as those with advanced human immunodeficiency virus (HIV) infection, CMV can reactivate and cause significant life- or sight-threatening disease. Approximately 25 to 40% of persons living with HIV and CD4 cell counts of $\leq 50 \times 10^6$/liter will develop CMV disease, and up to 90% will have CMV detected at autopsy (3, 7, 13).

Asymptomatic excretion of CMV in urine, saliva, and other body fluids occurs commonly in HIV-infected individuals, and thus the detection of CMV in these specimen types may not be clinically relevant. On the other hand, the demonstration of CMV viremia, which may be a marker of disseminated infection, correlates best with significant CMV disease (8, 19). Conventional laboratory culture methods for the detection of CMV viremia have had generally low sensitivity and often require days to weeks before a result is obtained. Recently, emphasis has been placed on the development of newer, rapid, and more sensitive diagnostic techniques. Assays such as the antigenemia assay, PCR, and DNA hybridization assays have not only proven to be more rapid and sensitive than conventional culture techniques but have also provided a means of quantitating the amount of activity of CMV in blood (2, 6, 10, 12, 14, 18, 20, 22–25, 27). These assays may be predictive of disease and may help guide preemptive therapy or management of relapsed disease.

We and others have previously reported on the use of the antigenemia assay and PCR in HIV-infected patients (1, 4, 11, 14). Recently, a new commercially available hybridization assay has been developed, the Digene Hybrid Capture System (DHCS) assay (Digene Diagnostics Inc., Silver Spring, Md.), but has not been fully evaluated. This assay is a solution hybridization assay for the quantitative detection of CMV DNA in leukocytes. Unlike PCR, this assay detects CMV DNA directly and does not require an amplification step. In this study, we compared the DHCS assay with the antigenemia assay and conventional culture techniques for the detection and quantitation of CMV in blood samples of HIV-infected persons.

(This work was presented in part at the 3rd Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1996.)

MATERIALS AND METHODS

Specimens. Blood samples were collected for the detection of CMV viremia by the DHCS assay, the antigenemia assay, and shell vial and tube cultures from HIV-infected patients with CD4 cell counts of $\leq 50 \times 10^6$/liter who had no prior history of clinical CMV disease. Only patients who developed confirmed clinical CMV disease during the study received CMV-specific therapy. Confirmed clinical CMV disease was defined by using the criteria described by Spector et al. (21). Briefly, CMV retinitis was diagnosed by ophthalmologists experienced in the diagnosis of this condition. CMV gastrointestinal disease was confirmed by endoscopy and biopsy. CMV pneumonia was confirmed by lung biopsy. Blood for the CMV antigenemia assay and shell vial and conventional cultures was collected in heparinized tubes. Blood for the DHCS assay was collected in EDTA tubes. All blood specimens were processed within 4 h of collection.

CMV antigenemia assay. This assay was performed according to the method of Gerna et al. (9) with only slight modification. Peripheral blood mononuclear leukocytes (PMNL) were separated from 5 ml of heparinized blood by using 1 ml of a 6% dextran solution. Contaminating erythrocytes were lysed by using a 0.8% NH_4Cl solution, and the PMNL were washed and resuspended in phosphate-buffered saline to yield 1.5×10^6 cells per ml. Duplicate cytospin slides were prepared by using 100 μl of the PMNL suspension per slide and a cytocentrifuge (Cytospin-3; Shandon Scientific, Pittsburgh, Pa.). Slides were air dried and then fixed in 5% formaldehyde and permeabilized with 0.5% Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.). Slides were stained by an indirect immunofluorescence technique by using mouse monoclonal antibodies directed against the pp65 antigen (Clonab CMV; Biotest AG, Dreieich, Germany) of CMV and

* Corresponding author. Mailing address: Department of Microbiology, Rm. 1485, 600 University Ave., Toronto, Ontario M5G 1X5, Canada. Phone: (416) 586-4695. Fax: (416) 586-8746.

fluorescein isothiocyanate-labelled goat anti-mouse monoclonal antibodies (Baxter, West Sacramento, Calif.). The number of antigen-positive cells was counted and expressed as the number of positive cells per 100,000 cells examined.

