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Comparison of Human Fibroblast Cells and Primary Rabbit Kidney Cells for Isolation of Herpes Simplex Virus

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Human foreskin fibroblast cells and primary rabbit kidney cells were compared for efficiency in isolation of herpes simplex virus from 1,100 clinical specimens. Of the fibroblast cultures, 265 were positive, whereas 268 primary rabbit kidney cultures were positive. The results indicate that either cell type is acceptable for diagnostic use.

Several factors have recently increased the demand for laboratory confirmation of suspected herpes simplex virus (HSV) infections. Among them are the prevalence of HSV infections, the increased interest of clinicians as well as the general public in HSV infections, and the availability of antiviral therapy.

Virus isolation is used by clinical virology laboratories as the most sensitive and accurate method of detecting HSV. HSV replicates well in a number of different cell types of human, monkey, rabbit, guinea pig, and mouse orgin (6). Most laboratories use some type of human fibroblast cell line such as WI-38 or MRC-5 for isolation of HSV. Several studies as well as routine laboratory experience indicate that human fibroblast cells are both sensitive and easily handled in the clinical laboratory (2, 4–7).

Two studies have compared the efficiency of human fibroblast cells to that of primary rabbit kidney (PRK) cells for the isolation of HSV. One study has reported that the human fibroblast cells detect only 83% of the cultures positive in PRK cells (3). In an earlier paper, it was reported that WI-38 cells detect only 65% of isolates positive by PRK cells (1). The results of these studies may indicate that human fibroblast cells are not as sensitive as PRK cells and would not be the cell type of choice for HSV isolation. However, the data from both of these studies were based on a low number of positive cultures from clinical samples. In addition, in one study the type of human fibroblast cells used (MRC-5, WI-38, and Flow 2000) was varied throughout the experiment making it difficult to draw conclusions concerning specific cell types (3).

Presented here is an evaluation of the efficiency of detection of HSV by using PRK cells and human fibroblast cells inoculated in parallel with a large number of clinical samples.

PRK cells were purchased from M. A. Bioproducts (Walkersville, Md.) and received weekly. A human foreskin fibroblast cell line (NHF) was established by the tissue explant method of Nichols et al. (5). NHF cells were originated and maintained in Eagle essential medium with Earle salts and 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (MEM) supplemented with 10% fetal bovine serum. NHF cells were split at a ratio of 1:3 every 5 to 7 days and could be preserved at −70°C by standard techniques (8). Culture tubes were prepared from passages 7 through 15. Generally, NHF cells were useful for up to passage 25.

All specimens were received in Virocult specimen tubes (Medical Wire and Equipment, Cleveland, Ohio). Of the specimens cultured, 81% were genital cultures, and the remainder were vesicle cultures from other areas of the body. A swab was placed in a tube with 4 ml of MEM containing 2% fetal bovine serum, 50 μg of gentamicin per ml, 100 U of penicillin per ml, and 10 μg of amphotericin B per ml and mixed on a vortex shaker. Swabs were discarded, and a tube of PRK cells and a tube of NHF cells were inoculated with 1 ml each, shaken slowly for 1 h at 37°C, and incubated at 37°C.

Cultures were examined daily for the presence of HSV-specific cytopathic effect. Cells from all suspected positive cultures were scraped from the tube, smeared, air dried, and acetone fixed on a glass slide. The presence of HSV was confirmed by staining with fluorescein-conjugated rabbit anti-HSV type 2 which reacted strongly with both HSV types (Dako Co., Santa Barbara, Calif.). NHF cultures were examined for 10 to 14 days. PRK cultures were examined for only 7 days, owing to the degeneration of the monolayer.

A total of 1,100 clinical specimens were inoculated into both NHF and PRK cells; of the total, 271 specimens were positive for HSV. The NHF cells detected 265 (24.1%) positive specimens, and the PRK cells detected 268 (24.4%) positive specimens. Three NHF positive results were not seen in PRK, and six PRK positive results were not seen in NHF. This difference was not statistically significant (Table 1).

Of the 271 positive specimens, 223 or 82% were detected on the same day in the two cell types. NHF cells detected 4.8% of the positive specimens a day or more earlier than did PRK cells, whereas the PRK cells detected 9.6% of the positive specimens a day or more earlier than the NHF cells (Table 1).

There were no significant differences in the time to detect positive results. Both cell types detected greater than 70% of

TABLE 1. Comparison of NHF and PRK cells for the detection of HSV from 1,100 clinical cultures

Cell type	No. positive (%)[a]	No. positive, 1 or more days faster[b] (% of positive results)
Human fibroblast	265 (24.1)	13 (4.8)
PRK	268 (24.4)	26 (9.6)

[a] The difference in the positive results is not significant ($P > 0.1$, chi-square).

