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Comparative Susceptibilities of Human Embryonic Fibroblasts and HeLa Cells for Isolation of Human Rhinoviruses

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The recovery of human rhinovirus (HRV) from nasal washings and nasal and pharyngeal swabs from volunteers with naturally acquired colds was compared in different cell types. Human embryonic lung fibroblast (HELF) strain WI-38 (sensitivity, 61 to 84%) and HeLa-I, an HRV-susceptible HeLa cell clone (sensitivity, 86 to 94%), were the most sensitive cell types used. HELF WI-38 cells showed a cytopathic effect earlier than the other cells used, and the different strains of HRV-susceptible HeLa cells varied in their sensitivities for HRV isolation. HRV was detected in a single cell type in 20 to 35% of the positive samples, suggesting that use of a combination of different HRV-susceptible cell lines is the best approach for the recovery of HRV. Although nasal washings tended to yield more HRV isolates than nasal and pharyngeal swabs, the two sampling methods were not found to be significantly different.

The routine laboratory diagnosis of human rhinovirus (HRV) infection is based on the recovery of HRV from respiratory secretions in cell culture. The presence of HRV is indicated by the typical cytopathic effect (CPE) in infected cells and is confirmed by the acid sensitivity of the isolate (9). In vitro propagation of HRVs has been accomplished in different cells of primate origin, such as human diploid fibroblasts, simian or human embryonic kidney cells, and human heteroploid HeLa cells, as well as in organ cultures of human respiratory epithelium. Of these, human embryo fibroblasts and HeLa cells have been most frequently used for the recovery of HRV from clinical specimens, because they are susceptible to a broader range of HRV serotypes (9).

Not all strains of HeLa cells are equally susceptible to HRV infection (5), and several clones of HeLa cells that are particularly supportive of HRV replication have been described (1, 5, 13–15). These have been used chiefly for the purposes of propagating HRV in vitro and preparing high-titer virus stocks. Correspondingly, different types of human fibroblasts also vary in their susceptibilities to HRV infection (2, 7, 20).

In addition, the optimal sampling method for HRV isolation from individuals with naturally occurring colds has not been determined. In studies of experimentally infected subjects, twice as many HRV isolates were recovered on nasal washings (NWs) as on nasal and pharyngeal swabs (NPSs) (3). Consequently, we conducted two studies to compare the sensitivity of HRV recovery from nasal NWs and NPSs from patients with naturally acquired colds in different cell strains.

Clinical studies and specimens. In the first study (September 1987) we analyzed 142 NWs collected from participants in the placebo arm of a previously published two-phase study on the prophylaxis of natural colds with beta$_{serine}$ interferon (18). In that study, subjects were prospectively monitored by daily recording of their symptoms. NWs were collected for virus isolation if the subject reported at least one respiratory symptom (excluding sneezing) on 2 consecutive days or two or more respiratory symptoms on 1 day.

In the second study (September and October 1994), adults with colds of less than 48 h were recruited through advertising. Volunteers were interviewed by a study nurse, and the presence of a cold was defined by the presence of at least two of the following symptoms: rhinorrhea of at least moderate severity, nasal stuffiness, sore throat, and cough. Both NWs (5 ml per nostril) and NPSs were obtained for virus isolation. Both studies were approved by the Human Investigation Committee of the University of Virginia, and written informed consent was obtained from all participants.

NWs were collected as described previously (11). In the second study, prior to collecting NWs, a vigorous deep nasal swab sample was collected through both nostrils and a second swab sample was collected from the throat with cotton-tipped wooden swabs. The two swabs were combined in one vial containing 2 ml of viral transport medium consisting of beef heart infusion broth with 1% bovine serum albumin and antibiotics. All samples were transported to the laboratory on ice.