DHCS assay. The assay kits were provided by the manufacturer, and the assay was carried out according to the manufacturer's instructions. Briefly, 3.5 ml of whole blood collected in an EDTA tube was lysed by using the manufacturer's lysis solution. The leukocytes were removed and transferred to hybridization tubes, pelleted, and then stored at −70°C until processing. Samples were denatured by using 50 μl of denaturing reagent (30 min at 70°C) and then transferred to fresh hybridization tubes and incubated for an additional 30 min. CMV probe (unlabelled RNA) was added, and the tubes were incubated for 2 h at 70°C. The contents of the hybridization tubes were then transferred to corresponding capture tubes coated with anti-RNA–DNA antibodies and placed on a rotary shaker for 30 min at 1,100 rpm. An alkaline phosphatase-conjugated antibody specific for RNA-DNA hybrids was then added, and the tubes were incubated for 30 min at room temperature. After the tubes were washed, substrate was added and the tubes were incubated. The tubes were subsequently read with the use of a luminometer. Quantitation of CMV DNA was obtained by comparing the relative light units (RLU) of specimens to the RLU of a calibration curve of standards and expressed as picograms per milliliter. For qualitative assessment, all specimens with a ratio of sample RLU to positive control RLU of ≥0.75 were considered positive for CMV DNA. Inter- and intra-assay variabilities were evaluated by using the manufacturer's positive control. Interassay variability was assessed by using the same positive control in duplicate in eight different assay runs. Intra-assay variability was assessed by testing the same positive control 20 times in the same assay run. Percent coefficients of variation for inter- and intra-assay results were calculated as previously described (17).

Conventional tube and shell vial cultures. Aliquots of 0.2 ml of the PMNL suspension prepared for the antigenemia assay were inoculated into two shell vials containing MRC-5 cells and one tube culture containing human foreskin fibroblasts. Shell vials were centrifuged at 3,500 × *g* for 15 min (5) and subsequently incubated at 37°C in 5% CO_2. Shell vials were stained for CMV at 24 and 48 h by using an indirect immunofluoresence technique and monoclonal antibodies directed to CMV immediate-early antigens (Bartels Diagnostic Division, Issaquah, Wash.). Conventional tube cultures were incubated at 37°C in 5% CO_2 for up to 4 weeks and examined twice weekly for the development of the characteristic CMV cytopathic effect. The presence of CMV was confirmed by using a direct immunofluorescence technique with fluorescein isothiocyanate-labelled CMV early and late monoclonal antibodies (Bartels Diagnostic Division).

Statistical analysis. Agreement (percent) between the DHCS assay and each of the other assays for the qualitative detection of CMV was calculated as follows:

$$\text{Agreement (\%)} = \frac{\text{Concordant positives} + \text{Concordant negatives}}{\text{Total number of specimens tested}} \times 100$$

Regression analysis and calculation of the correlation coefficient for the comparison of the quantitative results obtained by the DHCS assay and the antigenemia assay were carried out following standard methods (15, 16).

RESULTS

Two hundred thirty-four specimens from 72 HIV-infected patients were evaluated. Intra- and interrun precision of the DHCS assay by using the manufacturer's positive control gave coefficients of variation of 17.8 and 16.3%, respectively. Table 1 shows the results of the DHCS and antigenemia assays and the shell vial and tube cultures for CMV. The percent agreement (positive and negative) between the DHCS assay and each of the other three assays ranged from 83 to 86% (Table 1).

Of the 234 specimens tested, 73 were positive by the DHCS assay and were collected from 22 patients. Twelve of these 22 patients who had at least one positive sample by the DHCS assay developed confirmed clinical CMV disease (10 retinitis, 1 colitis, and 1 pneumonitis). All but 6 of the 73 specimens that were positive by the DHCS assay were also positive by at least one other assay. These six specimens were from six different patients, all of whom developed clinical CMV disease within 1 to 4 months following the specimen that was positive by the DHCS assay alone.

The mean peak titer of CMV DNA, as measured by the DHCS assay, was significantly greater ($P < 0.01$, Mann-Whitney U test) in the 12 patients who developed clinical CMV disease compared with the 10 patients who did not (176.5 pg/ml versus 33.1 pg/ml, respectively). At least one specimen was available for testing in 6 of the 12 patients following the start of ganciclovir therapy. CMV DNA levels decreased to undetectable levels in all six patients within one month of starting therapy. This correlated with each patient's clinical response.

