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Evaluation of CMV-vue Antigenemia Assay for Rapid Detection of Cytomegalovirus in Mixed-Leukocyte Blood Fractions

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The CMV-vue antigenemia assay was evaluated by using mixed-leukocyte (ML) blood fractions. Of 234 ML fractions studied, 32 (14%), 23 (10%), and 20 (8.5%) were positive in the CMV-vue assay and conventional and shell vial cultures, respectively. The CMV-vue assay was more sensitive than shell vial cultures for rapid detection of cytomegalovirus. ML fractions are appropriate specimens for the cytomegalovirus antigenemia assay.

In a previous study we showed that a commercially available CMV antigenemia assay (CMV-vue Kit; INCSTAR Corp., Stillwater, Minn.) was more sensitive than shell vial cultures for detection of cytomegalovirus (CMV) in polymorphonuclear leukocyte fractions of blood leukocytes (87.5 vs. 69%) (4). However, mixed-leukocyte (ML) blood fractions are routinely used in our laboratory for isolation of CMV in conventional and shell vial cultures. Therefore, we conducted a prospective study comparing the CMV-vue assay with shell vial cultures for the rapid detection of CMV using ML blood fractions.

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Blood specimens with sufficient blood volume to perform conventional cultures, shell vial cultures, and the CMV-vue assay were included in the study. The storage conditions of the specimens from collection to processing have been previously described (3). ML fractions were separated from 6 to 10 ml of EDTA-treated blood by gradient centrifugation with Polymorphoprep solution (Nycomed Pharma AS, Oslo, Norway). After centrifugation, all leukocyte bands were equally divided into two separate tubes. Cells from one tube were used for the CMV-vue assay, which was performed as previously described (4). Aliquots of ML suspensions containing 50,000 cells were placed in duplicate wells of microscope slides. Cells were fixed in acetone, incubated with a mixture of monoclonal antibodies directed against pp65, and stained as described previously (4). Slides were observed under a light microscope for the presence of red-brown nuclear or perinuclear staining indicative of CMV infection. Cells from the second tube were used to inoculate shell vial and conventional cultures. After three washes, ML fractions were resuspended in 2 ml of culture medium. Aliquots (0.3 ml) of these ML suspensions were inoculated (in triplicate) into shell vials containing coverslips covered with monolayers of human diploid fibroblasts. Shell vial cultures were processed according to previously described procedures (4). For conventional cultures, aliquots (0.2 ml) of ML suspensions were inoculated (in duplicate) into 24-well tissue culture plates containing confluent monolayers of human diploid fibroblasts. Plates were incubated for 3 weeks and observed at regular intervals under the light microscope for the appearance of cytopathic effect characteristic of CMV.

A total of 234 blood specimens from 152 immunocompromised patients (including bone marrow recipients, solid-organ recipients, and patients with AIDS) was studied. Overall, CMV was detected in 35 (15%) ML fractions, including 32 (14%) fractions positive in the CMV-vue assay, 23 (10%) fractions positive in conventional cultures, and 20 (8.5%) fractions positive in shell vial cultures. ML fractions positive in the CMV-vue assay included 16 fractions also positive in shell vial and conventional cultures, 3 fractions positive in shell vial cultures, 4 fractions positive in conventional cultures, and 9 fractions negative in shell vial and conventional cultures (Table 1). The nine ML fractions positive only in the CMV antigenemia assay were from nine patients, six (five solid organ transplant recipients, one AIDS patient) of whom had preceding and/or subsequent episodes of CMV viremia within 1 month of the CMV antigenemia-positive specimens. Of the remaining three patients, two had CMV isolated from other specimens (urine, bronchoalveolar lavage fluid) and one had positive cultures and a positive rise in serum CMV antibodies.