Cells and viruses. Monolayers of human embryonic lung fibroblast (HELF) strain WI-38 were purchased from Baxter Diagnostics, Bartels Division, Issaquah, Wash. (designated WI-38/B), and from BioWhittaker, Walkersville, Md. (designated WI-38/W). Monolayers of HELF strain MRC-5 were purchased from BioWhittaker. HeLa-M, an HRV-susceptible strain of HeLa cells contaminated with mycoplasma (19), originally used by Vincent Hamparian (5, 10), was a gift from David A. J. Tyrrell (Common Cold Unit, Medical Research Council, Salisbury, England). HeLa-H is an HRV-susceptible strain originally provided by V. V. Hamparian to R. R. Rueckert (14) and treated to eliminate mycoplasma contamination (12). HeLa-I is a HeLa cell clone that was originally a gift from James Darnell (Rockefeller University) to Bayer Research Center. The expression of ICAM-1 on the cell surface of HeLa-I was measured by fluorescence-activated cell sorter analysis with ICAM-1-binding monoclonal antibody 78.4 (8) following standard procedures with linearization of the mean peak channel output to allow for direct comparison of samples (16). HeLa-I cells expressed ICAM-1 at twice the level of HeLa cells, strain Ohio, and 52 times the level of HeLa cells, strain 229, a non-

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TABLE 1. Relative sensitivities of different cell lines for HRV recovery from nasal washings obtained from subjects with a common cold[a]

Specimen and cell line[a]	No. (%) of positive samples[b]		
	First study	Second study	
		First 90 samples	Subsequent 256 samples
All specimens tested	142	90	256
Specimens positive in any cell line	26 (100)	63 (100)	158 (100)
WI-38/B	NA[c]	45 (71)	119 (75)
WI-38/W	16 (61)	48 (76)	132 (84)
MRC-5	10 (38)	NA	NA
FT	10 (38)	NA	NA
HeLa-I	NA	54 (86)	149 (94)
HeLa-H	NA	26 (41)	NA
HeLa-M	16 (61)	40 (63)	NA

[a] See text for cell strain title abbreviations.
[b] Percentage of the total of positive samples in that study or subset.
[c] NA, not applicable.

ICAM-1-expressing strain. Subsequently, HeLa-I was shown to support the rapid propagation of HRV stocks (15). The HeLa cells were grown in minimal essential medium (MEM) with 10% bovine serum (5% fetal serum and 5% calf serum) and inoculated at 70 to 90% confluence. Fetal tonsil (FT) cells, originally a gift from Marion Cooney, University of Washington, Seattle (4), were also propagated in our laboratory. All monolayers were prepared in screw-cap roller tubes by standard techniques (17).

Specimen processing. In the first study, NWs were inoculated shortly after collection into duplicate monolayers of WI-38/W and MRC-5 cells, and the excess was stored frozen at −70°C. Two months after completion of the study, the stored NWs were inoculated into duplicate tubes of HeLa-M and FT cells.

In the second study, NWs and eluates from NPSs were frozen at −70°C for later processing. The 90 first consecutive NWs for the second study were inoculated into one tube each of WI-38/B, WI-38/W, HeLa-M, HeLa-H, and HeLa-I cells. The remaining 256 samples were inoculated into one tube each of WI-38/B and WI-38/W cells and two tubes of HeLa-I cells. In both studies, monolayers were inoculated with 0.2 ml of sample, absorbed in stationary racks for 1 h at 34°C, washed once with Dulbecco's phosphate-buffered saline (BioWhittaker), replenished with 1 ml of maintenance medium, and incubated at 34°C in rolling racks at 12 revolutions per h. Maintenance medium for HeLa cells was McCoy's medium (BioWhittaker) with 2% fetal bovine serum, 30 mM $MgCl_2$, and antibiotics. For HELFs, maintenance medium was MEM with 5% fetal bovine serum in the first study and 5% Fetalclone (HyClone, Logan, Utah) in the second study. Cultures were read every other day for 14 days for the presence of viral CPE. On day 7 after inoculation, HeLa cell monolayers were blind-passed and HELF cultures were decanted and refed with 1 ml of fresh maintenance medium. To pass HeLa cell cultures, monolayers were frozen and thawed and 0.2 ml of the supernatant was inoculated onto fresh monolayers. HRV isolates were confirmed by acid susceptibility testing (9).