TABLE 1. Comparison of the DHCS assay with the antigenemia assay and shell vial and tube cultures for the detection of CMV in 234 blood specimens from 72 HIV-infected patients

Assay and result	*n*	DHCS assay result		% Agreement[a]
		Positive	Negative	
Antigenemia				
Positive	94	67	27	
Negative	140	6	134	86
Shell vial				
Positive	47	41	6	
Negative	187	32	155	84
Tube culture				
Positive	50	42	8	
Negative	184	31	153	83

[a] Agreement (positives and negatives) between DHCS and each of the other three assays.

Of the remaining 50 patients who did not have a positive specimen for CMV DNA by the DHCS assay, 2 patients developed CMV retinitis. Only one specimen was available for each of these two patients. Both of these specimens had only one positive antigen cell per 10^5 cells examined by using the antigenemia assay.

Table 2 separates specimens based on the level of antigenemia and compares the ability of the DHCS assay and culture techniques to detect CMV at each level of antigenemia. The DHCS assay detected all specimens with an antigenemia level of ≥21 positive cells per 10^5 cells, whereas shell vial and tube culture detected only 59 and 74% of such specimens, respectively. For specimens with an antigenemia level between 1 and 20 positive cells per 10^5 cells, the DHCS assay detected 28 of 55 (51%) while shell vial and tube culture detected 44 and 33%, respectively.

Figure 1 shows the correlation between the DHCS assay and the antigenemia assay for the quantitative detection of CMV viremia. The graph is a log-log plot. The correlation coefficient for these two assays was 0.911 (95% confidence interval, 0.885 to 0.930; $P < 0.01$).

DISCUSSION

The importance of CMV as a major cause of morbidity and mortality in HIV-infected persons is well recognized. Although effective therapy is available for the treatment of CMV disease,

TABLE 2. Comparison of DHCS with CMV antigenemia assay and shell vial and tube cultures for the detection of CMV in 234 peripheral blood samples according to level of antigenemia

CMV antigenemia level [mean (range) positive PMNL/10^5]	*n*	Culture results (positive/tested)		DHCS results (positive/tested)
		Shell vial	Tube culture	
Negative	140	0/140	3/140	6/140
4 (1 to 10)	41	16/41	11/41	17/41
16 (11 to 20)	14	8/14	7/14	11/14
29 (21 to 50)	16	6/16	10/16	16/16
229 (>50)	23	17/23	19/23	23/23

