JOURNAL OF CLINICAL MICROBIOLOGY, May 1987, p. 755–757 Vol. 25, No. 5
0095-1137/87/050755-03$02.00/0
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Detection of Cytomegalovirus Infections in Specimens Other than Urine by the Shell Vial Assay and Conventional Tube Cell Cultures

CARLOS V. PAYA,[1] ARLO D. WOLD,[2] AND THOMAS F. SMITH[2]*

Department of Internal Medicine and Infectious Diseases[1] and Section of Clinical Microbiology,[2] Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

Received 3 November 1986/Accepted 15 January 1987

Blood, bronchoscopy-lavage, biopsy (lung, liver, kidney), sputum, and other (cecum, bone) specimens were inoculated into shell vials and conventional cell tube cultures seeded with MRC-5 cells over a 23-month period. Of 1,472 specimens, 182 (12.4%) yielded cytomegalovirus (CMV)-positive results from 81 patients. Significantly more CMV-positive specimens were detected in shell vials (n = 154; 84.6%) than in conventional tube cell cultures (n = 126; 69.2%) ($P < 0.01$). We found that 98 (53.8%) of the total 182 and 41 (42.7%) of the 96 blood specimens positive for CMV were detected by both the shell vial assay and conventional tube cell cultures. However, 56 (30.7%) of the total 182 and 31 (32.3%) of the 96 blood specimens positive for CMV were obtained exclusively in shell vials after detection with monoclonal antibody. Alternatively, 28 (15.4%) of the total 182 and 24 (25%) of the 96 blood specimens positive for the virus were isolated only in conventional tube cell cultures. Thus, although the shell vial assay was more sensitive and rapid than the conventional tube cell culture method, both systems must be used, especially for blood specimens, for the laboratory diagnosis of CMV infections.

Severe cytomegalovirus (CMV) infections are usually seen in congenitally infected infants and in immunocompromised hosts (6, 11). With the promise of effective treatment for CMV infections, rapid detection of this virus in the laboratory has become essential for the early initiation of antiviral chemotherapy (2, 12). Although DNA probe techniques are rapid, the methodology, reagents, and demonstrated efficacy of assays have not been extended into clinical laboratories for routine diagnosis of CMV infections (1, 10).

We previously reported the sensitive and specific detection of an early antigen of CMV with a monoclonal antibody by using 1-dram (ca. 3.7-ml) shell vials that contained a monolayer of MRC-5 cells on a 12-mm round cover slip followed by an immunofluorescence assay 16 h postinoculation (4, 13). These studies demonstrated the superiority of the shell vial assay over conventional tube cell cultures that consisted of screw-cap culture tubes (16 by 125 mm) containing a monolayer of MRC-5 cells; however, our laboratory experience was almost exclusively limited to use of urine specimens (5). In this study, we compared the detection of CMV in shell vials and conventional tube cell cultures for specimens other than urine over a 23-month period.

MATERIALS AND METHODS

Cells and virus. Human diploid cells (MRC-5) were obtained from Viromed Laboratories, Minneapolis, Minn. Eagle minimal essential medium containing 10% fetal bovine serum, penicillin, streptomycin, and gentamicin was used (4). Culture tubes (16 by 125 mm) containing monolayers were maintained with Eagle minimal essential medium until inoculation with specimens. Shell vials (1 dram [ca. 3.7 ml]), each containing a 12-mm round cover slip, were seeded with 50,000 MRC-5 cells per ml to produce a monolayer within 1 to 3 days. The AD169 strain of CMV (ATCC VR-538) was used as a positive control.

Specimens. (i) Collection and processing. Blood specimens (5 ml) were collected in heparinized tubes, and the leukocyte fraction was separated by Ficoll-Paque and Macrodex (Pharmacia, Inc., Piscataway, N.J.). Isolated lymphocytes and polymorphonuclear leukocyte fractions were combined, washed, and suspended in 2 ml of Eagle minimal essential medium (8). Tissue specimens were homogenized in a Stomacher Lab-Blender (no. 80; Tekmar Co., Cincinnati, Ohio) with nutrient broth added to make a 10 to 20% suspension. The homogenate was centrifuged at $700 \times g$, and the supernatant fraction was used for inoculation of cell cultures.

Bronchoalveolar lavage (B/BAL) specimens were collected with a flexible fiberoptic bronchoscope. The cells in the lavage fluid were adjusted to a concentration of 2.0×10^5 cells per 0.2 ml of saline by using a standard hemacytometer.

Culturette swabs (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) of throat or sputum were extracted into 2 ml of serum-free medium containing penicillin, streptomycin, and nystatin.

(ii) Inoculation and incubation. B/BAL, tissue, and swab extracts (0.2 ml) were inoculated into two shell vials and a conventional tube cell culture. The 2-ml combined suspension of lymphocytes and polymorphonuclear leukocytes was divided so that each of three shell vials received 0.3 ml of inoculum and a single cell culture tube received 1 ml. To avoid specimen toxicity, the sloughed leukocytes were removed from tube cell cultures the next day and replaced with fresh Eagle minimal essential medium. Shell vial and conventional tube cell cultures were incubated at 36°C as previously described (4).

Detection of CMV. Evidence of CMV infection in shell vials was detected with a monoclonal antibody to an early antigen (DuPont Specialty Diagnostics, Wilmington, Del.) in a fluorescence assay; evidence of CMV infection in conven-

* Corresponding author.