Effect of cell culture type. In the first study, HRV was isolated from 26 (19%) of 142 NWs. WI-38/W and HeLa-M cells each detected 61% of the positive NWs, whereas 38% of NWs were detected by MRC-5 and FT cells ($P = 0.17$) (Table 1). HRV was detected in a single cell type in 9 (35%) of 26 positive NWs, and 6 of those were positive only in HeLa-M cells. Seventeen (65%) of the 26 HRV isolates obtained in the first study were detected in WI-38 or MRC-5 cells inoculated with fresh NWs, whereas 20 (77%) were later detected in HeLa-M or FT cells upon thawing of frozen NWs.

In the second study, HRV was isolated from 63 (70%) of the first 90 NWs tested. Of the 63 positive NWs, 52 (83%) were positive in WI-38 cells and 55 (87%) were positive in one or more of the three HeLa cell strains used. Among the three strains of HeLa cells, HeLa-I cells detected 54 positive NWs, whereas 40 were detected by HeLa-M cells ($P = 0.006$) and only 26 were detected by HeLa-H cells ($P < 0.001$) (Table 1). Nineteen (30%) of the 63 positive NWs were positive in only one cell type, 11 of which were positive only in HeLa cells, and 10 of those were in HeLa-I cells. In this subset of 90 samples, it was notable that WI-38 cells detected 50% of the positive samples by day 2, 2 days earlier than HeLa-I cells and 4 to 8 days earlier than HeLa-H and HeLa-M cells. All three strains of HeLa cells generally showed a CPE later than WI-38 cells, but HeLa-I cells detected 50% of the positive NWs by day 4, and the cumulative frequency of detection by HeLa-I cells equaled that by WI-38 cells by day 7 (Fig. 1). All cell types showed a second rise in the number of isolates at about day 11, likely attributable to the blind passage of HeLa cells and feeding of WI-38 cells with fresh medium on day 7.

Given the superior sensitivity of HeLa-I and WI-38 cells for HRV isolation in the first subset of 90 samples, the remaining 256 samples of the second study were tested only in these two cell lines. HRV was isolated from 158 (62%) of these 256 NWs: 149 (94%) in HeLa-I cells and 137 (87%) in WI-38 cells. Thirty-one (20%) NWs were positive in only one cell line, and significantly more were positive in HeLa-I cells (22 of 31) than in WI-38 (9 of 31) fibroblasts ($P = 0.003$). The cumulative frequency of HRV isolation per day of incubation in the two cell types was comparable to that for the first subset of 90 samples (data not shown).

Of the 203 NWs positive in HeLa-I cells in the two subsets of the second study, 41 (20%) were detected only after blind passage of those cells on day 7.

Effect of sample type. To compare the sensitivities of the sample collection method, recovery rates from NWs and NPSs were compared for a subset of samples from 100 consecutive subjects from the second study. HRV was recovered in 69 of these samples. The proportions of positive NW (96%) and

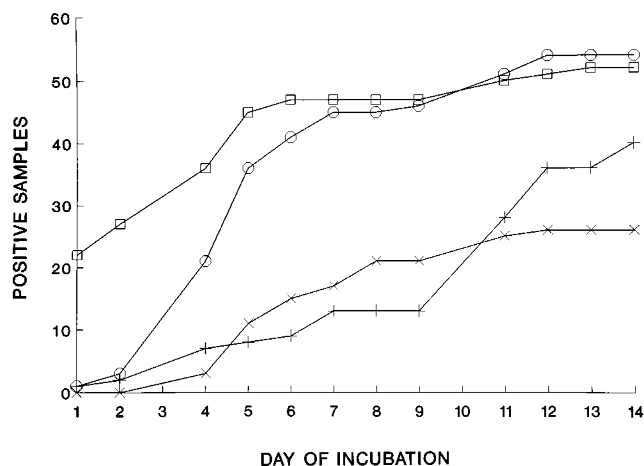

FIG. 1. Daily cummulative number of nasal washings ($n = 90$) positive for HRV CPE in HELF WI-38 (□), HeLa-I (○), HeLa-M (+), and HeLa-H (×) over 14 days of observation. On day 7, HeLa cells were blind passed and HELF were refed with fresh medium.

