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Novel DNA Assay for Cytomegalovirus Detection: Comparison with Conventional Culture and pp65 Antigenemia Assay

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We compared conventional cytomegalovirus (CMV) isolation, rapid viral culture, a CMV pp65 antigenemia assay, and a novel CMV DNA hybrid capture system (HCS). A total of 309 blood samples from individuals in different risk groups were assessed by at least two of the methods mentioned above. Leukocytes were recovered either after centrifugation in Leucosep tubes containing 1.080 Ficoll for pp65 assay or after simple differential lysis steps for DNA detection. HCS was based on DNA hybridization with a CMV RNA probe and its capture by antibodies to DNA-RNA hybrids. The CMV pp65 lower matrix protein was detected by fluorescence with c10-c11 monoclonal antibody in formalin-fixed leukocytes. Concordant results were observed for 92.9, 78.3, and 82.7% of the patients when comparing (i) viral culture and the pp65 antigenemia assay, (ii) viral culture and HCS, and (iii) the pp65 antigenemia assay and HCS, respectively. Discordant results were observed between a positive HCS result and negative culture and/or pp65 results. These results were associated with relatively low DNA levels (<20 pg/10^6 cells) and positive viruria. In conclusion, the pp65 antigenemia assay is a rapid and reliable method of detecting CMV and is preferable to culture, but the Murex HCS appears to be more sensitive for CMV detection.

Cytomegalovirus (CMV) infection is widespread in humans, with a 50 to 60% prevalence in rich countries and a 90 to 100% prevalence in poor countries. CMV infections are a major cause of mortality and morbidity in immunocompromised patients, such as organ or bone marrow transplant recipients, persons with AIDS, neonates, and older patients (1, 6, 13, 14). A prompt diagnosis is essential for efficient antiviral treatment in patients with severe or life-threatening CMV disease. Important efforts have been deployed to improve CMV detection. Consequently, several tests for CMV are now available, including PCR for CMV DNA detection (4), in situ hybridization (3), and monoclonal antibody (MAb)-based detection of CMV antigens (16, 18). Routinely, common methods such as cell culture for viral isolation and immunoglobulin M (IgM) serology are still used. Nevertheless, none of the current methods is entirely satisfactory in terms of sensitivity, specificity, and rapidity.

In our laboratory, diagnosis of CMV infection is achieved by the detection of CMV antigens in a rapid (48 h) cell culture for virus in which the culture is inoculated with patient leukocytes and is confirmed by conventional virus isolation. The aim of this study is to compare our diagnostic methods with (i) a pp65 antigenemia assay and (ii) a new, commercially available CMV DNA hybrid capture system (HCS; Murex Diagnostics Ltd., Dartford, United Kingdom).

MATERIALS AND METHODS

Patients. A total number of 198 patients with or at high risk of CMV disease were included in this study. Sixty-nine patients were from the Infectious Diseases Center (patients suffering from AIDS), 41 were from the Nephrology Center (kidney transplant recipients), 30 were from the Hematology Center (leukemic patients and bone marrow recipients), and 58 were suspected of having CMV disease. One of the AIDS patients who was undergoing antiviral (ganciclovir) therapy was monitored for 3 weeks for kinetic studies in terms of CMV pp65 antigen and CMV DNA levels in leukocytes. The IgG and IgM anti-CMV serological status of these patients was determined by enzyme immunoassay (CMV Enzygnost [Behring, Marburg, Germany] and CMV IMX [Abbott, North Chicago, Ill.]).

Blood samples. We analyzed 309 blood samples from the 198 patients. Peripheral blood samples were obtained in heparinized or EDTA-containing tubes, depending on the test method. Two cell culture-based methods were used (conventional CMV isolation and rapid viral culture), as were a pp65 antigenemia assay (254 samples) and a CMV DNA test (157 samples). When sufficient blood volumes were available, the last two tests were performed (133 samples). Blood samples were processed within 2 h following collection to minimize artifactual results (11). Blood cells were counted with an automatic cell counter (Technicon H.2 system; Bayer Diagnostics, Tarrytown, N.Y.).

