

## Evaluation of Three Types of Cell Culture for Recovery of Adenovirus from Clinical Specimens

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**The performance characteristics of HEK, HDF, and MK cells for adenovirus isolation were examined for eye and respiratory tract specimens. HEK cells were superior to HDF and MK cells in terms of both speed of virus detection and sensitivity.**

Adenoviruses are associated with acute respiratory disease and conjunctivitis in both adults and children (3, 5). Although isolation in cell culture is the primary method for demonstrating infection (7), limited information exists on the relative efficiency of different types of cell culture for adenovirus recovery. Therefore, we retrospectively examined our cell culture records (January 1980 to December 1985) to determine which of three cell culture types (primary monkey kidney [MK], human diploid fibroblast [HDF], and human embryonic kidney [HEK] cells) performed best in terms of speed and frequency of adenovirus isolation from eye and respiratory tract specimens.

All eye and respiratory tract specimens submitted for virus isolation were processed as per our laboratory protocol. Eye specimens were inoculated into one tube each of HEK cells (MA Bioproducts, Walkersville, Md.) and HDF

Department for confirmation and serotyping. All others were identified only by characteristic CPE. Specimens which required passage to confirm adenovirus CPE and those from which more than one virus was isolated were excluded. Statistical analysis of results was done by using the McNemar test for correlated proportions.

The cell culture findings of 150 adenovirus-positive specimens are presented in Table 1. In HEK cells virus was detected in 97% (59 of 61) of the adenovirus-positive eye specimens, whereas only 79% of positive specimens were detected in HDF cells ( $P < 0.01$ ). In addition, 75% (46 of 61) of eye specimens were positive for adenovirus earlier or only in HEK cells. The time (mean  $\pm$  standard deviation) to initial detection of CPE in HEK cells (Fig. 1) was  $4.2 \pm 2.2$  days, versus  $7.4 \pm 2.7$  days for HDF cells. HEK cells were also superior to both HDF and MK cells for the isolation of

TABLE 1. Detection of adenovirus in cell culture

Specimen type	No. of isolates	No. (%) of isolates positive for adenovirus in:			No. (%) of isolates in which adenovirus CPE was detected:			
					Earlier in <sup>a</sup> :		Only in:	
		HEK	HDF	MK	HEK	HDF/MK	HEK	HDF/MK
Eye	61	59 (97)	48 (79)	ND <sup>b</sup>	33 (54)	0	13 (21)	2 (3)
Respiratory tract	89	89 (100)	65 (73)	57 (64)	34 (38)	2 (2)	21 (24)	1 (1)

<sup>a</sup> Only eye isolates producing CPE in both HEK and HDF cells or respiratory tract isolates producing CPE in HEK, HDF, and MK cells were compared.

<sup>b</sup> ND, Not done.

cells (Flow Laboratories, Inc., McLean, Va.; MA Bioproducts). Respiratory tract specimens were inoculated into one tube each of HEK and HDF cells and one tube of MK cells (Flow Laboratories; MA Bioproducts). HEK and HDF cells were maintained in tubes containing modified Eagle medium with 0.3% sodium bicarbonate, 20 IU of potassium penicillin G, 50  $\mu$ g of gentamicin sulfate per ml, and 10% heat-inactivated fetal bovine serum. MK cells were maintained in serum-free Eagle medium. All tubes were incubated on roller drums at 0.2 rpm for 14 days at 36°C. The medium was changed on the same day that each culture was set up and on day 7 after inoculation. Cells were examined for cytopathic effect (CPE) daily for the first 5 days and every second to third day thereafter. Positive specimens were reported on the first day of observation of CPE characteristic of adenovirus in cell culture. Only isolates from patients with lower respiratory tract disease or conjunctivitis were sent to the New York State Health

adenovirus from respiratory tract specimens. All respiratory tract specimens positive for adenovirus (89 of 89) were detected in HEK cells, whereas only 73% (65 of 89) and 64% (57 of 89) of positive specimens were detected in HDF and MK cells, respectively ( $P < 0.01$ ). Of positive respiratory tract specimens, 62% were detected earlier or only in HEK cells. The mean time to detection of CPE in HEK cells compared with that for HDF and MK cells combined (Fig. 2) was  $4.8 \pm 2.8$  versus  $6.5 \pm 2.6$  days. No difference was seen in the average time to detection of CPE in MK or HDF cells when these cell types were examined separately.

