Evaluation of a Direct Fluorescein-Conjugated Monoclonal Antibody for Detection of Cytomegalovirus in Centrifugation Culture

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A fluorescein-conjugated murine monoclonal antibody (MAb) reactive with cytomegalovirus (CMV) was evaluated for the detection of CMV in centrifugation culture. Of 188 specimens, 90 were positive for CMV in centrifugation culture. The fluorescein-conjugated MAb detected CMV in 86 of 90 (95%) specimens at 16 h postinoculation, and 88 of 90 (98%) were positive at 36 h. The fluorescein-conjugated MAb can be used in a direct immunofluorescence assay that can be completed in 15 min following cover slip fixation. Use of this antibody in centrifugation culture provides a convenient and rapid assay for the identification of CMV.

Cytomegalovirus (CMV) can cause severe manifestations in congenitally infected infants and is a major clinical problem in immunocompromised hosts (8,11). The clinical diagnosis of CMV infection is difficult, and rapid laboratory diagnosis of CMV infection is often necessary for appropriate patient management. Recent developments in laboratory techniques such as centrifugation culture and improved immunohistological staining which were facilitated by the availability of murine monoclonal antibodies (MAb) specific for CMV proteins have dramatically decreased the time needed to detect CMV from various specimen sources (1, 2, 4, 5, 7, 12). In this report we describe the evaluation of a fluorescein-conjugated MAb for the detection of CMV in centrifugation culture by a direct immunofluorescence (IF) assay. Additionally, the sensitivity of this CMV MAb reagent was compared with that of a different CMV MAb reagent in an indirect IF assay.

The fluorescein-conjugated MAb used in the direct IF assay identifies the 72,000-dalton immediate-early nuclear protein of CMV (Ortho Diagnostics Systems, Carpinteria, Calif.) (9). MAb 2H2.4 also reacts with the 72,000-dalton immediate-early nuclear protein of CMV and was used in the indirect IF assay (Du Pont Diagnostics, Wilmington, Del.) (10). A total of 188 clinical specimens (74 throat, 55 tissue, 41 urine, 10 bronchoalveolar lavage, and 8 other types) obtained from marrow transplant patients were inoculated at a volume of 0.2 ml per vial into each of four 1-dram (3.887-g) shell vials (Fisher Scientific Co., Seattle, Wash.) containing cover slip cultures of MRC-5 cells (Viromed Laboratories, Minneapolis, Minn.). Centrifugation was performed at 700 × g for 40 min at 34°C as previously described (4, 10), after which 1.0 ml of Eagle minimal essential medium containing 2% fetal bovine serum, penicillin, streptomycin, and gentamicin was added to each vial. Cultures were then incubated at 36°C for 16 or 36 h. Following incubation the medium was removed by aspiration and cover slips were washed twice with phosphate-buffered saline-Tween 20 (pH 7.2) and fixed with cold acetone (−40°C) for 20 min. Control cultures were infected with the laboratory CMV strain AD169 and used as positive controls. Uninfected monolayers were used as negative controls. One cover slip was stained with each of the two separate MAb preparations following incubation for 16 or 36 h as follows.

For direct IF cover slips were removed from the vials and mounted cell side up onto glass slides by using a clear adhesive. Monolayers were then covered with 1 to 2 drops of the direct MAb reagent and incubated at room temperature for 15 min in a humidity chamber. Following incubation the coverslips were removed from the slides, rinsed gently in distilled water for 5 to 10 s, and mounted cell side down onto glass slides under 75% glycerol in phosphate-buffered saline (pH 8.5). The cover slips were then examined with a Zeiss epifluorescence microscope at a magnification of 250 × .

