Centrifugation-Shell Vial Technique for Rapid Detection of Herpes Simplex Virus Cytopathic Effect in Vero Cells

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A centrifugation-shell vial technique for herpes simplex virus detected the virus in 50 (24.6%) of 203 specimens by cytopathic effect at 48 h, compared with detection of the virus in 47 (23.2%) specimens by immunofluorescence at 24 h. The rapid detection of cytopathic effect may be useful to laboratories that are not interested in typing all herpes simplex virus specimens but that wish to reduce the cost of herpesvirus cultures.

The use of rapid techniques for detecting herpes simplex virus (HSV) has important clinical application, especially in the diagnosis of active infections in pregnant women or disseminated disease in immunocompromised patients (6, 7). Early diagnosis can aid in successful treatment of genital, ocular, and central nervous system infections (5, 12).

Recent improvements in the laboratory diagnosis of HSV infections have reduced turnaround time from an average of 5 to 7 days to less than 24 h. The direct antigen methods involve staining human cells with fluorescent-antibody (FA) conjugate reagents (16, 17), immunoperoxidase (IP) reagents (3, 16), and enzyme-linked immunosorbent assay reagents (18, 20). An advantage to these methods is that viral antigen is detected; therefore, viable virus does not have to be present. The disadvantages of direct methods are their lack of sensitivity (16, 20), dependence on a quality specimen being submitted (11), and the technical variables involved in distinguishing a positive from a negative specimen (11).

Tissue culture remains the "gold standard" by which other methods are compared (2). Cytopathic effect (CPE) caused by HSV is very characteristic on certain cell lines, especially when it occurs as soon as 1 to 2 days after inoculation; however, it is still best to confirm the CPE with FA, IP, or enzyme-linked immunosorbent assay reagents for HSV. We evaluated the correlation between visible CPE in shell vials versus FA staining of antigen or viable virus in shell vial tissue culture monolayers.

Most of the specimens were from genital sources and from females. The specimens were submitted in viral transport medium (Bartels Immunodiagnostic Supplies, Inc., Bellvue, Wash.) and kept at 4°C until set up or frozen at -70°C over the weekend. The procedure for setting up shell vial cultures and staining with monoclonal reagents was described previously (4). Briefly, specimens were inoculated to Vero cells grown on 12-mm cover slips at the bottom of a shell vial (Bartels) and centrifuged at 700 to 800 × *g* for 40 min at 30°C. After 1 h of absorption, the medium was changed, and two of the three shell vials were incubated for 16 to 24 h at 35°C. Vial 3 was incubated for 5 days at 35°C and observed daily for CPE.

After overnight incubation, the two vials were washed with phosphate-buffered saline, fixed in cold acetone, and stained with monoclonal reagents (Syva Co., Palo Alto, Calif.) for HSV type 1 (HSV-1) and HSV type 2 (HSV-2), respectively. The cover slips were mounted and observed for fluorescence with an American Optical microscope

equipped with a mercury fluorescence light source and incident illumination at ×200 magnification.

A total of 203 specimens were set up, and 50 (24.6%) produced typical CPE for HSV. Of these, 47 showed CPE at 24 h; all 50 showed CPE at 48 h. A total of 47 specimens were stained and typed with the monoclonal FA reagent. A total of 43 isolates (91.5%) were HSV-2, and 4 isolates (8.5%) were HSV-1. The three specimens negative by FA but positive by CPE showed at least 3- to 4+ CPE at 24 h, with typical swollen, refractile cells. Giant-cell formation was also seen. Two of these specimens eventually became positive after repeated culture and showed weak fluorescence with the HSV-1 conjugate, whereas the third was never positive by FA; however, it was identified as HSV-1 by a reference laboratory that uses the same technique and reagents. The relative sensitivity for CPE was 100 versus 94% for FA staining.

