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Effect of Delayed Processing of Blood Samples on Performance of Cytomegalovirus Antigenemia Assay

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This prospective, parallel, and blind study of 120 blood samples from immunocompromised patients examined the influence of delayed sample processing on cytomegalovirus antigenemia assay. Cytomegalovirus was detected in 49 samples (40.8%): 41 were processed within 2 to 4 h, and 40 were reprocessed the following day. Results were discrepant in 17 of the samples with the lowest positive cell counts. Differences between the two days did not reach statistical significance.

Cytomegalovirus (CMV) is a major cause of morbidity and mortality in immunocompromised patients, such as allograft recipients and those suffering from AIDS (6). Significant CMV infections may be difficult to differentiate from other opportunistic infections. In addition, new drugs active against CMV are now available. Reliable laboratory diagnostic methods are therefore required. Quantitative antigenemia assay (detection of CMV antigens in leukocytes) has emerged as a valuable and sensitive tool for early diagnosis and prognosis and for monitoring antiviral treatment (3, 4, 6, 9, 11–13). Although the test is easy to perform, there are a number of different methods, and several pitfalls have been described (1, 4, 7, 8, 11, 12, 14). More specifically, results about the influence of delayed processing for antigenemia assay have been contradictory (4, 7, 11–14). An 80% decrease in the detection rate when processing samples 6 h after collection has even been reported (4). This is important, because many laboratories have difficulty in processing specimens within 2 to 4 h of collection, and it could be a limitation for those referring specimens to other laboratories. In this prospective, parallel, and blind study, we examined the influence of overnight storage at 4°C of the blood samples on performance of the antigenemia assay.

In all, 120 heparinized blood samples from 62 immunocompromised patients (15 liver, 9 kidney, 8 heart, and 5 bone marrow transplant patients; 24 AIDS patients; and 1 patient with lupus erythematosus) were sent to the laboratory from July to August 1993. During the study period, there were eight episodes of CMV disease (five of symptomatic viremia, two of neumonitis, and one of hepatitis) which occurred in six patients (five kidney transplant recipients, one liver transplant recipient, and one AIDS patient). All were treated with ganciclovir, as was one seronegative liver transplant patient with asymptomatic viremia who had received an organ from a seropositive donor. Five heart transplant patients received prophylaxis with ganciclovir for CMV infection. A total of 33 blood specimens was obtained from these patients during antiviral agent administration.

One aliquot of each blood sample was processed for the antigenemia test, shell vial technique, and tube culture within 4 h of blood sampling (day 0). The samples were then stored overnight at 4°C, before complete and identical reprocessing of another aliquot of the same heparinized blood sample for an antigenemia test the day following collection (day 1). Shell vial procedures and conventional cultures were not performed on day 1.

For leukocyte extraction (mainly polymorphonuclear leukocytes), we followed basically the protocol described by Gerna et al. (4). Briefly, 3 ml of blood was added to 1 ml of 6% dextran solution in saline (Macrodex; Pharmacia, Uppsala, Sweden) and left at 37°C for 30 min. The enriched leukocyte supernatant was then transferred to another tube, washed in phosphate-buffered saline (PBS), and gently centrifuged. Contaminating erythrocytes were lysed with 2 ml of 0.8% ammonium chloride solution for 3 min. After further centrifugation, the pellet was washed again and resuspended in PBS, the leukocytes were counted in an automatic hematological counter, and the concentration was adjusted to about 10^6 cells per ml. For the tube and shell vial cultures, we used washed leukocytes (not treated to lyse erythrocytes) resuspended in Eagle's minimal essential medium with 3% fetal calf serum.