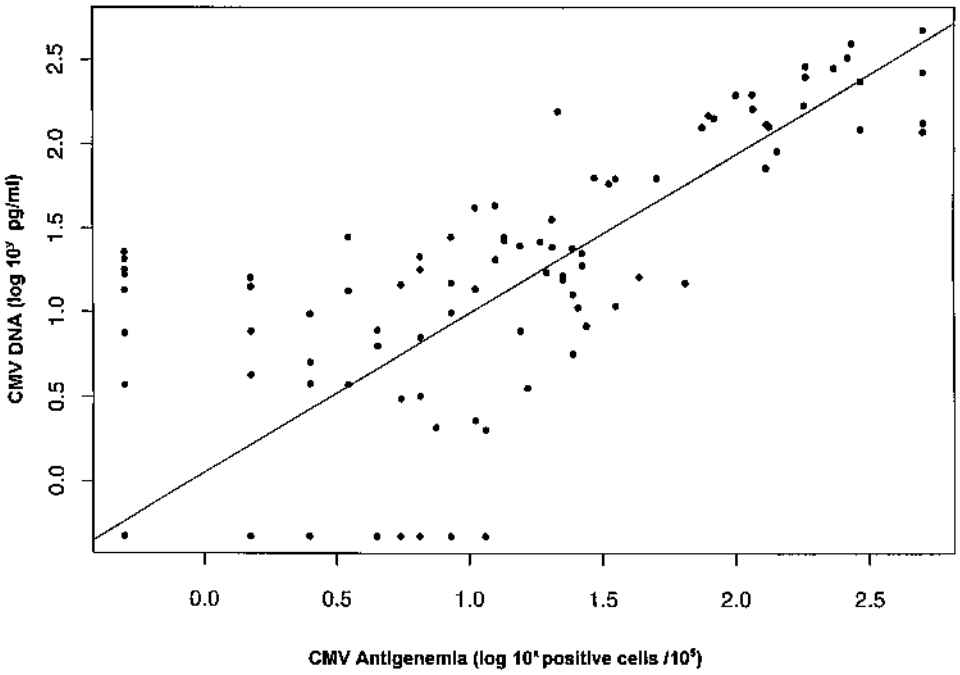

FIG. 1. Quantitative correlation of CMV DNA (pg/ml) as measured by the DHCS and CMV antigenemia assays. The line represents the result of a regression analysis. The correlation coefficient was 0.911.

emphasis has been placed on the development of rapid and sensitive laboratory assays that could be used to confirm a clinical suspicion of CMV disease. As well, there has been considerable interest in the development of quantitative assays for the measurement of viral load that could be used to predict which patients may develop end organ CMV disease and that could be used to monitor a patient's response to therapy once disease has been established. The DHCS assay, a new solution hybridization assay, is a rapid and sensitive assay that can quantitate the amount of CMV DNA present in leukocytes. Our results suggest that it is more sensitive than either shell vial or tube culture for the detection of CMV viremia. Although the DHCS assay appeared to be somewhat less sensitive than the antigenemia assay, it appeared to miss only those samples with relatively low levels of antigenemia (<20 positive cells per 10^5 cells).

The DHCS assay did detect CMV DNA in six samples from six different patients that were negative by all other assays used in this study. Review of the clinical data for these six patients showed that all six had positive samples for CMV by culture, antigenemia, and the DHCS assay within 1 month of the sample that was positive by the DHCS assay alone. All six patients developed clinical CMV disease 1 to 4 months following the positive sample. This suggests that these were not false-positive DHCS assay results but rather that the other assays gave false-negative results.

The inter- and intrarun variabilities of the DHCS assay of 16.3 and 17.8%, respectively, by using the manufacturer's positive control are relatively low compared with quantitative assays using PCR, which have reported variabilities of 30% or higher (26). The variability of the antigenemia assay has not been fully evaluated. Because quantitative assays may be used to monitor patients over time, relatively low coefficients of variation are required so that one can distinguish between assay variability and clinically significant changes in viral load or activity. Further evaluation of the DHCS assay is required to determine if similarly low coefficients of variation can be demonstrated by using clinical specimens and between different laboratories.

Figure 1 demonstrates a significant correlation between quantitative CMV antigen and CMV DNA as measured by the antigenemia assay and the DHCS assay, respectively. The strength of the relationship between these two assays is supported by the relatively high correlation coefficient of 0.911, which is statistically significant ($P < 0.01$) (15). Several studies have evaluated the usefulness of CMV quantitation by using the antigenemia assay for detecting clinically significant CMV disease and for monitoring response to antiviral therapy (2, 8, 12, 14). In general, with some exceptions, high-level antigenemia correlates with clinical disease, whereas low-level antigenemia is usually asymptomatic. The strong correlation between CMV antigen and DNA levels demonstrated in Fig. 1 suggests that the quantitative DHCS assay, like the antigenemia assay, can be used to measure the amount or activity of CMV in blood. This is also supported by our clinical data which showed that patients with high levels of CMV DNA as measured by the DHCS assay had clinical CMV disease whereas those with low or undetectable levels tended to be asymptomatic. Like the antigenemia assay, however, there were exceptions and low or negative CMV DNA did not exclude the possibility of CMV disease. Further study is needed to determine why some patients develop clinical CMV disease despite low or negative CMV antigen and DNA levels.