Indirect IF staining was done in the shell vials with MAb 2H2.4. The procedure was the same as that previously described (10). Briefly, 150 μl of MAb 2H2.4 was added to each vial and incubated for 30 min at 36°C. The monolayers were then washed twice with phosphate-buffered saline for 5 min each time with intermittent shaking, and 150 μl of goat antimouse fluorescein isothiocyanate-conjugated antibody (TAGO, Burlingame, Calif.) diluted in phosphate-buffered saline was added to each vial and incubated at 36°C for 30 min. Cover slips were washed, counter stained with 0.02% Evans blue, and mounted and examined as described for the direct IF assay. Cover slip cultures showing distinctive apple green fluorescence of the cell nuclei were considered positive for CMV in both assays. Both MAb reagents stained only cell nuclei.

Specimens were also inoculated at a volume of 0.25 ml per tube into two culture tubes (16 by 125 mm) containing human foreskin fibroblast monolayers. Cultures were maintained for 5 weeks and examined twice weekly for the first 2 weeks and then weekly for the next 3 weeks for CMV cytopathic effects.

CMV was detected in 90 of 188 specimens (48%) by centrifugation culture. Of the positive specimens, 89 were detected at 16 h postinoculation, and all 90 were positive at 36 h. In the direct IF assay 86 (95%) specimens were positive at 16 h and 88 (98%) were positive at 36 h. In the indirect IF assay 87 (96%) specimens were positive at 16 h, and 89 (99%) were positive at 36 h (Table 1). Of the 90 positive specimens, 84 (93%) were positive at 16 h and 88 (98%) were positive at 36 h with both MAb preparations. Table 2 describes the six specimens which were not positive with both MAb prepara-
tions at either 16 or 36 h. These included four throat and two 
urine specimens. Neither of the urine specimens was 
positive with the direct MAb reagent. Three of the six discrepant 
specimens, two throat and one urine, failed to yield CMV in 
standard cell culture.

CMV was isolated by standard cell culture from 82 of the 
90 (91%) specimens which were positive in centrifugation 
culture at a mean of 11.5 days. For the 82 specimens which 
were positive in standard cell culture, there was a significant 
(P < 0.0001) inverse correlation between the average number 
of foci in cubic centimeters at 16 h and the number of weeks 
to positivity in cell culture (r = −0.49, determined by 
the Spearman correlation coefficient test). Among the five 
specimens positive in centrifugation culture but negative in 
standard cell culture that were not described above, the 
average number of positive foci in centrifugation culture at 
16 h was 16.5. No specimen negative in centrifugation 
culture with both MAb reagents was positive in standard cell 
culture.

The fluorescein-conjugated MAb reagent detected CMV in 
88 (98%) of the 90 specimens which were positive in the 
centrifugation culture assay, as compared with 89 (99%) 
specimens detected by the indirect IF assay. Both specimens 
which were negative in the direct IF assay at both 16 and 36 
h but positive in the indirect IF assay were urine specimens. 
The first yielded a single positive focus at 16 h in the indirect 
IF assay, but the cover slips were unreadable at 36 h in both 
specimens, and this specimen was negative in standard cell 
culture. The second specimen was negative with both re-
agents at 16 h, and only two positive foci were found at 36 h 
by indirect IF. The standard cell culture was positive at 35 
days. It thus appears that the apparent (1%) difference in 
sensitivity between these two assays may have been due to 
sampling variability with specimens of low viral titer. For 
practical purposes these assays appear to be of equivalent 
sensitivity.

Eight specimens (four throat and four urine) that were 
positive for CMV in centrifugation culture were negative in 
standard cell culture. This difference in sensitivity has been 
described in previous studies by using centrifugal enhance-
ment (1, 3, 4, 6). Centrifugal enhancement appears to allow 
the detection of CMV beyond the limit of sensitivity of 
standard cell culture in specimens of low viral titer (1). The 
failure to isolate CMV in conventional culture may also have 
been due to sampling differences; six of the eight specimens 
had less than or equal to six foci on the cover slips.

