Rapid Isolation of Herpes Simplex Virus by Using Mink Lung and Rhabdomyosarcoma Cell Cultures

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Highly sensitive and rapid results can be obtained by isolating herpes simplex virus from clinical specimens in simple cell culture with rhabdomyosarcoma (RD) cells. In this study, 3,186 clinical specimens were inoculated into locally produced, equivalent-age RD and mink lung (ML) cells. Of 727 positive isolates, all (100%) were isolated from RD cells and only 691 (95%) were isolated from ML cells. Furthermore, 162 of the positive isolates (22%) were isolated in RD cells earlier than in ML cells. RD cells are continuous and can be cultivated in house without decreasing sensitivity as the passage number increases. They produce a highly distinguishable cytopathic effect in response to herpes simplex virus and maintain intense confirmatory staining patterns.

Herpes simplex virus (HSV) is probably one of the easiest and most common viruses to isolate and detect by cytopathic effect (CPE) in cell culture. Growth of HSV in cell culture remains the most rapid, sensitive, and cost-effective means of detecting HSV (1, 4, 7, 8, 16). A number of cell lines have been described for HSV isolation, most notably, mink lung (ML), MRC-5, primary rabbit kidney, and Vero cells. In general, the isolation times and sensitivities of these cell lines are comparable (2, 5, 15), although this varies from laboratory to laboratory and some reports have indicated that ML and rabbit kidney cells are more sensitive to HSV than are MRC-5 or Vero cells (1, 3, 6, 14, 16).

Use of locally produced rhabdomyosarcoma (RD) and ML cells in our laboratory has demonstrated that RD cells are better for detection of HSV than are ML cells (9). They are continuous cells which are easily cultivated, produce a better CPE, and are more sensitive for HSV isolation.

In this comparison of RD and ML cells for isolation of HSV, clinical specimens were gathered from clinics and hospitals in northeastern Wisconsin. The specimens were generally from female genital sources, although specimens from other sites on both males and females were submitted. The specimens arrived in the laboratory within 24 h of collection. The transport system consisted of an in-house-prepared medium containing Eagle minimum essential medium, antibiotics, and gelatin.

Each specimen was vortexed and inoculated (0.5 ml) into an RD or ML cell culture tube. These cell cultures were cultivated in our laboratory and were no older than 7 days postseeding. The cells ranged in passage number from 143 to 178. The tubes were then rotated (approximately 1 rpm) for 5 days at 35°C and observed daily for a CPE. Some specimens were kept longer if there were questionable morphological changes in the cell monolayer. Original RD cell cultures were obtained from the Wisconsin State Laboratory of Hygiene, Madison. Original ML cell cultures were obtained from Viromed Laboratories, Minneapolis, Minn.

Specimens displaying a positive HSV CPE were scraped for confirmatory staining with a fluorescein isothiocyanate-labeled monoclonal antibody (Syva Co., Palo Alto, Calif.). The staining procedure followed the manufacturer's protocol.

A total of 3,186 clinical specimens were evaluated in the dual-culture system. There were 727 positive specimens, of which 225 were type 1 and 502 were type 2. There were 2,459 HSV-negative specimens.

The RD cultures detected all 727 (100%) of the HSV-positive specimens. The ML cultures detected 691 (95%) of the HSV-positive specimens. In 162 (22%) of the positive cases, the RD cultures displayed a CPE 1 or more days before the corresponding ML culture tube. In only 25 (3%) of the positive cases, the ML culture tube displayed a CPE before the RD culture tube.

Chi-square analysis of these data showed a significant difference not only between the positive results obtained with the cell lines but, moreover, a significant difference between the speeds of detection by the two cell lines. This indicates that for isolation of HSV from clinical specimens, RD cells are not only more sensitive to clinical strains of HSV than are ML cells, but they also show a CPE more rapidly. The statistical data demonstrate that the efficiency of the RD cell line was significant and not due to inoculum distribution between the two tubes.

The overall pattern of positivity of the two cell lines is shown in Table 1. The RD cell line produced a CPE in 83% of the positive cases by 30 h postinoculation. By 54 h, 94% of the total number of positive isolates had been identified in RD cells. At 54 h, only 74% of the total number of positive isolates had been identified in ML cells.

The cell cultures were produced locally to maximize sensitivity to the virus (6, 9), so the comparison was made between cells of exactly the same age. Results of other studies in our laboratory have also indicated that RD cells are highly sensitive to HSV (9, 10). There are further considerations, however, that need to be made when selecting a cell line for HSV isolation.

Production of cells in our laboratory keeps our cost much lower than if commercial cells were purchased. This was possible because of available personnel and volume. It was more cost effective to use a continuous heteroploid cell line because there was no need to replace it because of decreasing sensitivity during serial passage, as with MRC-5 cells (16).

Quality assurance evaluations were made routinely, however, to ensure continued sensitivity of the continuous cell lines. The RD cells were easy to grow, and the CPE was very distinguishable. Enlarged balloon cells were produced in large, distinct foci. In our experience and that of others (5),
the CPE produced in ML cells was relatively small and initially less apparent than that produced in RD cells. We have also observed that RD cells were somewhat more resistant to toxic factors than were ML cells, although no data were collected in this regard.

Along with the sensitivity of the RD cell line to the virus, it maintained a high level of susceptibility to staining. It has been indicated that different cell lines are not necessarily comparable in the ability to produce equivalent staining intensities (6). Random observations during this evaluation indicated that ML cells can produce a lower intensity of staining than RD cells when scraped from a positive tube for confirmation with a fluorescein isothiocyanate-labeled monoclonal antibody (Syva).

The range of infectivity with other viruses was also better in the RD cell line than in the ML cell line. Primary rabbit kidney cells have been shown to be highly sensitive to HSV and comparable to ML cells (3, 5, 6, 8, 14, 16), but neither rabbit kidney nor ML cells readily detect a CPE caused by viruses other than HSV (2, 12, 13, 15, 16). The RD cell line has been shown to be susceptible to other viruses, especially echoviruses (11).

The final decision on which types of cells a laboratory should use needs to be based on many factors, including sensitivity, speed, cost, flexibility, and interpretability of the cell line. In our laboratory, the RD cell line has not only proven to be more sensitive and rapid than the ML cell line in detecting HSV, but it is easy to grow, it shows an easily distinguishable CPE and intense staining patterns, it stands up well with toxic specimens, and it can be used to detect viruses other than HSV.

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**LITERATURE CITED**