Conventional CMV isolation. Confluent monolayers of MRC-5 cells (BioMerieux, Lyon, France) in 25-cm^2 flasks were inoculated with 1 ml of buffy coat cells isolated from 6 ml of heparinized blood samples. The estimated number of blood cells was 6×10^6 per flask, which was higher than the recommended minimum number (2). After 1 h of absorption, the inoculum was replaced with 10 ml of minimum essential medium (MEM) containing 10% fetal calf serum (FCS). Cell cultures were maintained at 37°C for 3 weeks. The cultures were checked every other day for the appearance of the characteristic cytopathic effects of CMV.

Rapid viral culture. MRC-5 cells grown to confluency on coverslips in flat-bottom Bijou containers (Bibby Sterilin Ltd., Stone, United Kingdom) were inoculated with 0.3 ml of the buffy coat cells mentioned above and were centrifuged at room temperature for 45 min at $600 \times g$. The inoculum was then removed, and 1 ml of MEM containing 10% FCS was added to each tube. Cell cultures were placed in an incubator at 37°C with 5% CO_2. After 48 h of incubation, the cells were fixed in acetone and the coverslips were transferred onto slides. Fifty microliters of a 100-fold-diluted murine MAb directed against CMV immediate-early antigens (E13 clone; Biosys, Compiègne, France) was added for 30 min at 37°C in a moist chamber. The cells were stained with fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG and IgM (BioSource International, Camarillo, Calif.). A green fluorescent nuclear staining was observed for CMV-infected cells. This procedure for blood samples was also applied to urine samples.

pp65 antigenemia assay. The pp65 antigenemia assay is based on the direct detection of the CMV pp65 phosphoprotein, which is the viral lower matrix protein predominantly found in leukocytes during active CMV infections (17). Three milliliters of 1.080 Ficoll for polymorphonuclear cell isolation (Eurobio, Les Ullis, France) was added to a Leucosep tube (Esquire Chemie AG, Zurich, Switzerland). These tubes contained a porous membrane that covered the Ficoll after centrifugation. Approximately 6 ml of heparinized blood was poured onto the membrane, and the tubes were centrifuged at $800 \times g$ for 15 min. The leukocytes were separated from the erythrocytes by the membrane and were easily recovered from the Ficoll upper layers by another centrifugation step

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TABLE 1. Comparison of CMV culture methods and pp65 antigenemia assay

pp65 antigenemia assay result	No. of samples with the following culture method result:		
	+	−	Total
+	38	13	51
−	5	198	203
No. of samples	43	211	254

TABLE 2. Comparison of culture methods and CMV DNAemia assay result by HCS

CMV DNA result by HCS	No. of samples with the following culture method result:		
	+	−	Total
+	23	34	56
−	0	100	101
No. of samples	23	134	157

($800 \times g$ for 10 min). The supernatant (plasma) was discarded, and the pellet of leukocytes was washed once with calcium-magnesium-free phosphate-buffered saline (PBS), resuspended in 1 ml of PBS, and counted on Kova slides (Hycor Biomedica Inc., Irvine, Calif.). The cell concentration was adjusted to 10^6/ml with PBS. A volume of 0.2 ml of the leukocyte suspension (2×10^5 cells) was cytocentrifuged onto glass slides at 500 rpm for 5 min (Cytospin 3; Shandon Ltd., Runcorn, United Kingdom). The slides were air dried for at least 30 min. The cells were fixed with 5% paraformaldehyde–2% sucrose in PBS for 10 min at room temperature. They were permeabilized with 0.5% Nonidet P-40–10% sucrose–1% FCS in PBS for 5 min at room temperature and were air dried for the staining procedure.

Fixed cells were incubated at 37°C for 30 min with 50 μl of diluted MAb specific to CMV pp65. We used either a fivefold-diluted Clonab CMV C10-C11 MAb (Biotest Diagnostic Corp., Dreieich, Germany) or a 20-fold-diluted 1C3-AYM1 MAb (Argene, Varilhes, France). The cells were washed twice in PBS and were incubated for 30 min at 37°C with 50 μl of FITC-labelled goat anti-mouse antibody containing Evans blue counterstain. The slides were washed in PBS and mounted for examination of the fluorescent cells. The antigenemia level was semiquantified by counting the number of pp65-positive cells per 2×10^5 leukocytes and was reported to 1×10^6 cells. In accordance with Gerna et al. (8) and Landry and Ferguson (10), the presence of fewer than 50 positive cells (per 10^6 cells) was considered a low-positive result, the presence of 50 to 249 positive cells was considered an intermediate result, and the presence of 250 or more positive cells was considered a highly positive result.