Both of these MAB reagents are reactive with the 72,000-
dalton immediate-early protein of CMV. Both reagents 
showed only nuclear staining patterns, which could not be 
distinguished from each other. However, after cover slip 
fixation, the direct IF assay with the conjugated MAb

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Total no.</th>
<th>Direct IF at:</th>
<th>Indirect IF at:</th>
<th>Standard cell culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat</td>
<td>74</td>
<td>42 44 42 44 44 40</td>
<td>16 h 36 h 16 h 36 h</td>
<td>40 42 42 42 42 42</td>
</tr>
<tr>
<td>Tissue</td>
<td>55</td>
<td>22 22 22 22 22 22</td>
<td>16 h 36 h 16 h 36 h</td>
<td>22 22 22 22 22 22</td>
</tr>
<tr>
<td>Urine</td>
<td>41</td>
<td>19 19 19 20 20 17</td>
<td>16 h 36 h 16 h 36 h</td>
<td>17 17 17 17 17 17</td>
</tr>
<tr>
<td>BAL</td>
<td>10</td>
<td>2 2 2 2 2 2</td>
<td>16 h 36 h 16 h 36 h</td>
<td>2 2 2 2 2 2</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>1 1 1 1 1 1</td>
<td>16 h 36 h 16 h 36 h</td>
<td>1 1 1 1 1 1</td>
</tr>
</tbody>
</table>

a One specimen was toxic and not readable at 36 h.

The detection of CMV by direct and indirect IF assays in 
centrifugation culture

TABLE 2. Specimens yielding discrepant results with the two 
MAb preparations

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Reading time (h)</th>
<th>No. of positive foci</th>
<th>Standard cell culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>16 36</td>
<td>0 N/C 1</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throat</td>
<td>16 36</td>
<td>0 0 1</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>5 6</td>
<td></td>
</tr>
<tr>
<td>Throat</td>
<td>16 36</td>
<td>0 2 4</td>
<td>Positive at 21 days</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>2 6</td>
<td></td>
</tr>
<tr>
<td>Throat</td>
<td>16 36</td>
<td>0 2 4</td>
<td>Positive at 14 days</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>1 2</td>
<td></td>
</tr>
</tbody>
</table>

a N/C, No cells.

The urine specimens. culture first yielded 90% of urine specimens. culture with the Spearman correlation test. Among the five specimens positive in centrifugation culture but negative in standard cell culture that were not described above, the average number of positive foci in centrifugation culture at 16 h was 16.5. No specimen negative in centrifugation culture with both MAb reagents was positive in standard cell culture.

The fluorescein-conjugated MAb reagent detected CMV in 88 (98%) of the 90 specimens which were positive in the centrifugation culture assay, as compared with 89 (99%) specimens detected by the indirect IF assay. Both specimens which were negative in the direct IF assay at both 16 and 36 h but positive in the indirect IF assay were urine specimens. The first yielded a single positive focus at 16 h in the indirect IF assay, but the cover slips were unreadable at 36 h in both assays, and this specimen was negative in standard cell culture. The second specimen was negative with both reagents at 16 h, and only two positive foci were found at 36 h by indirect IF. The standard cell culture was positive at 35 days. It thus appears that the apparent (1%) difference in sensitivity between these two assays may have been due to sampling variability with specimens of low viral titer. For practical purposes these assays appear to be of equivalent sensitivity.

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Both of these MAb reagents are reactive with the 72,000-dalton immediate-early protein of CMV. Both reagents showed only nuclear staining patterns, which could not be distinguished from each other. However, after cover slip fixation, the direct IF assay with the conjugated MAb

reagent could be performed in 15 min, as compared with 90 min for the indirect IF assay, and had only one step, as compared with two steps for the indirect IF assay. In some circumstances CMV can be detected in centrifugation culture as early as 4 to 6 h following inoculation (4). Used with the technique of centrifugation culture, this MAb reagent provides a more convenient and more rapid assay for the identification of CMV in clinical virology laboratories.

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LITERATURE CITED
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