TABLE 1. Comparison between conventional tube cell culture method and the shell vial assay in 182 clinical specimens

Result of detection method		No. of specimens:							
Tube cell culture	Shell vial assay	Blood	B/BAL	Lung	Sputum	Liver	Throat	Other[a]	Total
+	+	41	30	17	3	4	1	2	98
+	−	24	3	0	0	0	0	1	28
−	+	31	15	2	5	0	3	0	56
Total		96	48	19	8	4	4	3	182

[a] Other: intestine, kidney, and bone marrow (conventional tube cell culture positive, shell vial assay negative).

tional tube cell cultures was detected by the recognition of specific cytopathic effect (CPE) characteristic of the virus.

RESULTS

A total of 1,472 blood, B/BAL, sputum, biopsy (lung, liver, and kidney), and other (cecum and bone) specimens were submitted over a 23-month period; CMV-positive results were obtained from 182 (12.4%) specimens from 81 patients. Of the total 182 CMV-positive specimens, 154 (84.6%) were positive by the shell vial assay compared with only 126 (69.2%) in conventional tube cell cultures (Table 1). Thus, although 98 (53.8%) of the CMV-positive results were positive by both conventional tube cell cultures and the shell vial assay, 56 (30.7%), and 28 (15.4%) were positive exclusively by conventional tube cell cultures or in shell vials, respectively ($P < 0.01$; sign test).

Over one-half ($n = 96$; 52.7%) of the 182 CMV-positive results in our study were obtained from blood specimens (Table 1). The majority of the CMV viremic infections in blood specimens ($n = 41$; 42.7%) were detected by both diagnostic methods; however, 55 of these were found by conventional tube cell cultures ($n = 24$; 25%) or by the shell vial assay ($n = 31$; 32.3%) ($P = 0.1$ [not significant]; sign test). With the exception of blood specimens, only 4 of 86 (4.7%) CMV isolates were detected by conventional tube cell cultures alone, whereas 24 of 86 (28%) were detected exclusively by the rapid shell vial assay. CMV isolates from B/BAL specimens were detected more often in shell vials than in conventional tube cell cultures ($P < 0.1$; sign test).

Microbial contamination of the conventional tube cell culture occurred in 8 (15%) of the 55 specimens (from 39 patients) that yielded positive CMV results by the shell vial assay but were negative for the virus by CPE. In specimens from eight of these patients, CMV was detected only by the shell vial assay, and no additional specimens were submitted. However all 31 remaining patients had CMV detected in the same or other body fluids or tissues ($n = 21$; 68%) or in urine ($n = 10$; 32%) within a 2-month period.

DISCUSSION

Recognition of the specific CPE produced in conventional tube cell cultures by CMV has been regarded as the standard for the laboratory diagnosis of these infections. However, because of the long time required for the development of the CPE (average, 9 days), rapid techniques were sought so that prompt positive reports of CMV could be available for the medical management, including antiviral chemotherapy, of these severe infections (7). For this purpose, we have used the shell vial assay in our laboratory for almost 3 years.

Early experience with 555 urine specimens inoculated into shell vials showed that 109 (19.6%) were positive for CMV 16 h postinoculation. CMV was isolated in conventional tube cell cultures from only 77 (13.8%) of the 555 specimens. Interestingly, all 77 isolates that were recovered in the conventional tube cell cultures were also detected by the rapid shell vial assay. Because of the sensitive and specific detection of CMV in shell vials, our laboratory has routinely processed urine specimens exclusively by this rapid method. However, in a limited analysis of 15 CMV-positive results from 189 blood and lung specimens, the shell vial assay was not positive in all instances in which the virus was detected by CPE in conventional tube cell cultures.

Extended evaluation of blood, B/BAL, and tissue specimens over a 23-month period indicates that 84 (46.1%) of the total 182 CMV-positive specimens were detected in only one assay system. The results for blood specimens were the most striking. Of a total of 96 positive blood specimens, 55 (57.3%) were found only in shell vials ($n = 31$; 32.3%) or only in conventional tube cell cultures ($n = 24$; 25%). To a lesser extent, 3 of 48 (6.3%) B/BAL specimens would have been reported as false-negative if the shell vial assay only was used. One explanation for these discrepant results might be based on the level of virus present in the specimen. For example, the mean time required for recognition of specific CPE in conventional tube cell cultures with the 71 specimens that were positive by both methods was 7.6 days compared with 9.5 days for 24 specimens that were conventional tube cell culture positive but shell vial assay negative ($P < 0.05$; two-sample t test). Therefore, specimens with high titers of CMV would presumably be detected in conventional tube cell cultures more rapidly than those with lower levels of CMV present, thereby favoring detection in both systems. Similar results have been described when the shell vial assay was used with bronchoscopy specimens (3, 9).

Inoculation of the leukocyte fraction of blood specimens is technically different from the processing of other specimens submitted for the diagnosis of CMV infections by the shell vial assay. Of the 2-ml suspension of leukocytes, 0.3 ml is inoculated into each of three shell vials, whereas 1.0 ml of the specimen is added to a single tube cell culture. Nevertheless, despite the larger volume of cells in the tube cell culture, the shell vial assay yielded more positive CMV results (31 versus 24) than the CPE detection method did.

In summary, we found that the rapid shell vial assay was more sensitive than the use of conventional cell culture tubes for the diagnosis of CMV from specimens other than urine. However, especially for blood specimens, both systems must be used for maximal detection of the virus.

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