Comparison of Antibodies for Rapid Detection of Cytomegalovirus

DOMENICK N. RANDAZZO AND FRANK J. MICHALSKI*
MetPath, Inc., Corning Laboratory Sciences, Teterboro, New Jersey 07608

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Antibodies were compared for use in the spin-amplified shell vial method for rapid cytomegalovirus detection. Commercial antibodies by Du Pont Co., Whittaker M.A. Bioproducts, Bartels Immunodiagnostic, Virostat, and Serono Diagnostics were compared at incubation times of 16 to 48 h on 22 patient specimens. Only the Du Pont antibody showed 100% sensitivity and specificity and no nonspecific reactions.

The spin-amplified shell vial method developed by Gleaves et al. in 1984 (2) has gained wide acceptance as the new standard for laboratory diagnosis of cytomegalovirus (CMV) infection (3, 5–7). Since then, several commercially available monoclonal antibodies to CMV have been developed which can replace the antibodies used by Gleaves. These antibodies are now distributed by the Du Pont Co., Wilmington, Del., and other companies. This study compared the performance of these antibodies in the shell vial assay for CMV.

A stock preparation of CMV isolated from urine and passaged in MRC-5 cells was used in this study. Samples were frozen at a ratio of 1:1 in sucrose-phosphate freezing medium (4) at −70°C. The titer in MRC-5 cells was 10⁴ fluorescent nuclei 50% infectious doses per ml (FNID₅₀/ml). Patient specimens, urine samples, or bronchoalveolar lavages, from which CMV had been isolated, were also used. Two urine samples had been frozen at −70°C; all other specimens were stored at 4°C until use. Urine titers ranged from 10⁰ to 10⁴ FNID₅₀/ml.

The following monoclonal antibodies to CMV were used in the comparison fluorescence studies: (i) chromatographically purified mouse monoclonal anti-CMV early-nuclear-protein antibody immunoglobulin G2A (Du Pont) at a 1:20 dilution, the same antibody used in the original study by Gleaves (2), with fluorescein-conjugated affinity-purified goat anti-mouse immunoglobulin G (heavy- and light-chain specific) (Cooper Biomedical, Inc., Malvern, Pa.) at a 1:50 dilution (Du Pont indirect reagent); (ii) mouse monoclonal anti-CMV antibody (Whittaker M.A. Bioproducts, Inc., Walkersville, Md.) undiluted with the same second antibody described above (M.A. indirect reagent); (iii) fluorescein-conjugated mouse monoclonal anti-CMV antibody with Evans blue counterstain (Whittaker) undiluted (M.A. direct reagent); (iv) fluorescein-conjugated affinity-purified mouse monoclonal anti-CMV antibody with Evans blue counterstain (Bartels Immunodiagnostic, Bellevue, Wash.) undiluted (Bartels direct reagent); (v) fluorescein-conjugated mouse monoclonal anti-CMV antibody with Evans blue counterstain (Virostat, Portland, Maine) at 1:20 dilution (Virostat direct reagent); and (vi) mouse monoclonal anti-CMV early-nuclear-protein antibody undiluted with fluorescein-conjugated sheep anti-mouse immunoglobulin G with Evans blue counterstain (Serono Diagnostics, Norwell, Mass.) at 1:10 dilution (Serono indirect reagent).