CMV DNA HCS. The CMV DNA HCS, a new, commercially available kit (Murex), detects CMV DNA with an RNA probe (which shares 16% of the CMV genome) and two anti-RNA-DNA hybrid antibodies. In short, 3.5 ml of fresh whole blood collected in EDTA-containing tubes was incubated with a lysis buffer for 15 min at 20 to 25°C. After centrifugation (15 min at $1{,}000 \times g$), the supernatant was discarded and the pellet was resuspended in 1.5 ml of the lysis buffer. The cells were then counted on Kova slides. After a second centrifugation step, the leukocyte pellet that was obtained either was kept at −20°C for up to 3 months or was immediately processed by HCS.

The diluent and denaturing reagents were added to the whole pellet, and the mixture was incubated at 70°C for 50 min. Hybridization of the target DNA was carried out with a specific CMV RNA probe for 2 h at 70°C. RNA-DNA hybrids were transferred to specific antibody-coated tubes and were revealed with a second antibody, labelled for a chemiluminescence reaction. CMV DNA was quantified after plotting a calibration curve of standards on a luminometer (Leader TM 50; MGM Instruments Inc., Hamden, Conn.) and is reported to 10^6 leukocytes (not recommended by Murex).

Statistical analysis. The culture, pp65 antigenemia assay, and CMV DNA methods were compared, and statistical analysis was performed by the Cohen concordance kappa (κ) test. The absence of concordance was observed for a κ value of <0.6. The three pp65 groups (low, mild, and high levels of antigenemia, as defined above) were compared with the medium and range of CMV DNA levels for each group. Statistical analysis of the three groups was performed by variance analysis by the Fisher F test and the Student *t* test, taking the groups two by two. A significant variation was noted for a *P* value of <0.05. A correlation *r* test was applied to the cell counting results: for leukocytes, automatic measure with whole blood compared with leukocyte counting after erythrocyte lysis in the HCS kit. No correlation was noted for an *r* value of <0.6.

RESULTS

Conventional culture allows for the specific and sensitive detection of CMV. Thus, it is generally considered the "gold standard." In our laboratory, rapid viral culture is combined with conventional culture. Since the results for all samples were concordant by these assays (data not shown), the assays were designed as "culture methods" in this study.

A 92.9% concordance ($\kappa = 0.765$) was observed between the culture methods and the pp65 antigenemia assay (Table 1). The sensitivity of the pp65 antigenemia assay compared with the results of the culture methods was 88.4%. The 18 patients with discordant results were under antiviral treatment (ganciclovir), and the numbers of pp65-positive cells were low (<50/10^6 cells), in accordance with Gerna et al. (8). A 78.3% concordance ($\kappa = 0.441$) was observed between culture methods and the CMV DNAemia assay by HCS (Table 2). Among the 34 samples with discordant results, 11 had very high DNA levels (>20 pg/10^6 cells, with an average value of 44.9 pg/10^6 cells), and 23 had lower DNA levels (<20 pg/10^6 cells, with an average value of 7 pg/10^6 cells). An 82.7% concordance ($\kappa = 0.553$) was observed between the pp65 antigenemia assay and the CMV DNAemia assay (Table 3). Among the 23 samples with discordant results, only 2 blood samples were CMV DNA negative, with a low number of pp65-positive cells (<50/10^6 cells). For the 21 pp65-negative samples, 4 had very high DNA levels (>20 pg/10^6 cells, with an average value of 31.7 pg/10^6 cells) and 17 had lower DNA levels (<20 pg/10^6 cells, with an average value of 8.4 pg/10^6 cells). These positive DNA samples with discordant results that were also negative by the blood culture methods were all positive for CMV, as determined by culture of a urine specimen taken at the same time (data not shown). Finally, the results of CMV IgM detection in this study were not concordant with either culture, pp65 antigenemia assay, or DNAemia assay results (61.7%; $\kappa = 0.227$; $n = 47$). Its sensitivity was 39% compared with the results of the other methods. Therefore, this serological marker cannot be used for the screening of replicative CMV in immunocompromised patients.

