Comparison of Conventional and Shell Vial Cultures for Detecting Cytomegalovirus Infection

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We tested 606 specimens for cytomegalovirus infection. Fifty-two specimens were positive by conventional culture versus forty-three by pre-cytopathic effect detection. Twenty-nine specimens were positive by both methods. Because of toxicity, specimens should be set up on both conventional and shell vial culture systems to achieve maximum sensitivity.

Most cytomegalovirus (CMV) infections in immunocompetent children and adults are asymptomatic or subclinical and self-limited. The virus has, however, been implicated in a wide spectrum of diseases, such as inclusion disease of the newborn, heterophil-negative mononucleosis-like syndrome, encephalitis, posttransfusion syndrome, and pneumonia (2). In immunosuppressed patients, especially those with suppression of T-lymphocyte function, CMV infection is a major clinical problem, often resulting in disseminated infection (6).

The standard method for diagnosing CMV infection is by culture (4); however, the growth of CMV is a slow process, and occasionally specimens with low amounts of virus may be falsely negative. New antiviral agents for the treatment of CMV infection have recently become available for clinical trials (3, 10). Because of the increasing importance of CMV in clinical medicine, rapid routine detection methods are needed for identifying this pathogen. For this purpose, the use of monoclonal antibodies to detect the viral antigen in cultures can substantially reduce the time for diagnosis. This rapid method is called pre-cytopathic effect (pre-CPE).

A total of 606 clinical specimens (245 blood samples, 92 bronchoalveolar lavage [BAL] samples, 86 urine samples, 75 gastrointestinal tract biopsies, 51 respiratory specimens from sources other than BAL, 41 cerebrospinal fluid samples, and 16 other specimens [including six bone marrow biopsies, two semen samples, two pericardial fluid samples, two lymph node samples, two placenta samples, one kidney biopsy, and one cervix sample]) were processed by the usual methods (8).

For conventional tube cultures, human diploid cells were obtained from Viromed Laboratories, Minneapolis, Minn., and maintained in minimal essential medium containing fetal bovine serum, penicillin, streptomycin, and gentamicin. Shell vials containing MRC-5 cells were also obtained from Viromed. After inoculation of the specimen (0.3 ml) in each of two shell vials, centrifugation was performed at 700 × g for 40 min. Afterwards, 2 ml of minimal essential medium containing fetal bovine serum, penicillin, streptomycin, and gentamicin was added to each vial. Cultures were then incubated at 36°C. Two conventional cell culture tubes inoculated with 0.3 ml of specimen were maintained for 4 weeks and were examined daily for the first 2 weeks and twice weekly for the next 2 weeks for CMV CPE.

After incubation, the medium of the shell vial was removed by aspiration and the cover slips were washed twice with phosphate-buffered saline (pH 7.2) and fixed with acetone for 10 min. Control cultures were infected with a laboratory CMV strain and used as positive controls. Uninfected monolayers were used as negative controls.

Indirect immunofluorescence staining was done on the cover slips. After the fixative was removed, the cover slips were washed with phosphate-buffered saline, taken from the vials, and placed cell side up onto glass slides. Thirty microliters of anti-CMV monoclonal antibody to both immediate early antigen and early antigen (Syva, Palo Alto, Calif.) was then added to each cover slip, and they were then incubated for 30 min at 36°C in a humidity chamber. The monolayers were washed in phosphate-buffered saline, 30 μl of the goat anti-mouse immunoglobulin G reagent fluorescein isothiocyanate-conjugated antibody (Syva) was added, and the cover slips were incubated at 36°C for 30 min in a humidity chamber.

Cover slips were washed, mounted cell side down onto glass slides under Syva mounting fluid, and examined with a Leitz epifluorescence microscope. We looked for specific immunofluorescence confined to the regular outlined shape of the nucleus (immediate early antigen) or small clusters of dense, brighter fluorescence within the nucleus (early antigen). The time between inoculation and staining ranged from 15 to 64 h for the early reading and 36 to 90 h for the late reading.

Of the 606 specimens processed by these two methods, 66 were positive by at least one of them. Fifty-two specimens were positive by conventional culture versus forty-three by pre-CPE detection (Table 1). Twenty-nine specimens were positive by both methods. Seventeen were detected by pre-CPE after the first incubation time (mean, 18.8 h), whereas twelve were detected at the second reading (mean, 48.7 h). Of the 43 positive specimens in the shell vial system, 14 of them failed to yield CMV in the conventional culture (3 blood samples, 4 BAL samples, 5 urine samples, and 2 others).

Of the 52 specimens which were positive in standard cell culture, 23 were negative by the rapid method (6 blood samples, 9 BAL samples, 4 urine samples, 2 gastrointestinal samples, and 2 others).

Of twenty-three specimens positive by conventional culture but negative by pre-CPE, the second pre-CPE reading time was more than 60 h for ten. In thirteen of these twenty-three, the fibroblast monolayer in one or both shell vials exhibited toxicity.

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TABLE 1. Comparison of pre-CPE immunofluorescence versus conventional culture on positive specimens

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of samples with the following test result:</th>
<th>Culture positive and pre-CPE positive (n = 29)*</th>
<th>Culture negative and pre-CPE positive (n = 14)*</th>
<th>Culture positive and pre-CPE negative (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ER LR</td>
<td>ER LR</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td>3 2</td>
<td>1 2</td>
<td>6</td>
</tr>
<tr>
<td>BAL</td>
<td></td>
<td>3 3</td>
<td>2 2</td>
<td>9</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td>6 5</td>
<td>3 2</td>
<td>4</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td>1 1</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td>4 1</td>
<td>2 0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td></td>
<td>0 0</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td>0 0</td>
<td>0 0</td>
<td>2</td>
</tr>
</tbody>
</table>

* ER, Early reading (mean, 18.8 h); LR, late reading (mean, 48.7 h). Figures in the two columns are mutually exclusive.

Besides the 52 CMVs, the conventional cultures of the 606 specimens yielded seven herpes simplex viruses, two respiratory syncitial virus, two enteroviruses, one adenovirus, one influenza B virus, and one parainfluenza 3 virus; none of these reacted in the pre-CPE method. Lately, various laboratories have described a method of accelerating the detection of CMV in cell culture (5, 9). In most of the studies, this technique was as sensitive or more sensitive than routine culture. In our study, 14 specimens that were conventional culture negative were positive by the rapid detection method, but 23 specimens were culture positive and pre-CPE negative.

Pre-CPE-positive, conventional culture-negative specimens may have resulted from (i) low viral titers in the samples, resulting in random positivity; (ii) detection of viral particles inducing immediate early or early antigens but not CPE or viable virus (7); or (iii) detection of CMV beyond the limit of sensitivity of standard cell cultures in specimens of low viral titer as a result of centrifugation (9).

On the other hand, pre-CPE-negative, conventional culture-positive specimens may have resulted from increased tissue culture toxicity in centrifuged specimens (1). Of the twenty-three specimens positive by the conventional culture and negative by the pre-CPE method, the fibroblast monolayer in one or both shell vials was damaged in thirteen (56%). Toxicity of the specimen (and therefore false-negative pre-CPE results) may be a greater problem with specimens such as blood, BAL, and gastrointestinal samples and less of a problem with urine samples. Because of the toxicity of certain specimens, it is advisable to set up conventional and shell vial culture systems in parallel to achieve maximum detectability of CMV infections.

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LITERATURE CITED