Comparison of Two Rapid Culture Methods for Detection of Cytomegalovirus in Clinical Specimens

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The conventional virus isolation technique was compared with a 24-h shell vial centrifugation culture technique and with a 48-h tube culture method for the detection of cytomegalovirus (CMV) in MRC-5 cells. Of 200 clinical specimens tested, 41 were positive for CMV by at least one procedure. Indirect immunoperoxidase staining was positive for 32 (78.0%) of 41 specimens in the tube culture method and for 30 (73.2%) of 41 specimens in the shell vial centrifugation method. CMV was detected in 23 (56.1%) of 41 specimens by the development of cytopathic effect within 14 days.

Cytomegalovirus (CMV) infection is associated with diseases such as cytomegalic inclusion disease of the newborn and heterophil-negative mononucleosis syndrome and with disseminated infections in immunocompromised patients (8). Laboratory diagnosis of CMV infection has traditionally been accomplished by cell culture methods (9, 12). Several reports have recently described the use of cell culture methods combined with immunologic methods for detection of CMV in clinical specimens within 16 to 48 h after inoculation (1, 2, 4, 6, 11, 14). The use of monoclonal antibodies for detection of CMV by shell vial centrifugation (1, 4), standard tube culture (14), and slide chamber systems (13) has been reported to be more rapid and at least as sensitive as the development of CMV cytopathic effect (CPE) in conventional tube cell cultures. In the present study, we compared the shell vial centrifugation and standard tube culture methods for the rapid detection of CMV in clinical specimens using a monoclonal antibody in an indirect immunoperoxidase (IPA) staining procedure.

A total of 200 clinical specimens was processed and treated with antibiotics by standard methods (9, 12) and then passed through a 0.45- μ m-pore-size filter to remove debris as previously described (14). For conventional tube cell cultures, 0.2 ml of the filtered specimen was inoculated into each of two MRC-5 tubes (16 by 125 mm; Whittaker M.A. Bioproducts, Walkersville, Md.) containing 2 ml of Eagle minimal essential medium, and the cultures were incubated at 36°C. After 48 h, one of the inoculated monolayers was stained by the IPA procedure. The remaining MRC-5 tube was examined every 2 to 3 days for up to 14 days for the development of typical CMV CPE as previously described (5). For shell vial centrifugation cell cultures, the medium was removed from a 1-dram MRC-5 shell vial (Whittaker M.A. Bioproducts), and 0.2 ml of the filtered specimen was placed directly onto the monolayer. The shell vials were then centrifuged at 700 $\times$ g (AccuSpin; Beckman Instruments, Inc., Palo Alto, Calif.) for 45 min at 36°C as previously described (10). After centrifugation, 1 ml of Eagle minimal essential medium was added to each vial, and the cultures were incubated at 36°C. After 24 h, the inoculated monolayer was stained by the IPA procedure. IPA staining was per-

formed in each tube culture or shell vial as described previously (14) with a monoclonal antibody against a CMV early nuclear antigen (10) obtained from Du Pont Specialty Diagnostics, Wilmington, Del. After IPA staining, the tube cultures were examined under an inverted microscope at a magnification of $\times 40$ for the presence of any darkly stained nuclei. The shell vial cover slips were mounted on glass slides and examined under a standard microscope at a magnification of $\times 100$ for the presence of any stained nuclei. A specimen yielding at least one typically stained nucleus in either the tube culture or the cover slip monolayer was considered positive for CMV. The IPA stain was always interpreted before the results of conventional virus isolation were known.

Of 200 specimens processed in tube cultures and in shell vials and stained by the IPA procedure, 32 were positive for CMV in tube cultures at 48 h postinfection, 30 were positive in shell vials at 24 h, and 23 were positive in standard tube cell cultures by detection of typical CPE within 14 days, with a mean of 10.1 days (Table 1). An additional seven CMV isolates were obtained when 18 IPA-positive specimens were subcultured after they failed to produce CPE within 14 days. CMV was detected in 41 of the 200 specimens by at least one of the three procedures. For the 22 specimens that were positive by IPA staining in both the shell vial centrifugation method and the tube culture method, the mean number of stained nuclei was about 20 times greater in the shell vial (2,000 nuclei) than in the tube culture (100 nuclei). Fewer than 10 stained nuclei were observed in seven of the eight

TABLE 1. Comparison of the shell vial centrifugation method with the tube culture method for rapid detection of CMV by IPA staining

Specimen source (n)	No. (%) positive by IPA		No. (%) positive by CPE within 14 days ^a	Total no. (%) positive
	24-h shell vial centrifugation	48-h tube culture		
Urine (133)	15 (11.3)	17 (12.8)	16 (12.0)	23 (17.3)
Bronchial washings (55)	9 (16.4)	10 (18.2)	5 (9.1)	12 (21.8)
Other (12)	6 (50.0)	5 (41.7)	2 (16.7)	6 (50.0)
Total (200)	30 (15.0)	32 (16.0)	23 (11.5)	41 (20.5)

^a An additional seven specimens were positive by CPE upon subculture after an initial incubation period of 14 days.

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TABLE 2. Specimens which were toxic in the shell vial centrifugation method and in the tube culture method for rapid detection of CMV by IPA staining

Specimen source (n)	No. (%) of toxic specimens	
	24-h shell vial centrifugation	48-h tube culture
Urine (133)	14 (10.5)	1 (0.8)
Bronchial washings (55)	18 (32.7)	2 (3.6)
Other (12)	1 (8.3)	0 (0.0)
Total (200)	33 (16.5)	3 (1.5)

specimens that were IPA positive in the shell vial but IPA negative in the tube culture. The rate of cytotoxicity was much higher in the shell vial centrifugation method than in the tube culture method. Of the 200 specimens tested, 33 (16.5%) could not be evaluated by the shell vial centrifugation method because of toxicity to the fibroblast monolayer (Table 2). This high rate of cytotoxicity accounted for the failure of IPA staining to detect CMV in the shell vial in 6 (4 urine specimens and 2 bronchial washings) of the 10 specimens that were IPA positive in the tube culture alone. Only 3 (1.5%) of the 200 specimens were toxic in the tube culture method.

IPA staining in tube cultures after 48 h, combined with the development of CPE within 14 days by conventional virus isolation, detected 36 (87.8%) of 41 CMV-positive specimens. IPA staining in shell vials after 24 h, combined with the development of CPE within 14 days by conventional virus isolation, identified 37 (90.2%) of 41 CMV-positive specimens. Thus, the combination of either one of the rapid IPA staining procedures and conventional virus isolation resulted in detection of nearly all of the CMV-positive specimens. The 10.5% toxicity rate that we observed for urine specimens in the shell vial centrifugation method was similar to that reported by others (1, 3), but the 32.7% toxicity rate associated with bronchial wash specimens has not been previously reported. The use by other investigators of bronchoalveolar lavage specimens in the shell vial centrifugation method was not reported to be associated with a high rate of toxicity (7). However, others have also observed moderate to high rates of toxicity in the shell vial centrifugation method with various types of respiratory specimens (M. J. Miller and V. Fiocco, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1987, C43, p. 330). The present study confirmed that IPA staining in either the shell vial centrifugation method or the tube culture method is more rapid and is at least as sensitive as conventional virus isolation. We found the IPA staining procedure in tube cultures to be more practical than in shell vials, because the centrifugation step, the manipulation of cover slips, and the problem of toxicity were all avoided.

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