For the pp65 antigenemia assay, the test with the 20-fold-diluted 1C3-AYM1 Argene MAb appeared to be as good as the test with the Biotest Clonab MAb diluted 5-fold, with reduced background signals.

When comparing CMV pp65- and DNA-positive blood samples, we observed that low, mild, and high levels of pp65 antigenemia were positively correlated with the presence of CMV DNA (Fig. 1).

The leukocyte count was well correlated after erythrocyte lysis in the HCS kit with the count obtained by automatic numeration with whole blood ($n = 70$; $2.560 \times 10^6 \pm 1.239 \times 10^6$ versus $2.680 \times 10^6 \pm 1.222 \times 10^6$ leukocytes per ml; $r = 0.959$). No significant variation was observed between the different groups of patients.

TABLE 3. Comparison of pp65 antigenemia assay and CMV DNAemia assay result by HCS

CMV DNA result by HCS	No. of samples with the following pp65 antigenemia assay result:		
	+	−	Total
+	22	21	43
−	2	88	90
No. of samples	24	109	133

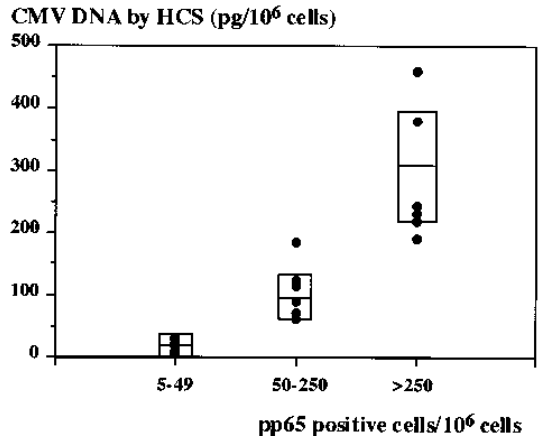

FIG. 1. Evaluation of CMV DNA quantified by HCS (picograms per 10^6 cells) versus pp65-positive cell numbers (per 10^6 cells) for 22 patients positive for CMV DNA by both methods. Mean DNA values were 19 ± 9, 107 ± 44, and 307 ± 96 pg/10^6 cells for 5 to 49, 50 to 250, and >250 pp65-positive cells per 10^6 cells, respectively. The three groups were significantly different, since a global mean comparison gave a value of 24.77 by the Fisher F test. The three mean values taken two by two were also different (low versus mild, $t = 2.268$ and $P < 0.05$; mild versus high, $t = 5.155$ and $P < 0.001$; low versus high, $t = 7.425$ and $P < 0.001$).

DISCUSSION

We have described here a new, commercially available CMV DNA HCS (Murex) and compared it with more conventional methods of CMV detection, such as viral culture and pp65 antigenemia assays.

The pp65 antigenemia assay was performed as described by Gerna et al. (7), with some modifications. Mixed leukocytes were obtained with a Ficoll-Leucosep tube system instead of polymorphonuclear leukocytes isolated by a dextran method. This modification enabled us to simplify the procedure for isolating leukocytes from whole-blood samples for the pp65 assay, since the conventional dextran sedimentation method (7), based on differential erythrocyte lysis, required many centrifugations and washings. To our knowledge, the Ficoll-Leucosep system has not yet been described. This simple and rapid method yielded mixed leukocytes of high purity without contamination with erythrocytes and appeared as suitable as the dextran method for a pp65 antigenemia assay, in agreement with Erice et al. (5). However, a Giemsa coloration showed that the mixed leukocytes consisted of approximately 60% polymorphonuclear leukocytes and 40% other leukocytes (monocytes and lymphocytes). Moreover, we found that optimal results were obtained when (i) cells were formalin fixed and permeabilized with Nonidet P-40 and (ii) infected cells were detected by an immunofluorescence assay and not by an immunoperoxidase assay (7). Under these conditions, our pp65 antigenemia assay was as sensitive as the culture methods. Thus, compared with conventional CMV culture, the pp65 antigenemia assay exhibited a slightly better sensitivity than the CMV-vue kit used by Erice et al. (5). However, Landry and Ferguson (10) and Van der Bij et al. (17), who used dextran-isolated polymorphonuclear leukocytes, claimed that the pp65 antigenemia assay is more sensitive than culture methods. It was not established whether the pp65 antigenemia assay was more sensitive or CMV culture was less efficient in their studies than in our study.