Overall, the CMV-vue antigenemia assay was significantly more sensitive than shell vial cultures for rapid detection of CMV (32 vs. 20 positive ML fractions; $P < 0.002$, McNemar's test) and for detection of CMV in ML fractions positive in conventional cultures (87 vs. 74%; $P = 0.4$, McNemar's test). The number of positive cells in the CMV-vue assay and the

TABLE 1. Comparison of shell vial cultures and CMV antigenemia assay with conventional cultures for detection of CMV in ML fractions

Result of:		No. of conventional cultures	
CMV antigenemia assay	Shell vial culture	Positive ($n = 23$)	Negative ($n = 211$)
+	+	16	3
+	−	4	9
−	+	1	0
−	−	2	199

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TABLE 2. Cells inoculated in conventional and shell vial CMV cultures of ML fractions positive in the CMV antigenemia assay

Results of:		No. of specimens	No. of positive cells (CMV antigenemia assay)[a]	No. leukocytes (10^6) inoculated in:	
Conventional culture	Shell vial culture			Conventional culture[b]	Shell vial culture[b]
+	+	16	23	0.5 (0.1–1.9)	0.7 (0.3–2.8)
+	−	3	11	0.9 (0.3–2.1)	1.4 (0.5–3.2)
−	+	4	13	2.5 (0.8–4.9)	3.7 (1.2–7.3)
−	−	9	9	1.0 (0.2–3.9)	1.5 (0.3–5.9)

[a] Numbers represent average of positive cells per 50,000 leukocytes.
[b] Numbers represent average values; ranges are given in parentheses.

number of cells inoculated in conventional and shell vial cultures are shown in Table 2. Overall, negative conventional and shell vial cultures were not inoculated with lower numbers of cells.

The results of this study indicate that ML fractions can be successfully used in the CMV-vue antigenemia assay. As in our previous study that used polymorphonuclear leukocytes instead of ML fractions (4), the CMV-vue assay was more sensitive than shell vial cultures for the rapid diagnosis of CMV viremia (defined by the isolation of CMV in conventional cultures). Nine (4%) ML fractions (from nine patients) were positive only in the CMV antigenemia assay. Most likely, these were not false-positive results of the CMV-vue assay, as evidenced by the occurrence of preceding and/or subsequent episodes of CMV viremia (six patients), isolation of CMV in conventional and/or shell vial cultures of other clinical specimens (three patients), or serological evidence of active CMV infection (one patient). A potential limitation of this comparative study is that shell vial and conventional cultures were inoculated with variable numbers of blood leukocytes, whereas the same number of blood leukocytes was always used in the CMV-vue assay. The optimal number of blood leukocytes required for isolation of CMV in cultures is not known. A recent study recommends inoculating at least two shell vials with 2×10^6 blood leukocytes per vial for detection of CMV viremia (2). The effect of inoculating variable numbers of leukocytes in cultures in the results of our study is not known. However, conventional and shell vial cultures of all culture-positive and culture-negative ML fractions that were positive in the CMV-vue assay were inoculated with similar numbers of cells (Table 2).

The higher sensitivity of the CMV-vue assay makes it preferable to shell vial cultures for rapid detection of CMV in blood leukocytes of patients suspected to have CMV viremia. One advantage of the shell vial cultures is that they can be used for detection of CMV in other clinical specimens such as urine, respiratory secretions, and tissues (1, 5). Whether the CMV antigenemia assay can be used with those specimens is not known. Finally, not all patients demonstrated viremic by isolation of CMV in conventional cultures in our study were identified by the CMV-vue assay or shell vial cultures. Therefore, negative results with these methods should probably be verified with conventional cultures.

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REFERENCES

1. **Arens, M., J. Owen, C. M. Hagerty, C. A. Reed, and G. A. Storch.** 1991. Optimizing recovery of cytomegalovirus in the shell vial culture procedure. Diagn. Microbiol. Infect. Dis. **14:**125–139.
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. **Erice, A., M. A. Holm, and H. H. Balfour, Jr.** 1993. Specimens for cytomegalovirus antigenemia assay. J. Clin. Microbiol. **31:**1400–1401. (Letter.)
4. **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.
5. **Paya, C. V., A. D. Wold, and T. F. Smith.** 1987. Detection of cytomegalovirus infections in specimens other than urine by the shell vial assay and conventional tube cell cultures. J. Clin. Microbiol. **25:**755–757.