JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1988, p. 222–224
0095-1137/88/020222-03$02.00/0

Vol. 26, No. 2

Comparison of Primary Rabbit Kidney and MRC-5 Cells and Two Stain Procedures for Herpes Simplex Virus Detection by a Shell Vial Centrifugation Method

ELLENA M. PETERSON,* BRUCE L. HUGHES, SANDRA L. AARNAES, AND LUIS M. DE LA MAZA

Department of Pathology, Division of Medical Microbiology, University of California Irvine Medical Center, 101 City Drive, Orange, California 92668

Received 16 July 1987/Accepted 10 November 1987

By using a conventional tissue culture method as a standard, four shell vial centrifugation culture (SVC) formats were compared for herpes simplex virus (HSV) detection in 300 clinical samples. Both MRC-5 and primary rabbit kidney (PRK) cells were used in the conventional and SVC systems. In addition, both a direct monoclonal fluorescent antibody to HSV (MAb-FA; Syva Corporation, Palo Alto, Calif.) and an indirect HSV polyclonal antibody-horseradish peroxidase stain (poly-HRP; Difco Laboratories, Detroit, Mich.) were used to stain shell vials of both cell types. Conventional tubes were incubated for up to 7 days with daily examination for cytopathic effect, which was confirmed as HSV by staining with an MAb-FA. Shell vials were inoculated, centrifuged, incubated for 16 to 24 h, and stained directly with MAb-FA or indirectly with a poly-HRP stain. Of the 300 specimens examined, 82 (27%) were HSV positive by conventional tissue culture. PRK cells detected 81 (99%) positive specimens, compared with 74 (90%) specimens detected with MRC-5 cells. Of the 82 positive specimens by conventional culture, the SVC formats detected 68 by MRC-5 and MAb-FA, 74 by MRC-5 and poly-HRP, 64 by PRK and MAb-FA, and 77 by PRK and poly-HRP. Therefore, PRK stained by an indirect method with poly-HRP was the most sensitive of the SVC formats tested, detecting 94% of the positive specimens.

One of the major challenges of the diagnostic virology laboratory has been the rapid detection of herpes simplex virus (HSV) from clinical specimens. The standard for comparison of any new rapid method is tissue culture isolation. With this method, cultures are usually held for 7 days, and depending on the cell line, approximately 60 to 80% of all positive specimens are detected by day 3 of culture. Numerous cell lines have been examined in the search for a more sensitive and rapid cell line, but even under optimal conditions, conventional cultures cannot be considered rapid (1, 3, 5, 6).

Recently, a variation of the traditional cell culture has been described which combines culture in shell vials with centrifugation (4). The shell vial centrifugation culture (SVC) technique for the detection of HSV has significantly added to our ability to detect this virus accurately and rapidly (within 24 h). To date, this technique shows great promise when used with HEp-2 (2), mink lung (8), MRC-5 (4), and Vero (7) cells. However, in our experience and that of others, primary rabbit kidney (PRK) cells have proven to be the most sensitive and rapid cell line for HSV detection when used in a conventional tissue culture format (1, 3, 6). Therefore, we compared PRK cells in shell vials alongside MRC-5 cells. Conventional tissue culture, which was used as the reference standard, was also performed with both cell lines. In addition, direct monoclonal fluorescent staining and indirect polyclonal antibody-horseradish peroxidase (poly-HRP) stains have never been compared with one another in the shell vial technique. Therefore, in addition to the comparison of cell lines, a comparison of the different staining formats was undertaken.

* Corresponding author.

MATERIALS AND METHODS

Specimens. The 300 clinical specimens included in this study were submitted for HSV isolation to the Medical Microbiology Laboratory at the University of California Irvine Medical Center. Specimens were mainly from genital sources, although specimens from oral, rectal, and dermal sites were included. HSV cultures were received as swabs or, in some cases, fluid from vesicles. Before culture, all specimens were placed in 3 ml of Eagle minimal essential medium containing 1.0% fetal bovine serum and gentamicin (50 μg/ml) and vortexed for 1 min. Specimens were tested within 24 h of collection or were previously frozen at −70°C.

Conventional tissue culture. Two tubes each of MRC-5 cells (Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash., or Viromed Laboratories, Inc., Minneapolis, Minn.) and PRK cells (Difco Laboratories, Detroit, Mich.) were inoculated with 0.2 ml of sample and placed at 37°C for 1 h, after which time 2 ml of minimal essential medium–2% fetal bovine serum was added. Tubes were incubated at 37°C for up to 7 days and inspected daily for HSV cytopathic effect (CPE). All positive cultures were typed as either HSV-1 or HSV-2 with fluorescein-tagged monoclonal antibodies (MAb-FA) to HSV-1 and HSV-2 (Syva Corporation, Palo Alto, Calif.). Slides were read by using an Olympus (Japan) fluorescence microscope equipped with epi-illumination.

SVC. Two vials each of MRC-5 cells (Bartels or Viromed) and PRK cells (Difco) grown on 12-mm cover slips in 1-dram (1.772-g) glass vials were inoculated with 0.2 ml of specimen. Vials were centrifuged at 700 × *g* at 30°C for 45 min. Immediately after centrifugation, 1 ml of minimal essential medium–2% fetal bovine serum medium was added to the vials. All vials were incubated at 35°C for 16 to 24 h. One vial of each cell type was stained by each method, and each was read blindly by at least two readers. The following staining methods were used.