Compared with HCS, both culture methods and the pp65 antigenemia assay had low sensitivities (39 and 51%, respectively). The sensitivity of HCS was reported to be close to that of PCR (12). Its limit has been evaluated to be 8 pg/ml, or about 3×10^3 CMV DNA copies per ml of whole blood. We have expressed the concentration of DNA in our study in picograms per 10^6 leukocytes and not the amount per volume of blood, since cell levels may vary considerably in immunocompromised patients, as shown in this work. An average value of 3×10^6 leukocytes per ml of whole blood was found, which was lower than usual. High variations (from 5×10^5 to 1×10^7) were observed between the patients and even in blood from the same patients taken at different times. We have shown that automatic enumeration of leukocytes in whole blood may be used to calculate the amount of CMV DNA and reported as the number of cells.

HCS detects a chemiluminescence intensity proportional to the quantity of CMV DNA. Thus, it allows an objective quantification of the viral load and avoids (i) the laborious screening of fluorescent cells either in the pp65 antigenemia assay or in rapid viral culture and (ii) the mistakes in counting the numbers of infected cells. We tried to evaluate the equivalence of the number of pp65-positive cells and the related amount of DNA detected by HCS. Discordant pp65 and DNA levels were observed for several reasons. First, fluorescent cell counts were only approximate, and the results could be given only in three intensity groups (low, mild, and high levels of antigenemia). Consequently, pp65 antigenemia levels may not be reliable. Second, the pp65 antigenemia assay evaluated the number of CMV-infected cells, i.e., the numbers of cells expressing pp65 antigens during active CMV infection, whereas HCS detected all CMV genomes from these cells. Since infected cells harbor greater or fewer numbers of CMV genomes, pp65 antigenemia levels can correspond to either very high or low DNA levels. Furthermore, HCS may detect defective CMV (15) which is not able to replicate in cell cultures. If pp65 antigenemia reflects active CMV infection and HCS detects both CMV replication and the latent form of CMV, then it is expected that HCS will not correlate with clinical symptoms. In fact, Lazzarotto et al. (12) found that the DNA level by HCS correlated as well as the level of pp65 antigenemia with clinical symptoms. According to Imbert-Marcille et al. (9), a DNA level below 23 pg/ml of blood was predictive of asymptomatic CMV infection in renal transplant recipients, whereas a higher viral load was always associated with CMV disease. However, these low DNA levels associated with the asymptomatic form of CMV infection did not exclude the possibility of active CMV replication. Indeed, all of our patients with samples with CMV DNA-positive results but with negative results by both culture and the pp65 antigenemia assay were positive for viruria. The meaning of long-lasting positive viruria is still under discussion, but it may reflect low-level CMV replication in infected leukocytes, below the sensitivity of blood culture and the pp65 antigenemia assay. The specificity of CMV DNA detection in some of these patients was also assessed by positive CMV PCR results (data not shown), as indicated by Lazzarotto et al. (12).

In conclusion, the pp65 antigenemia assay is a more rapid and reliable method than CMV culture for CMV detection. Nevertheless, quantification of infected cells on the basis of the pp65-positive cell count is exhaustive for a large number of samples and is unsuitable for the precise measurement of the level of CMV in cells. On the other hand, the Murex CMV DNA HCS appears to be a promising method for CMV detection, in particular for patients with a low viral load. It is also well adapted for the therapeutic follow-up of patients under antiviral treatment. Indeed, for our AIDS patients on ganciclovir therapy, we observed a rapid decrease in the level of CMV DNAemia by day 4 and viral disappearance by day 12 using this method.

