Use of A-549 Cells in a Clinical Virology Laboratory

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A-549 cells were compared with other cell lines for virus recovery, except from specimens submitted specifically for detection of cytomegalovirus. Of 589 specimens submitted specifically for detection of herpes simplex virus (HSV), 163 (28%) were positive for HSV—159 (75.7%) in A-549 cells and 156 (96%) in primary rabbit kidney cells. HSV cytopathic effect was identified an average of 0.6 day earlier in A-549 cells. Virus was recovered from 194 (11%) of 1,790 specimens submitted for general virus isolation. Of 40 HSV isolates, 85% were positive in A-549 cells, 72.5% were positive in MRC-5/WI-38 cells, and 42.5% were positive in HEP-2 cells. With adenovirus, 96% of 45 isolates were detected in A-549 cells, 62% were detected in HEP-2 cells, 38% were detected in MRC-5/WI-38 cells, and 31% were detected in PMK cells. Of the 76 enterovirus-positive specimens, 71% were positive in PMK cells, 62% were positive in A-549 cells, and 62% were positive in MRC-5/WI-38 cells. None of 12 respiratory syncytial virus, 14 rhinovirus, or 7 influenza A virus isolates were detected in A-549 cells. Of the cell lines examined, A-549 cells performed optimally for recovery of HSV and adenovirus, they allowed good growth of many of the enterovirus isolates, but they did not allow recovery of any of the respiratory syncytial virus, rhinovirus, or influenza A virus isolates.

In recent years, emphasis in the clinical virology laboratory has been focused on rapid diagnosis and cost containment. The shell vial centrifugation method (5), by allowing detection of cytomegalovirus in 16 h, has significantly improved turnaround time and patient management, especially since the advent of ganciclovir (1). This technique has been adapted with various degrees of success to allow rapid detection of other viruses (2, 3, 6). Inconsistent results have been reported for herpes simplex virus (HSV) (6, 13), and for detection of adenovirus (3) and influenza virus (2), the sensitivity of the shell vial assay at 24 h was unacceptably low. Consequently, conventional tissue cell culture remains the method by which most viruses are identified in the clinical virology laboratory. Given that viral isolation is both time consuming and costly, it is essential that the cell lines used are not only sensitive but also versatile, allowing detection of several different viruses. For initial recovery of certain viruses, primary cell lines are the most sensitive tissue culture system. For example, primary human embryonic kidney cells are the cell line of choice for isolation of adenovirus (9). Primary rabbit kidney (PRK) cells are both sensitive and specific for HSV, and primary monkey kidney (PMK) cells provide optimal recovery of influenza viruses and also allow good growth of many enteroviruses. Disadvantages of primary cell lines include the variable quality of the cells and the cost.

Recently, in an evaluation conducted in an Army Medical Center that focused on monitoring upper respiratory viral agents (in particular, adenovirus epidemics) in military personnel, Smith et al. demonstrated that the A-549 cell line, a human lung carcinoma continuous cell line (4, 10), was an efficient and economical cell line for recovery of adenovirus and HSV (12). They also observed that various enteroviruses and varicella-zoster virus were recovered in A-549 cells; however, the numbers were too small to allow firm conclusions. To determine the utility of A-549 cells in our institution (a university hospital virology laboratory that serves as a reference center for much of eastern Nebraska), we examined their use for all specimens with the exception of those submitted specifically for detection of cytomegalovirus, since Smith and colleagues found that cytomegalovirus did not grow in A-549 cells.

Autopsy and biopsy tissues, sputum, and stool specimens were homogenized in Hanks balanced salt solution (Hazelton Dutchland, Inc., Denver, Pa.) by using a sterile mortar and pestle to make a 10 to 20% suspension and then centrifuged at 8,000 × g for 30 min. The cell extract was used for virus recovery. Swabs (throat, nasopharyngeal, cervical, urethral, skin, rectal, and conjunctival) were extracted into 2 ml of Hanks balanced salt solution with gelatin. Bronchoalveolar lavage fluid, urine, cerebrospinal fluid, and nasopharyngeal and throat washings were not manipulated before inoculation. An antibiotic suspension, consisting of penicillin, gentamicin, and amphotericin B (Fungizone), was added to all of the above-described specimens except cerebrospinal fluid before inoculation of cell cultures.