In summary, the DHCS assay appears to be a sensitive and specific assay for the detection and quantitation of CMV DNA in leukocytes. Whole blood samples to be tested by the DHCS assay can be held for up to 6 days at 4 to 8°C before processing. This provides an advantage over the antigenemia assay, for which it has generally been recommended that samples be processed within 4 h of collection (12). The assay allows for batch testing of up to 48 samples per run and provides results within 6 to 8 h. To obtain quantitative results, several standards

must be included in each test run so that a calibration curve can be generated. This may make the testing of small numbers of samples per run less attractive and more costly than batch testing, thus making this assay best suited for large-volume laboratories. Although the DHCS assay did not detect as many low positives as the pp65 antigenemia assay, it did provide an objective measure of the amount of CMV DNA present in leukocytes with good reproducibility. The strong correlation between CMV antigen and DNA levels suggests that the quantitative DHCS assay can provide information similar to that of the antigenemia assay and that high levels of CMV DNA likely reflect clinical CMV disease and low levels likely reflect asymptomatic infection.

ACKNOWLEDGMENTS

This work was supported by a grant from the Ontario Ministry of Health.

We thank Anna Smargiasso for preparation of the manuscript, Denise Cosani and Jennifer Clark-Diprata for the collection of patient samples, and Keith O'Rourke for statistical analysis.