Shell vials of MRC-5 cells were received weekly from Bartels and used within 1 week. After cells were examined, the medium was decanted from the vials, and the vials were inoculated with 0.1 ml of stock virus or 0.2 ml of patient specimen. The vials were centrifuged at 700 x g for 1 h at room temperature and then refed with 1 ml of Eagle minimum essential medium containing 2% fetal bovine serum, gentamicin, and amphotericin B (Fungizone). After incubation for 16 to 24 or 44 to 48 h at 35°C, the medium was decanted from the shell vial, and the cells were washed twice with sterile phosphate-buffered saline. The monolayer was then fixed with cold acetone for 20 min. After the acetone was decanted, the monolayer was air dried and then rehydrated with deionized water before staining. The proper reagent (30 μl) was added to each vial, and the vial was recapped and incubated at 35°C for 30 min on a shaker platform (Macro Use Card Test Rotator; Hynson, Westcott, Dunning). After the shell vial was rinsed twice with deionized water, the cover slip was removed and mounted. The cover slips were examined under a UV microscope with a 25× or 40× dry objective (Laborlux KW-Ploempak incident-light fluorescence illuminator with a 50-W mercury lamp; Leitz/Opto-Metric Div. of E. Leitz Inc., Rockleigh, N.J.). Cover slips with a minimum of three cells showing specific green nuclear fluorescence were considered positive. Non-specific fluorescence was defined as cytoplasmic or perinuclear fluorescence which made specific nuclear fluorescence difficult to observe.

Results are summarized in Table 1. A total of 22 patient specimens (18 urine specimens and 4 bronchoalveolar lavages) in which CMV had previously been detected and 2 stock CMV preparations were compared by the shell vial method with different antibodies at 16 to 24 or 44 to 48 h after infection. Noninfected cells were stained as controls. Stock virus was detected by all reagents, but only the Du Pont indirect reagent detected the virus in all the clinical specimens. Nonspecific fluorescence was detected in uninfected, stock-virus-infected, and clinical-specimen-infected cells by the M.A. direct, M.A. indirect, and Bartels direct reagents. The percentage of fluorescent nuclei detected by the Du Pont indirect reagent was little affected by incubation time, but a change from negative at 16 to 24 h to positive at 44 to 48 h was found in 11 of 19 (58%) positive comparisons with the other reagents. In two urine specimens, toxicity due to contamination at 48 h made the detection of fluorescent nuclei at this time impossible. Counterstain in the Virostat direct reagent was in such high concentration that a small amount of nuclear fluorescence could have been masked, and in the Serano indirect reagent, the counterstain fluoresced green, making it difficult to distinguish nuclear from cytoplasmic fluorescence.

These results show that the Du Pont indirect antibody...
method is highly specific and sensitive. The virus titer was an important factor for detectability, since the high-titer stock virus preparations were detected by all reagents in almost every case. Some urine specimens with high virus titers were detected by reagents other than the Du Pont reagent. The Du Pont indirect reagent worked best with the shorter incubation time, while other reagents that yielded positive results favored the longer incubation time. The shorter incubation time yielded faster results and less chance of contamination. Of the direct and indirect reagents and methods (three each) compared in this study, only the indirect reagent by Du Pont gave acceptable results. There has been speculation that the direct reagents do not perform well because, in the process of conjugation, the monoclonal antibodies may be functionally damaged. Unpublished data from our laboratory showed that a direct CMV conjugate that is no longer available worked almost as well as the Du Pont indirect antibody. It has also been reported that a direct conjugate works well in a study of 188 specimens (1). Several companies have monoclonal antibodies to CMV under development which were not available for use in our comparative study. This study shows that the testing of these new reagents should be performed not only on high-titer stock CMV but also on clinical specimens to determine the ability of the reagents to detect low-titer CMV. In conclusion, the Du Pont indirect monoclonal anti-CMV antibody is best for the rapid detection of CMV.

LITERATURE CITED


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### TABLE 1. Comparison of antibody reagents for detecting CMV in shell vials

<table>
<thead>
<tr>
<th>Reagent</th>
<th>No. of positive samples</th>
<th>No. of samples with nonspecific fluorescence</th>
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<tr>
<td></td>
<td>Stock virus prepn (n = 2)</td>
<td>Clinical specimens (n = 22)</td>
</tr>
<tr>
<td>Dupont indirect</td>
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<td>Virostat direct</td>
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<tr>
<td>Serono indirect</td>
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* See text for reagent descriptions.