Detection of Herpes Simplex Virus by 8 h in Shell Vial Cultures with Primary Rabbit Kidney Cells

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Shell vial (SV) cultures for herpes simplex virus using primary rabbit kidney cells stained at 8 h after inoculation were compared with 20-h SV cultures as well as conventional tissue culture. Of the 326 clinical specimens examined, conventional culture detected 67, and of these, 61 (91%) and 42 (63%) were detected by 20-h SV cultures and 8-h SV cultures, respectively.

In the past few years we have seen the traditional "gold standard" for herpes simplex virus (HSV) detection, namely, tissue culture held for 3 to 7 days, challenged by the 16- to 24-h shell vial (SV) culture (1, 3, 8–11). Similar to conventional culture, depending on the cell line and stain used, this method offers rapid detection as well as reported sensitivities ranging from 88 to 100% (1–3, 8–11). In addition, recently several rapid nonculture techniques have been described which include direct staining with fluorescence-labeled monoclonal antibodies (5), DNA probes used in DNA-DNA hybridizations (7), enzyme-linked immunosorbent assay (6), and latex agglutination (4). These rapid techniques offer results within minutes to hours and sensitivities which range from 50% for latex agglutination (4) to 92% for an HSV biotinylated DNA probe used in a high-prevalence setting (7). In an effort to reduce the time of the SV culture method, we compared 8-h SV cultures with 20-h cultures using primary rabbit kidney (PRK) cells and an indirect stain using polyclonal rabbit anti-HSV antisem. We chose this cell line and stain because we have found it to be the most sensitive combination in a 20-h SV culture (9).

A total of 326 clinical specimens submitted for HSV culture to the Medical Microbiology Laboratory at the University of California Irvine Medical Center were included in this study. Specimens came primarily from genital sources, although some were from oral, rectal, and dermal sites. HSV cultures were received as swabs or fluid from vesicles. Upon receipt, specimens were placed in 3 ml of Eagle minimal essential medium containing 1% fetal calf serum and gentamicin (50 μg/ml) and vortexed for 1 min. Samples were held at 4°C and cultured within 24 h of receipt.

Two vials of PRK cells (Difco Laboratories, Detroit, Mich.) grown on 12-mm cover slips in glass vials were each inoculated with 0.2 ml of specimen. Vials were centrifuged at 700 × g at 30°C for 1 h, after which 1 ml of Eagle minimal essential medium–2% fetal calf serum containing gentamicin (50 μg/ml) was added to the vials. All vials were incubated at 35°C for either 8 or 20 h and then stained with an indirect rabbit anti-HSV–horseradish peroxidase system; reagents and the procedure in the Cellmatics anti-HSV detection system (Difco) were used for SV culture staining. At 8 or 20 h postinoculation, medium was decanted from the vials and the cells were fixed in 3.7% buffered formaldehyde. Cells were rinsed twice with 3.0 ml of distilled water, after which time rabbit polyclonal anti-HSV serum (0.1 ml) was added. Vials were incubated for 15 min at 37°C and then washed three times in 3 ml of distilled water. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (0.1 ml) was then added, and the vials were incubated and washed as before. Substrate (0.024% H2O2 and chromogen [4 mM 3-amino-9-ethyl carbazole and 9.4 mM 4-chloro-1-naphthol in dimethyl sulfoxide–ethanol, 2:1]) was added, and the vials were incubated and washed as above. Cover slips were mounted in glycerol and read with a light microscope.

Conventional cultures were inoculated with 0.2 ml of specimen and incubated at 37°C for 1 h followed by the addition of 2 ml of Eagle minimal essential medium–2% fetal calf serum. Tubes were incubated at 37°C and observed daily for 7 days for HSV cytopathic effect (CPE). Isolates were typed from cultures by using fluorescence-tagged monoclonal antibodies to HSV type 1 (HSV-1) and HSV-2 (Syva Corp., Palo Alto, Calif.). Slides were read with an Olympus fluorescence microscope equipped with epi-illumination.

Of the 326 specimens included in this study, 67 were positive for HSV by conventional tissue culture for an isolation rate of 21%. Among these, 16 (24%) were HSV-1 and 51 (76%) were HSV-2. Only 21 (31.3%) of the positive specimens demonstrated CPE at 24 h by conventional culture, while CPE was demonstrated by day 3 in 64 (95.5%) of the 67 specimens. The mean time for detection of CPE was 2.0 days.