The detection of 93% positive specimens at 24 h and 100% positive specimens at 48 h demonstrates an improvement over previous studies in detecting CPE. Miller and Howell (9) detected only 23% of HSV isolates at 24 h using WI38 and African green monkey kidney cell tubes, and Moore (10) detected 29% of HSV-positive specimens at 24 h using primary rabbit kidney (PRK) cell tubes. Salmon and co-workers (13) reported improved results using mink lung cell tubes to detect CPE. They found 26 (65%) of 40 specimens positive by CPE at 24 h. In a companion study (14), they compared mink lung cell tubes to Vero cell tubes and found positive isolation rates of 51 and 34%, respectively, at 24 h. Callihan and Menegus (2) used a combination of human embryonic lung fibroblasts and PRK cell tubes to improve isolation but were able to detect only 59.7% of HSV isolates at 24 h and 84.6% at 48 h. Using a centrifugation technique and shell vial tissue cultures, Salmon et al. (15) reported a 72% HSV-positive rate by CPE at day 1. Perhaps the sensitivity of mink lung cells was responsible for the lower isolation rate.

Methods other than CPE have been used to improve isolation rates as well as turnaround time. The use of a direct IP stain on human newborn foreskin fibroblast (HFF) cells in shell vials at 24 h detected 40 of 40 specimens positive for HSV (9); however, in another study, a commercial IP kit detected only 19 (48%) of 40 specimens at 48 h (13). The quality of reagents and the use of shell vials versus tubes may account for the variation in these results (8).

Gleaves and co-workers (4) were among the first to advocate using low-speed centrifugation and shell vial cultures to improve turnaround time and HSV isolation rates.

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Their study noted a sensitivity of 100% and a specificity of 95.6% for the shell vial technique in comparison with MRC-5 cell tubes. Using a similar technique, but staining with IP, Salmon et al. (15) detected 96% of all HSV isolates at day 1. In their study, the centrifugation speed was increased to $3,500 \times g$, but the time was reduced to 15 min. The use of FA or IP stains is particularly advantageous, especially when only one to two foci of CPE are present throughout the monolayer.

The two specimens which required repeat culture and FA testing before showing fluorescence and the one specimen that never stained by FA despite 3- to 4+ CPE present problems in diagnosis. Ideally, the FA conjugates should be used to stain cells which have up to 2+ CPE; however, with HSV this may not always be possible. Barnard et al. (1) also noted problems in staining clinical isolates with commercial reagents and believed this resulted from the very restrictive epitope range of the monoclonal antibody. In their study, most of the isolates which did not stain with Syva reagents were HSV-2, whereas in our study, all three isolates were identified as HSV-1 by another reference laboratory. Further studies may reveal the need to expand the epitope range in commercial kits.

The improvement in detecting CPE in this study may be attributed to several factors. Several studies (4, 15, 19) have shown the advantages of centrifugation, especially to increase the number of foci showing CPE (15). A second factor is the concentration of specimens onto the small area of Vero cells on the cover slip. With tubes, dilution of the specimen occurs over a larger area, resulting in scattered areas of CPE which are harder to find. The smaller area of the cover slip allows easier scanning for CPE, either with an inverted scope or with a regular light microscope, by viewing shell vials upside down with the condenser lowered all the way down. Finally, Vero cells are excellent for differentiating normal aging from viral CPE. Also, the CPE of HSV is very characteristic on this cell line, so that even one focus of CPE can readily be seen under the microscope.

In this era of diagnostic related groups, an advantage of screening shell vials for CPE is that only positive specimens need be stained for confirmation or typing. The College of American Pathologists manual for laboratory workload recording lists 4 min of labor per cover slip for staining and reading, which could be saved. In addition, a reagent cost of \$10.50 per specimen would be realized. Therefore, for an average of 10 specimens plus two controls, a labor savings of approximately 48 min and a monetary savings of \$84 (through the elimination of the staining of 8 specimens) would be realized per run. In addition, the detection of more positive specimens may prevent higher hospital costs that result from complications in delivery or from not starting viral therapy soon enough.

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