Between 1 January 1986 and 31 March 1987, 589 specimens (70% genital) were submitted specifically for recovery of HSV. One tube each of A-549 cells (ATCC CCL185; American Type Culture Collection, Rockville, Md.) at passage 77 to 110 and PRK cells (M.A. Bioproducts, Walkersville, Md.) was inoculated with 0.2 ml of each specimen and examined daily for HSV-specific cytopathic effect (CPE) for 7 days (14 days for brain biopsies). Isolates were typed by using monoclonal antibodies (Syva, Palo Alto, Calif.).

Between 1 January 1986 and 15 September 1987, 1,790 specimens (respiratory, 1,304; oral cavity, 3; central nervous system, 188; rectal, 237; urine, 10; pancreas, 1; liver 47) of the specimens submitted for general virus isolation were included in the study. One tube each of A-549 cells, MRC-5 (ATCC CCL171) cells or WI-38 (ATCC CCL75) cells, HEP-2 (ATCC CCL23) cells, and PMK cells (M.A. Bioproducts) was inoculated with 0.2 ml of specimen, incubated at 37°C (35°C for respiratory specimens), and examined three times per week for 2 weeks for virus-specific CPE. Isolates were confirmed by direct immunofluorescence with monoclonal antibodies (HSV, Syva Co.; respiratory syncytial virus.

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Ortho Diagnostics, Inc., Raritan, N.J.) or by indirect immunofluorescence with monoclonal antibodies (adenovirus, Chemicon International Inc., El Segundo, Calif.; influenza A, Centers for Disease Control, Atlanta, Ga.). An attempt was made to type all isolates identified by CPE as an enterovirus. Neutralization was performed by standard procedures (11) with the Lim Benyesh-Melnick pools of antisera, obtained through the World Health Organization. Rhi

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toviruses were distinguished from enteroviruses by CPE, growth characteristics, and acid sensitivity (7). The data were analyzed by binomial distribution.

Of the 589 specimens submitted for detection of HSV, 163 (28%) were positive for the virus. Fifty-four (33%) of the isolates were type 1 and 109 (67%) were type 2. Of the genital isolates, 82% were type 2, and 85% of oral isolates were type 1. Of the 163 HSV-positive isolates, 159 (97.5%) were positive in A-549 cells and 156 (96%) were positive in PRK cells. There was no significant difference in the detection of HSV between A-549 and PRK cells, although CPE was recognized an average of 0.6 day sooner in A-549 cells. Of the 1,790 specimens submitted for general virus isolation, HSV was detected in 40 (2%) of them (97.5% were type 1). The number of HSV isolates recovered in each cell line and the average number of days to recognition of CPE are shown in Table 1. Eighty-four percent of HSV isolates were positive in A-549 cells, 72.5% were positive in MRC-5/WI-38 cells, and 42.5% were positive in HEp-2 cells. Ten HSV isolates were recovered only in A-549 cells. Significantly more HSV isolates were detected in A-549 cells than in HEp-2 cells (P < 0.001). There was no significant difference between A-549 and MRC-5/WI-38 cells in detection of HSV. The average time to recognition of CPE was 4 days for all three cell lines.

Forty-five specimens (2.5%) were positive for adenovirus. Of the 45, 43 (96%) were positive in A-549 cells, 28 (62%) were positive in HEp-2 cells, 17 (38%) were positive in MRC-5 or WI-38 cells, and 14 (31%) were positive in PMK cells (Table 1). Seventeen isolates were recovered only in A-549 cells. A-549 cells recovered significantly more adenovirus isolates than did HEp-2 cells (P < 0.001), MRC-5/WI-38 cells (P < 0.001), and PMK cells (P < 0.001). The average times to detection of CPE (Table 1) were approximately 8 days in A-549, HEp-2, and PMK cells and 9 days in MRC-5/WI-38 cells.