In the present study, we compared the performances of HEK, HDF, and MK cells for the isolation of adenovirus from eye and respiratory tract specimens and demonstrated superior virus recovery in HEK cells. HEK cells not only were more sensitive than HDF and MK cells for the isolation of adenovirus but also performed better in terms of speed of virus detection. In a similar study with HDF, MK, and HeLa cells, Herrmann (6) found that only 66% of clinical specimens positive for adenovirus were detected in HDF cells by

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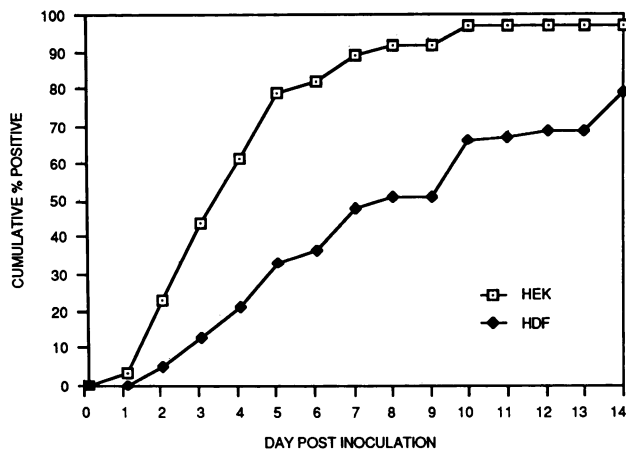


FIG. 1. Time required for recovery of adenovirus from eye specimens in HEK and HDF cells.

day 10, whereas 80% were positive in MK and HeLa cells. We found MK cells comparable to HDF cells for the cultivation of adenovirus from respiratory tract specimens. However, non-human cell cultures such as MK, although permissive for adenovirus growth, are said to produce low yields of infectious particles, which may limit successful subpassage (8). Continuous cell lines such as HeLa, HEp-2, and KB cells are useful for adenovirus recovery but may be difficult to maintain in good enough condition for the easy recognition of viral CPE (6, 10). Vargosko et al. (10) found that in conventional cell culture with KB and HEp-2 cells, CPE was detected in only 18% of adenovirus-positive respiratory tract specimens by day 4 postinoculation, and Herrmann (6) reported that CPE was seen in 33% of adenovirus-positive specimens inoculated into HeLa cells by day 4. In contrast, greater than 50% of both positive eye and respiratory tract specimens detected in the present study were

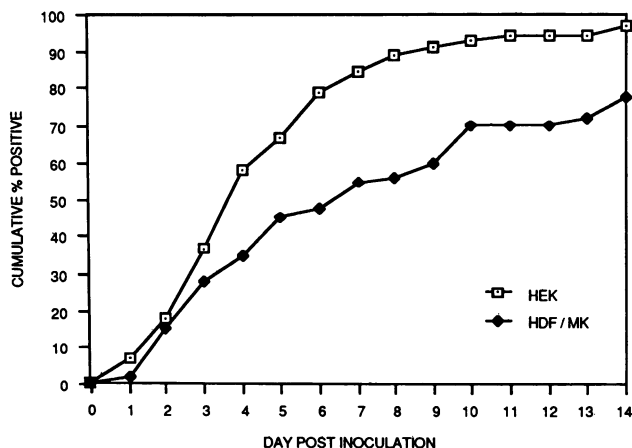


FIG. 2. Time required for recovery of adenovirus from respiratory tract specimens in HEK, HDF, and MK cells.

detected by day 4 in HEK cells. Recent reports (1, 2, 4, 9) evaluated A549 cells, a human lung carcinoma cell line, for the isolation of adenovirus from respiratory tract specimens, and 293 cells, a continuous line of transformed HEK cells, for the isolation of both enteric and nonenteric adenoviruses. Each cell line appeared promising. Additional comparisons of these continuous lines with HEK cells may establish one of them as the new "gold standard." Unfortunately, at present, information on the time to detection of CPE in 293 and A549 cells and on their relative sensitivity is limited.

The high isolation rate and the decreased time to initial detection of CPE realized in HEK cells enables the laboratory to report the presence of adenovirus in clinical specimens with increased efficiency. In addition, the highly distinctive CPE produced by adenoviruses in HEK cells facilitates presumptive identification of virus in cell culture. For many laboratories doing diagnostic virology, the cost and limited availability of HEK cells may make their routine use prohibitive. However, until a more sensitive method is found, isolation in HEK cells should serve as the "gold standard" in the evaluation of new culture and rapid diagnostic techniques for the detection of adenovirus in clinical specimens.

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