A total of 200 μl of the adjusted cell suspension (about 2×10^5 cells) was spotted onto one slide by means of a cytocentrifuge (Cytospin-2, Shandon Scientific, Runcorn, England) at 700 rpm for 7 min, allowed to dry, and then fixed in a solution containing 5% formaldehyde and 2% sucrose in PBS for 10 min (4). The slides were rinsed in PBS for 3 min, dried at room temperature, and then stained by an indirect immunofluorescence assay with a commercially available monoclonal antibody directed against CMV pp65-68 (Monofluokit CMV; Diagnostics Pasteur, Marnes-la-Coquette, France). The same reagent was used in each matched experiment, and all batches were controlled for quality before their use. Only one slide per specimen was made in each antigenemia assay on days 0 and 1. The slides were screened at a magnification of ×160 and confirmed at a magnification of ×400. Readings were always taken blind by two experienced observers (J.L.P. and J.N.). Only cells showing well-defined nuclear fluorescence were recorded, and the results were expressed as positive cells per 10^5 total leukocytes. Vial and tube cultures were processed in MRC-5 fibroblasts in accordance with standard procedures (5, 10). Briefly, vials (one per specimen) were inoculated with approximately 3×10^5 to 4×10^5 leukocytes, incubated at 37°C for 18 h, and then stained with a monoclonal antibody directed against the 72-kDa immediate-early CMV antigen (E 13; Biosoft, Paris, France). The same inocula were used for tube cultures, which were incubated for at least 15 days and read 4 days a week for the typical cytopathic effect.

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TABLE 1. Antigenemia assay: comparison of two different days of processing in 120 matched experiments

Day after collection[a]	No. of specimens		Mean positive cell count/10^5 leukocytes
	Positive (% of total)	Positive only on indicated day (% of total positive tests)	
0	41 (83.7)	9 (18.4)	18.1
1	40 (81.6)	8 (16.3)	15.7
Total	49 (100.0)	17 (34.7)	

[a] Day 0, 2 to 4 h after collection; day 1, 24 h later.

Table 1 gives the assay results. CMV was detected by antigenemia assay in 49 of the 120 samples (40.8%), 41 on day 0 and 40 on day 1. There were 32 concordant positive and 71 concordant negative results, whereas 17 samples (34.6% of positive) gave discrepant results. Of these, nine were positive only when processed on the day of collection; conversely, eight had detectable CMV antigen only when reprocessed after overnight storage. Differences were not statistically significant, either for proportions ($P = 1$; McNemar test) or for the mean positive cell counts ($P = 0.181$; paired t test). All discrepancies occurred in samples with the lowest positive counts, 1 to 10 cells per 10^5 leukocytes; seven of the discrepancies were in samples from patients with ganciclovir treatment or prophylaxis. All patients with confirmed CMV disease showed antigenemia counts equal to or higher than 20 cells per 10^5 leukocytes (range, 21 to 250), in contrast to those with asymptomatic viremia and/or antigenemia (range, 1 to 13). Consequently, discrepancies between antigenemia tests performed on days 0 and 1 were not clinically relevant. In our opinion, many of these discrepancies could be due to the inherent variability of the antigenemia assay. Of course, performing duplicate or multiple slides could reduce discrepancies, but this practice should be analyzed from the cost-effectiveness point of view.

CMV was isolated in culture or shell vial in 23 samples (19 by shell vial, 15 by tube culture, and 11 by both methods). All except three were positive for antigenemia. Thus, there were 29 antigen-positive samples not confirmed by either culture method. Of these, 16 specimens were obtained from patients treated with ganciclovir. The remaining 13 discordant results between antigenemia and culture methods were observed in samples with the lowest antigenemia counts (equal to or fewer than 3 positive cells per 10^5 total leukocytes). A higher sensitivity of antigenemia assay in comparison with isolation procedures has been reported in the literature (3, 11, 13) and is a constant in our experience (data not shown).

Hospitals can expect an increasing number of immunocompromised patients; hence, there is a need for sensitive, specific, easy-to-perform, and inexpensive methods for diagnosing and monitoring CMV infections. The antigenemia assay has these characteristics (4, 6, 9, 11–13), which could make it the method of choice. We emphasize here that the test could be done by most hospital laboratories without sophisticated facilities. Nevertheless, the disagreements and controversial results published in the literature (8, 11, 14) could discourage its use. For practical reasons, the need for rapid processing of samples would be a major limitation. However, our prospective study shows that there were no differences, either qualitatively or quantitatively, in assaying samples kept overnight. Although we used a specific laboratory protocol, we believe that our conclusions would be applicable to other laboratories and procedures. In their indirect and retrospective study on the influence of delayed processing, Erice et al. came to similar conclusions (2). In any case, a small-scale comparison done by each laboratory with their particular procedures would be advisable before adoption of delayed processing.

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