An enterovirus was recovered from 76 specimens (4%). Of these 76 isolates, 65 could be further identified as follows: 18 echovirus 6, 10 echovirus 11, 6 echovirus 18, 4 echovirus 4, 3 echovirus 9, 2 echovirus 7, 1 echovirus 27, 9 coxsackievirus B5, 3 each coxsackievirus B3 and B4, 1 each coxsackievirus B2 and B6, and 2 each poliovirus 1 and 2. Overall, 54 specimens (71%) were enterovirus positive in PMK cells, 47 (62%) were positive in A-549 cells, and 47 were positive in WI-38 or MRC-5 cells (Table 1). There was no significant difference between A-549 and WI-38/MRC-5 cells or between A-549 and PMK cells for the detection of enteroviruses. Enterovirus CPE became apparent an average of 1 day sooner in PMK cells than in A-549 or WI-38/MRC-5 cells. Type B coxsackieviruses, A-549 cells performed as well as PMK cells and significantly better than WI-38 cells (P < 0.01), whereas for echoviruses A-549 cells were somewhat, but not significantly, less sensitive than both PMK and WI-38 cells.

Rhinovirus, respiratory syncytial virus, and influenza A virus were recovered from 14, 12, and 7 specimens, respectively. All respiratory syncytial virus-positive specimens were detected in HEp-2 cells, whereas questionable CPE developed in A-549 cells in only two cases. Neither rhinovirus nor influenza A virus was recovered in A549 cells.

In summary, our results supplement those of Smith and colleagues, since we examined different cell lines. We found that for recovery of adenovirus and HSV, A-549 cells performed the best of the cell lines tested. Consequently, for recovery of HSV, we have replaced PRK cells with A-549 cells. Moreover, as suggested by Smith et al. (12), in our study many enterovirus isolates were recovered in A-549 cells. Because we had no varicella-zoster virus isolates, further comparative studies should be performed to determine the sensitivity of A-549 cells to that virus.

We did find, however, that there were limitations to A-549 cells. Neither respiratory syncytial virus, rhinovirus, nor influenza A virus was recovered in A-549 cells. In neutralization testing conducted to identify the enteroviruses, A-549 cells did not perform as well as did WI-38, MRC-5, or PMK cells. Moreover, the susceptibility of A-549 cells to both adenovirus and HSV decreased after passage 115. Smith and colleagues reported a loss of sensitivity of A-549 cells to adenovirus at passage 120 (12). Consequently, they did not use A-549 cells after passage 100, and they included controls to ensure sensitivity. We, likewise, recommend including controls, but in our experience A-549 cells performed well up to passage 110.

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table 1. Comparison of virus recovery and time to isolation for four cell lines

| Virus (total no. of isolates) | No. of isolates recovered (avg no. of days to CPE) with the following cell line: | A-549 | HEp-2 | PMK | WI-38/MRC-5-

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| HSV (40) | 34 (4) | 17 (4) | —** | 27 (4) |
| Adenovirus (45) | 43 (8) | 28 (8) | 14 (8) | 17 (9) |
| Echovirus (44) | 25 (6) | — | 31 (5) | 33 (6) |
| Coxsackie B17 (17) | 12 (5) | — | 11 (6) | 3 (5) |
| Poliovirus (4) | 4 (4) | — | 4 (3) | 3 (3) |
| Unknown (41) | 6 (7) | — | 8 (6) | 8 (6) |
| Total enteroviruses (76) | 47 (6) | — | 54 (5) | 47 (6) |

** — Cell line not evaluated.

LITERATURE CITED