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REFERENCES

1. **Adler, S. P.** 1988. Molecular epidemiology of cytomegalovirus: viral transmission among children attending a day care center, their parents, and caretakers. J. Pediatr. **112:**366–372.
2. **Buller, R. S., T. C. Bailey, N. A. Ettinger, M. Keener, T. Langlois, J. P. Miller, and G. A. Storch.** 1992. Use of a modified shell vial technique to quantitate cytomegalovirus viremia in a population of solid-organ transplant recipients. J. Clin. Microbiol. **30:**2620–2624.
3. **Dankner, W. M., J. A. McCutchan, D. D. Richman, K. Hirata, and S. A. Spector.** 1990. Localisation of human cytomegalovirus in peripheral blood leukocytes by in situ hybridization. J. Infect. Dis. **161:**31–36.
4. **De Rossi, 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. **Erice, A., M. A. Holm, M. V. Sanjuan, D. L. Dunn, P. C. Gill, and H. H. Balfour.** 1995. Evaluation of CMV-vue antigenemia assay for rapid detection of cytomegalovirus in mixed-leukocyte blood fractions. J. Clin. Microbiol. **33:**1014–1015.
6. **Gerna, G., M. Parea, E. Percivalle, D. Zipeto, E. Silin, G. Barbarini, and G. Milanesi.** 1990. Human cytomegalovirus viraemia in HIV-1-seropositive patients at various clinical stages of infection. AIDS **4:**1027–1031.
7. **Gerna, G., M. G. Revello, E. Percivalle, and F. Morini.** 1992. Comparison of different immunostaining techniques and monoclonal antibodies to the lower matrix phosphoprotein (pp 65) for optimal quantification of human cytomegalovirus antigenemia. J. Clin. Microbiol. **30:**1232–1237.
8. **Gerna, G., D. Zipeto, M. Parea, M. G. Revello, E. Silini, E. Percivalle, M. Zavattoni, 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.
9. **Imbert-Marcille, B.-M., D. Cantarovich, S. Boedec, L. Vernoux, J. P. Soulillou, and S. Billaudel.** 1995. Evaluation of a new quantification method for cytomegalovirus DNA in leukocytes: clinical value in renal transplant recipients. *In* Proceedings of the Fifth International Cytomegalovirus Conference.
10. **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.
11. **Landry, M. L., D. Ferguson, S. Cohen, K. Huber, and P. Wetherill.** 1995. Effect of delayed specimen processing on cytomegalovirus antigenemia test results. J. Clin. Microbiol. **33:**257–259.
12. **Lazzarotto, T., B. Campisi, S. Galli, P. Guglielmi, P. Dal Monte, and M. P. Landini.** 1995. A quantitative test (HCMV-Hybrid Capture™) to detect human cytomegalovirus DNA in the blood of immunocompromised patients compared with antigenemia and polymerase chain reaction. In Proceedings of the Fifth International Cytomegalovirus Conference.
13. **Meyers, J. D., N. Flournoy, and E. D. Thomas.** 1986. Risk factors for cytomegalovirus infection after human marrow transplantation. J. Infect. Dis. **153:**478–488.
14. **Peterson, P. K., H. H. Balfour, S. C. Marker, D. S. Fryd, R. J. Howard, and R. L. Simmons.** 1980. Cytomegalovirus disease in renal allograft recipients: a prospective study of the clinical features, risk factors and impact on renal transplantation. Medicine (Baltimore) **59:**283–300.
15. **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.
16. **The, T. H., W. van der Bij, A. P. van der Berg, M. van der Giessen, J. Weits, H. G. Sprenger, and W. J. van Son.** 1990. Cytomegalovirus antigenemia. Rev. Infect. Dis. **12:**S737–S744.
17. **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.
18. **Woods, G. L., A. Young, A. Johnson, and G. M. Thiele.** 1987. Detection of cytomegalovirus by 24-well plate centrifugation assay using a monoclonal antibody to an early nuclear antigen and by conventional cell culture. J. Virol. Methods **18:**207–214.