Effect of Dexamethasone on Detection of Herpes Simplex Virus in Clinical Specimens by Conventional Cell Culture and Rapid 24-Well Plate Centrifugation

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During a 4-month period, two methods for rapid detection of herpes simplex virus (HSV) were examined: (i) pretreatment of A549 cells with dexamethasone for conventional tissue culture (277 specimens) and (ii) 24-well plate centrifugation using A549 cells with and without dexamethasone pretreatment and staining with serotype-specific monoclonal antibodies (Syva Co., Palo Alto, Calif.) after incubation for 16 to 18 h (153 specimens). By conventional tube cell culture, both with and without dexamethasone, HSV was identified in 88 of 277 (32%) specimens. Significantly more specimens were positive for HSV at 24 h (46 versus 27 specimens) and at 48 h (a total of 72 versus 59 specimens) \( P < 0.0001 \) in dexamethasone-treated A549 cells. Of the 153 specimens tested by conventional culture and 24-well plate centrifugation, HSV was detected in 44 (29%) by conventional culture, and by 24-well plate centrifugation with and without dexamethasone, HSV was detected in 32 (21%) and 30 (20%) specimens, respectively. The sensitivity, specificity, and positive and negative predictive values of 24-well plate centrifugation with A549 cells for detection of HSV were 73 (71%) without dexamethasone, 100, 100, and 90%, respectively. In conventional tube cell culture, pretreatment of A549 cells with dexamethasone results in more rapid detection of HSV. Centrifugal inoculation of dexamethasone-treated and untreated A549 cells in 24-well plates and staining with monoclonal antibodies after incubation for 16 to 18 h is an insensitive means to detect HSV in clinical specimens and should not replace conventional tube cell culture.

Much emphasis in the clinical virology laboratory has been placed on rapid viral diagnosis, especially with the advent of effective antiviral agents. The shell vial centrifugation method provides early detection of cytomegalovirus in clinical specimens (2, 3, 6). Results of shell vial centrifugation for rapid detection of herpes simplex virus (HSV) are controversial (4, 7, 10, 11). Preliminary data suggests that pretreatment of tissue culture cells with dexamethasone allows earlier detection of HSV (P. G. West, B. Aldrich, R. Hartwig, and G. J. Haller, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C-150, p. 348). To confirm that dexamethasone does permit more rapid recovery of HSV and to determine whether dexamethasone could increase the sensitivity of HSV detection by rapid centrifugation, we compared the sensitivity of A549 cells, with and without dexamethasone pretreatment, to HSV by both conventional tube cell culture and 24-well plate centrifugation, which is a modification of shell vial centrifugation (9, 12).

During a 4-month period, 277 clinical specimens from various sources (60% genital, 26% oral, 5% skin, 1% brain biopsy tissue, 4% other, 10% not specified) were obtained from patients with suspected HSV infection. Of the 277 specimens, 153 were processed for both conventional culture and 24-well plate centrifugation, and for the remainder, only conventional tube cell culture was performed. Approximately 30% of these specimens were received from hospitals and clinics outside the University of Nebraska Medical Center. Cotton swabs were agitated vigorously and extracted into 2 ml of Hanks balanced salt solution with 0.5% gelatin. Brain tissue was homogenized in Hanks balanced salt solution by using a sterile mortar and pestle to make a 10 to 20% suspension, and after centrifugation at 8,000 \( \times g \) for 30 min, the cell-free extract was used for virus recovery. To all specimens, an antibiotic suspension consisting of penicillin (100 U/ml), gentamicin (50 \( \mu \)g/ml), and amphotericin B (Fungizone) (40 \( \mu \)g/ml) was added. After arriving in the laboratory (usually within 12 h of collection), specimens were stored at 4°C until further processing (most often within 8 h of receipt).

The 24-well plate centrifugation method has been described in detail elsewhere (9). Briefly, sterile circular cover slips (10-mm diameter) were placed in sterile 24-well plates (Costar, Gaithersburg, Md.) and seeded with A549 cells (ATCC CCL 185; American Type Culture Collection, Bethesda, Md.) suspended in Leibovitz medium (Hazelton Dutchland, Inc., Denver, Pa.) supplemented with 1 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; Irvine Scientific, Santa Ana, Calif.) (L-H) and containing 10% fetal calf serum (HyClone, Logan, Utah). After a confluent monolayer had formed, the media from 12 wells were aspirated and replaced with L-H containing \( 10^{-5} \) M of dexamethasone. The plates were then incubated at 36°C for 24 h. Four wells (two with and two without dexamethasone) were inoculated with 0.2 ml of each specimen. Plates were centrifuged at 700 \( \times g \) for 40 min at 30°C, 1.0 ml of L-H (without dexamethasone) was added to each well, and plates were incubated at 36°C for 16 to 18 h. Cover slips were washed twice with phosphate-buffered saline, fixed in cold methanol for 10 min, and then stained with serotype-specific fluorescein isothiocyanate-labeled monoclonal antibodies (Syva Co.). For each specimen, two cover slips (one with and one without dexamethasone) were stained with each

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TABLE 1. Comparison of dexamethasone-treated\(^a\) and untreated A549 cells for detection of HSV by conventional cell culture\(^b\)

<table>
<thead>
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<th>Treatment</th>
<th>No. (%) of specimens positive for HSV(^c) at time (h):</th>
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<tbody>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>46 (52)</td>
</tr>
<tr>
<td>No dexamethasone</td>
<td>27 (31)</td>
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</tbody>
</table>

\(^a\) Incubation of cells in media containing \(10^{-5}\) M dexamethasone for 24 h prior to inoculation.
\(^b\) A total of 277 specimens were examined.
\(^c\) A total of 88 dexamethasone-treated and 88 untreated specimens were positive.

Our results suggest that for detection of HSV, centrifugal inoculation of specimens followed by staining with serotype-specific monoclonal antibodies (Syva) should not serve as a substitute for culture. Gleaves et al. reported that shell vial centrifugation with MRC-5 cells and staining with monoclonal antibodies allowed identification of all specimens that were positive for HSV by tissue culture (4). Because of these excellent results, we attempted to adapt the procedure to 24-well plates. In our hands, for detection of HSV, the 24-well plate centrifugation method with MRC-5 cells and staining with monoclonal antibodies had a sensitivity of only 70% (11). Peterson et al. likewise could not repeat the results of Gleaves and colleagues. They reported a sensitivity of 84% for detection of HSV by using shell vial centrifugation, MRC-5 cells, and staining with monoclonal antibodies (7). The A549 cell line, a human lung carcinoma continuous cell line (1, 5), has been shown to be an efficient and economical cell line for recovery of HSV (8, 11a; M. J. Jagua-Stewart and J. Tichota-Lee, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C-151, p. 348). Therefore, in our opinion, an evaluation of the use of this cell line for rapid detection of HSV by 24-well plate centrifugation was warranted. Compared with tissue culture, the sensitivity of 24-well plate centrifugation with A549 cells and staining with monoclonal antibodies for detection of HSV was 73%, which is similar to what we found for MRC-5 cells (11). Peterson and colleagues reported a higher sensitivity when staining with a polyclonal antibody than with monoclonal antibodies (7); therefore, it is possible that the use of a polyclonal antibody would likewise increase the sensitivity of our 24-well plate centrifugation test.

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LITERATURE CITED

Int. J. Cancer 17:62–70.