TABLE 2. Comparison of NWs and NPSs for the recovery of HRV from 100 subjects with a common cold

Specimen and cell line	No. (%) of positive samples/ sampling method	
	NW	NPS
Positive specimens	66[a]	60[b]
Specimens positive in WI-38	56 (85)	49 (82)
WI-38/B	53 (80)	40 (67)
WI-38/W	56 (85)	41 (68)
Specimens positive in HeLa-I	62 (94)	54 (90)
Tube 1	60 (91)	52 (87)
Tube 2	62 (94)	49 (82)

[a] Ninety-six percent of 69 total positive samples.
[b] Eighty-seven percent of 69 total positive samples.

NPS (87%) samples were not significantly different (Table 2). However, discrepancies were noted in 12 (17%) of the positive samples. In nine (75%) samples, only the NW was positive, and in three (25%) samples, only the NPS was positive. In addition, a higher frequency of disagreement was found between duplicate tubes inoculated with NPSs than with NWs for both HELFs and HeLa-I cells (Table 2).

Comment. The present studies found that the sensitivities of susceptible cell lines are not uniform for the isolation of HRV strains from clinical samples.

The overall rate of HRV recovery was relatively low in the first study, likely as a consequence of the design of that particular study. However, comparisons between the relative contribution of each cell type can be made. The evident lack of superiority of FT cells over WI-38, MRC-5, and HeLa-M cells for HRV recovery in the present study is in contrast to previous observations (4, 6, 7). This finding and the existence of more readily available HRV-susceptible cell lines make the routine use of FT cells for HRV isolation from clinical samples unnecessary.

The fact that HRV was recovered in only one cell type for 20 to 35% of the positive NWs supports the use of combinations of cell types in clinical studies when multiple unknown HRV serotypes are expected to occur. In addition, the finding that most of the HRVs isolated in a single cell line were obtained in HeLa cells, particularly in HeLa-I cells, highlights the value of these cells for the primary isolation of HRV. Also, the fact that 20% of the HRV isolates isolated in HeLa-I cells were detected only after the blind passage performed on day 7 underscores the importance of blind passing of these cells for optimizing the recovery of HRV from clinical samples.

The sensitivities of NWs and NPSs for HRV isolation were not significantly different, indicating that in circumstances when it may be impractical to obtain an NW, NPSs could be used without a significant loss of sensitivity. However, the higher frequency of disagreement between tubes inoculated with NPSs compared with that with tubes inoculated with NWs suggests that NW samples contain higher virus titers and thus should be preferable when only one cell monolayer is used for HRV recovery.

The recovery of HRV from frozen-thawed NWs in HeLa-M and FT cells was actually higher than that from fresh NWs in MRC-5 and WI-38 cells in the first study. While the results were obtained with different cell lines, this observation indirectly suggests that freezing-thawing is not too deleterious for the recovery of HRV. This is in keeping with the results from a previous study of 33 subjects experimentally infected with HRV strain Hanks. In that study, similar proportions of HRV recovery were obtained on days 2 through 6 postinfection with both frozen-thawed NWs (91, 79, 79, 61, and 64%, respectively) and fresh NWs (85, 76, 64, 52, and 61%, respectively) (10a).

In summary, we have found that the NW and NPs sampling methods are not significantly different as sampling methods for HRV recovery and that the cell types commonly used are not uniformly sensitive for primary isolation. HeLa-I, an HRV-susceptible clone of HeLa cells and HELF strain WI-38 were the two best cell lines for HRV recovery.

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