REFERENCES

1. **Bek, B., M. Boeckh, J. Lepenies, B. Bieniek, K. Arasteh, W. Heise, K.-M. Deppermann, G. Bornhoft, M. Stoffler-Meilicke, I. Schuller, and G. Hoffken.** 1996. High-level sensitivity of quantitative pp65 cytomegalovirus (CMV) antigenemia assay for diagnosis of CMV disease in AIDS patients and follow-up. J. Clin. Microbiol. **34:**457–459.
2. **Boeckh, M., R. A. Bowden, J. M. Goodrich, M. Pettinger, and J. D. Meyers.** 1992. Cytomegalovirus antigen detection in peripheral blood leukocytes after allogeneic marrow transplantation. Blood **80:**1358–1364.
3. **Drew, W. L.** 1992. Cytomegalovirus infection in patients with AIDS. Clin. Infect. Dis. **14:**608–615.
4. **Drouet, E., A. Biobieus, S. Michelson, et al.** 1995. Polymerase chain reaction detection of cytomegalovirus DNA in peripheral blood leukocytes as a predictor of cytomegalovirus disease in HIV-infected patients. AIDS **7:**665–668.
5. **Engler, H. D., and S. T. Selepak.** 1994. Effect of centrifuging shell vials at 3,500 $\times$ *g* on detection of viruses in clinical specimens. J. Clin. Microbiol. **32:**1580–1582.
6. **Erice, A., M. A. Holm, P. C. Gill, S. Henry, C. L. Dirksen, D. L. Dunn, R. P. Hillam, and H. H. Balfour, Jr.** 1992. Cytomegalovirus (CMV) antigenemia assay is more sensitive than shell vial cultures for rapid detection of CMV in polymorphonuclear blood leukocytes. J. Clin. Microbiol. **30:**2822–2825.
7. **Gallant, J. E., R. D. Moore, D. D. Richman, et al.** 1992. Incidence and natural history of cytomegalovirus disease in patients with advanced human immunodeficiency virus treated with zidovudine. J. Infect. Dis. **166:**1223–1227.
8. **Gerna, G., M. Parea, E. Percivalle, D. Zipeto, E. Silini, G. Barbarini, and G. Milanesi.** 1990. Human cytomegalovirus viremia in HIV-1 seropositive patients at various clinical stages of infection. AIDS **4:**1027–1031.
9. **Gerna, G., M. G. Revello, E. Percivalle, and F. Morini.** 1992. Comparison of different immunostaining techniques and monoclonal antibodies to the lower matrix phosphoprotein (pp65) for optimal quantitation of human cytomegalovirus antigenemia. J. Clin. Microbiol. **30:**1232–1237.
10. **Gerna, G., M. G. Revello, E. Percivalle, M. Zavattoni, M. Parea, and M. Battaglia.** 1990. Quantification of human cytomegalovirus viremia by using monoclonal antibodies to different viral proteins. J. Clin. Microbiol. **28:**2681–2688.
11. **Krajden, M., S. Walmsley, P. Shankaran, R. Chua, and T. Mazzulli.** 1995. Detection of cytomegalovirus (CMV) viremia in HIV-infected patients by pp65 antigenemia and semi-quantitative PCR using the Digene Sharp Signal assay (DSSA), abstr. C-219, p. 38. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
12. **Landry, M. L., and D. Ferguson.** 1993. Comparison of quantitative cytomegalovirus antigenemia assay with culture methods and correlation with clinical disease. J. Clin. Microbiol. **31:**2851–2856.
13. **Leach, C. T., J. D. Cherry, P. A. English, et al.** 1993. The relationship between T-cell levels and CMV infection in asymptomatic HIV-1 antibody-positive homosexual men. J. Acquired Immune Defic. Syndr. **6:**407–413.
14. **Mazzulli, T., R. H. Rubin, M. J. Ferraro, R. T. D'Aquila, S. A. Doveikis, B. R. Smith, T. H. The, and M. S. Hirsch.** 1993. Cytomegalovirus antigenemia: clinical correlations in transplant recipients and in persons with AIDS. J. Clin. Microbiol. **31:**2824–2827.
15. **Munro, B. H.** 1986. Correlation, p. 63–85. *In* B. H. Munro, M. A. Visintainer, and E. Button Page (ed.), Statistical methods for health care research. J. B. Lippincott Co., Philadelphia.
16. **Munro, B. H.** 1986. Descriptive statistics, p. 86–126. *In* B. H. Munro, M. A. Visintainer, and E. Button Page (ed.), Statistical methods for health care research. J. B. Lippincott Co., Philadelphia.
17. **Noe, D. A.** 1985. Variability in laboratory measurement, p. 19–30. *In* The logic of laboratory medicine. Urban and Schwarzenberg, Baltimore.
18. **Rasmussen, L., S. Morris, D. Zipeto, J. Fessel, R. Wolitz, A. Dowling, and T. C. Merigan.** Quantitation of human cytomegalovirus DNA from peripheral blood cells of human immunodeficiency virus-infected patients could predict cytomegalovirus retinitis. J. Infect. Dis. **171:**177–182.
19. **Salmon, D., F. Lacassin, M. Harzic, C. Leport, C. Perronne, F. Bricaire, F. Brun-Vezinet, and J. L. Vilde.** 1990. Predictive value of cytomegalovirus viraemia for the occurrence of CMV organ involvement in AIDS. J. Med. Virol. **32:**160–163.
20. **Souza, I. E., D. Nicholson, S. Matthey, et al.** 1994. Detection of human cytomegalovirus in peripheral blood leukocytes by the polymerase chain reaction and a non-radioactive probe. Diagn. Microbiol. Infect. Dis. **20:**13–19.
21. **Spector, S. A., G. F. McKinley, J. P. Lalezari, et al.** 1996. Oral gangiclovir for the prevention of cytomegalovirus disease in persons with AIDS. N. Engl. J. Med. **334:**1491–1497.
22. **Spector, S. A., J. A. Rua, D. H. Spector, and R. McMillan.** 1984. Detection of human cytomegalovirus in clinical specimens by DNA-DNA hybridization. J. Infect. Dis. **150:**121–126.
23. **Stöckl, E., T. Popow-Kraupp, F. X. Heinz, F. Mühlbacher, P. Balcke, and C. Kunz.** 1988. Potential of in situ hybridization for early diagnosis of productive cytomegalovirus infection. J. Clin. Microbiol. **26:**2536–2540.
24. **The, T. H., M. Van Der Ploeg, A. P. Van Den Berg, A. M. Vlieger, M. Van Der Giessen, and J. Van Son.** 1992. Direct detection of cytomegalovirus in peripheral blood leukocytes—a review of the antigenemia assay and polymerase chain reaction. Transplantation **54:**193–198.
25. **Zipeto, D., F. Baldanti, D. Zella, M. Furione, A. Covicchini, G. Milanesi, and G. Gerna.** 1993. Quantification of human cytomegalovirus DNA in peripheral blood leukocytes of immunocompromised patients by the polymerase chain reaction. J. Virol. Methods **44:**45–56.
26. **Zipeto, D., S. Morris, C. Hong, A. Dowling, R. Wolitz, T. C. Merigan, and L. Rasmussen.** 1995. Human cytomegalovirus (CMV) DNA in plasma reflects quantity of CMV DNA present in leukocytes. J. Clin. Microbiol. **33:**2607–2611.
27. **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.