Compared with conventional results, SV cultures incubated up to 20 h had a sensitivity of 91% (61 of 67), while the sensitivity of cultures incubated for 8 h was 63% (42 of 67). The specificities of the 8- and 20-h SV cultures were 99 and 97%, respectively. If results of 8-h SV cultures were compared with those of 20-h SV cultures, the sensitivity of the 8-h SV method was 62% and the specificity was 99%.

Table 1 illustrates results with specimens that gave discrepant findings. With the exception of the 20 negative 8-h SV cultures for which 20-h SV cultures were positive, there was no one pattern of results that was significant among the discrepant culture results. Of the SV cultures missed at 8 h, 72% of these had ≤5% of the cells positive by 20-h SV cultures and the mean detection time for CPE by conventional culture for these specimens was 2.6 days. Of the specimens detected by both 8- and 20-h SV cultures, there were approximately 20% more cells staining positive for HSV from monolayers incubated for 20 h as compared with 8 h. For the five positive specimens in which both 8- and 20-h SV culture formats were negative, CPE in the conventional culture was not detected until day 3 in three specimens and
day 2 in two specimens. There were eight cultures that were negative by conventional culture but positive by either one or both SV culture formats. Of these eight, one was positive by both 8- and 20-h SV formats, with both cultures exhibiting ≤5% of the entire monolayer staining positive. Also among these eight, seven (88%) had ≤5% of the entire monolayer staining positive, while one demonstrated 100% staining of the 20-h SV culture. It is interesting to note that with this latter specimen, although the PRK conventional culture was negative, a tube of MRC-5 cells set up at the same time in our laboratory was positive for HSV on day 4. Therefore, by conventional culture with MRC-5 cells, owing to the delay in CPE, it is likely that the original viral titer was low. This may explain the negative PRK conventional culture, but in our laboratory we have consistently found PRK cells to be more sensitive than MRC-5 cells in conventional culture (2, 9). In addition, even if this failure of the conventional PRK cell culture was due to a low virus titer, this would be inconsistent with the 100% infection seen by the 20-h SV culture. Therefore, with this specimen the findings are not consistent and technical error could be a possibility. The other SV-positive, conventional culture-negative specimens may also be the result of a low virus titer, since the number of infected cells was low, with the few virus particles unevenly distributed among the cultures. This could also be the reason for conventional culture-positive, SV-negative cultures. However, since the SV culture is not the reference standard, these SV culture-positive specimens should be considered false-positives.

Salmon et al. (11) in a study of 431 specimens reported that when cultured for HSV, 91 were detected by conventional cultures held 5 days and 107 were positive by SV cultures. Of the 16 SV-positive, conventional culture-negative specimens, 14 either came from patients with a history of genital HSV infection or had the virus isolate confirmed by cell culture passage or reculture of the original specimen. Therefore, these researchers concluded that the SV discrepant samples were true positives, thus making the SV method more sensitive than conventional culture.

For the 61 SV culture-positive and conventional culture-positive specimens, 22 (36%) had 100% of the cells stained positive for HSV with the 20-h SV culture. Of these 22, 10 (45%) also had 100% of the cells stained positive for HSV at 8 h, and the mean percentage of positive cells for these was 85%. Furthermore, the appearance of cells staining positive at 8 h differed to various degrees depending on the original viral inoculum as concluded by CPE detection time in tissue culture and the percentage of positive cells in the 20-h SV culture format (12). When the original viral inoculum was high, CPE was detected as early as day 1 in conventional culture and cells in both the 20- and 8-h SV culture demonstrated dark nuclear and cytoplasmic staining. However, when the viral inoculum was lower and CPE was not detected until day 2 or later, the cells in the positive 20-h SV culture stained as described above, but cells of the positive 8-h SV cultures exhibited a different appearance with a much lighter cytoplasmic color and predominant nuclear staining. For those 8-h SV cultures that demonstrated this type of cell staining, the mean percent positivity of cells at 20 h was 46%.

Although the sensitivity of the 8-h SV culture method is higher than that of direct latex agglutination, it is lower than that reported for direct smear examination with monoclonal antibodies or HSV DNA probes (4, 5, 7). In our study, however, we did not break down the data as to the origin of the specimen in terms of stage of the lesion. However, the higher sensitivities obtained with some of the direct methods were derived from lesions from a high-incidence population (5, 7). It has been clearly demonstrated that depending on the stage of lesion and also the original virus titer, the sensitivity of direct smears as well as culture will vary greatly (5, 7, 12). Since the 8-h SV culture was positive in most instances when there appeared to be a large number of infecting virions, it is necessary to determine whether 8-h SV cultures have a higher sensitivity with specimens from early vesicles. However, at this point we cannot recommend this procedure for the routine detection of HSV.

**LITERATURE CITED**