[b] The difference in earlier detection is significant ($P < 0.05$, chi-square).

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TABLE 2. Speed of detection of 271 positive HSV cultures

Cell type	% Positive on day after inoculation[a]						
	1	2	3	4	5	6	>6
Human fibroblast	27	72	85	94	95	96	98
PRK	29	74	88	96	97	99	99

[a] There was no significant difference in the percent positive at any day ($P > 0.1$, chi-square).

the positive specimens by day 2 and greater than 90% of the positive specimens by day 4 (Table 2). Qualitatively, the HSV cytopathic effect was easier to detect in the NHF cells, owing to the regularity of the cell monolayer. No viral cytopathic effect could be detected in PRK cells after 6 days of incubation, thus the examination of cultures inoculated on PRK cells for longer than this was not productive.

The results presented here contradict the results of the two previous studies which compared the sensitivity of human fibroblast and PRK cells for HSV isolation (1, 3). The data indicate that there is no significant difference in the total number of positive HSV specimens that can be detected by using human foreskin fibroblast or PRK cells. In this study, 82% of the cultures were positive on the same day in both cell types. Faster detection by PRK cells accounted for less than 10% of the total positive results. This is much less of a difference than previously reported where 56% of 48 positive HSV cultures were positive the same day in PRK and human fibroblast cells, and the remaining 44% were detected first by PRK cells (3).

The NHF cells used here were produced in our laboratory, whereas the PRK cells were obtained commercially. There are, however, many types of fibroblast cells commercially available. It is very likely that some of these cell types are at least as sensitive as the foreskin fibroblast cells used here. This could be determined by parallel virus titrations and comparison of isolation rates with identical clinical specimens.

It was noted during the course of the experiments that one of the eight lots of PRK cells used had a much lower sensitivity than the NHF cells or other lots of PRK cells. This lot-to-lot variation in sensitivity may be a problem when using commercially prepared primary cells. Each lot of primary cells can come from a different preparation using different animals, whereas a fibroblast cell line is produced from the same frozen stock. This can result in a greater chance of lot variation in primary cells than in a cell line.

Since the difference in efficiency of detection of HSV was slight between the two cell types studied, other considerations can be addressed when selecting a cell type for use in the laboratory. Human fibroblast cells are also a good substrate for varicella-zoster and cytomegalovirus; PRK cells are not. This cell trophism can be an advantage depending on whether other viruses besides HSV are being sought. The cost, availability, and continued lot-to-lot quality of a cell type also must be considered when selecting a cell type for use in laboratories. The results presented here indicate that either human fibroblast cells or PRK cells are acceptable for clinical use in isolation of HSV.

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LITERATURE CITED

1. **Douglas, R. G., and R. B. Couch.** 1970. A prospective study of chronic herpes simplex virus infection and recurrent herpes labialis in humans. J. Immunol. **104**:289–295.
2. **Herrmann, E. C.** 1967. Experiences in laboratory diagnosis of herpes simplex, varicella-zoster, and vaccinia virus infections in routine medical practice. Mayo Clin. Proc. **42**:744–753.
3. **Landry, M. L., D. R. Mayo, and G. D. Hsiung.** 1982. Comparison of guinea pig embryo cells, rabbit kidney cells, and human embryonic lung fibroblast cell strains for isolation of herpes simplex virus. J. Clin. Microbiol. **15**:842–847.
4. **Moseley, R. C., L. Corey, D. Benjamin, C. Winter, and M. L. Remington.** 1981. Comparison of viral isolation, direct immunofluorescence, and indirect immunoperoxidase techniques for detection of genital herpes simplex virus infection. J. Clin. Microbiol. **13**:913–918.
5. **Nichols, W. W., D. G. Murphy, V. J. Cristofalo, L. H. Toji, A. E. Greene, and S. A. Dwight.** 1977. Characterization of a new human diploid cell strain, IMR-90. Science **196**:60–63.
6. **Rawls, W. E.** 1979. Herpes simplex virus types 1 and 2 and herpes virus simiae, p. 309–360. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for: viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.
7. **Reeves, W. C., L. Corey, H. G. Adams, L. A. Vontver, and K. K. Holmes.** 1981. Risk of recurrence after first episode of genital herpes. N. Engl. J. Med. **305**:315–319.
8. **Schmidt, N. J.** 1979. Cell culture techniques for diagnostic virology, p. 65–139. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for: viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.