TABLE 1. SVC results compared with combined conventional tissue culture results with PRK and MRC-5 cells[a]

SVC	No. pos	Sensitivity (%)	Specificity (%)	Predictive value (%)	
				Pos	Neg
MRC-5–MAb-FA	68	83	100	100	94
MRC-5–poly-HRP	74	90	100	100	96
PRK–MAb-FA	64	78	100	100	92
PRK–poly-HRP	78[b]	94	99	99	98

[a] 82 specimens were positive when results are combined for both cell lines. Pos, Positive; Neg, negative.
[b] One of the 78 positive specimens was negative by conventional culture.

(i) Monoclonal fluorescent staining. Medium was decanted from vials and the cells were washed with phosphate-buffered saline (PBS; 0.01 M, pH 7.4), after which they were fixed with acetone for 10 min and again washed with PBS. Subsequently, 0.1 ml of a 1:1 mixture of HSV-1 and HSV-2 MAb-FA was added and incubated for 30 min at 35°C. Vials were washed three times with PBS with agitation, and the cover slips were mounted in glycerol and read.

(ii) Poly-HRP. The Cellmatics HSV detection system (Difco) provided the recommended staining procedure and reagents. After the medium was decanted, the vials were fixed in 3.7% buffered formaldehyde, rinsed two times with distilled water, and given a final wash with PBS. Rabbit polyclonal anti-HSV serum (0.1 ml) was added, and vials were incubated for 15 min at 37°C. Vials were then washed three times with distilled water; 0.1 ml of peroxidase-conjugated goat anti-rabbit immunoglobulin G was added, and the vials were incubated and washed as before. Substrate (0.024% H_2O_2 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, incubated, and washed as above. Cover slips were mounted in glycerol and read with a light microscope.

Data analysis. The chi-square formula and modified chi-square analysis for a 2-by-2 contingency table were used to analyze the data.

RESULTS

Conventional culture. Of the 300 specimens entered into the study, 82 were positive for HSV by conventional tissue culture, for an overall positive isolation rate of 27%. Of the 82 positive specimens, 13 (16%) were HSV-1 and the remaining 69 (84%) were HSV-2. Of the 82 positive cultures, 81 (99%) were positive by PRK cells but only 74 (90%) were positive by MRC-5 cells ($P < 0.05$). PRK cells were not only more sensitive but also more rapid in time of detection. By 48 h, PRK cells had detected 63% of the specimens, compared with the 41% found to be positive by MRC-5 cells ($P < 0.005$).

Of the eight specimens negative by MRC-5 cells, four were HSV-1 and four were HSV-2. Half of those not detected by MRC-5 cells were positive in only one of the two PRK cell tubes inoculated. All eight were detected late by PRK cells: two by day 3, five by day 5, and one by day 7.

The one specimen missed by PRK cells was an HSV-2 isolate that was detected on day 3 in only one of the two MRC-5 cell cultures. This specimen was also negative in three of four shell vials inoculated, being detected at a level of <1% of the monolayer in an MRC-5 SVC stained with the poly-HRP stain.

SVCs. The overall results of the four SVC formats compared with conventional culture by using results of both cell lines combined can be seen in Table 1. None of the SVC systems were as sensitive as the conventional culture when both MRC-5 and PRK cells were used. The most sensitive method of the SVC systems was PRK cells stained with poly-HRP. Even this system was less sensitive than conventional culture ($P < 0.1$). All SVC specificities and predictive positive values were 100%, excluding PRK–poly-HRP-stained vials, for which both values were 99%. This lower value was due to one specimen which was positive for PRK–poly-HRP SVC and negative by conventional culture.

Since most laboratories use only one cell type in their tissue culture method for isolating only HSV, we also compared the SVC results with the conventional tissue culture results, considering each cell line separately. When sensitivity and specificity with MRC-5 conventional culture alone as the standard were calculated, all SVCs were less sensitive (Table 2). This difference was statistically different ($P < 0.01$) for both SVC formats stained with MAb-FA but not for vials of either cell type stained with poly-HRP ($P > 0.05$). PRK–poly-HRP actually detected 78 positive specimens, compared with the 74 detected by conventional MRC-5 culture. Three of the four positive specimens detected by PRK–poly-HRP and not by MRC-5 conventional tissue culture were also positive by PRK conventional culture, and one was negative by all systems. For data analysis, however, with MRC-5 conventional culture as the only standard, these four specimens were considered false positives.

When PRK conventional culture alone was used as the standard to compare SVCs, none of the SVCs were as sensitive as the conventional culture (Table 2). The PRK–poly-HRP approached the sensitivity of the PRK conventional culture. However, the other SVC formats were significantly ($P < 0.05$) less sensitive than conventional PRK cell results.

Of the two staining systems in SVC formats, the indirect poly-HRP stain was the most sensitive. When SVC MRC-5 cells were used, the MAb-FA stain detected 68 positive specimens, whereas the poly-HRP detected 74 positive specimens. Likewise, 64 and 77 of the 82 specimens positive by conventional culture were detected with PRK cells stained with MAb-FA and poly-HRP stains, respectively. The percentage of positive cells staining with either one of the two stains was the same in approximately two-thirds of

TABLE 2. Overall results of SVC formats compared with conventional culture results[a]

SVC	No. pos	Result (%) with MRC-5 alone (74 pos)		Result (%) with PRK alone (81 pos)	
		Sensitivity	Specificity	Sensitivity	Specificity
MRC-5–MAb-FA	68	88	99	84	100
MRC-5–poly-HRP	74	93	98	90	99
PRK–MAb-FA	64	82	99	79	100
PRK–poly-HRP	78[b]	96	97	95	99

[a] Pos, Positive.
[b] One of the 78 positive specimens was negative by conventional culture.

the positive specimens, whereas with one-third of the positive specimens the poly-HRP stain exhibited greater than a 10% increase in the amount of the cell monolayer staining compared with the MAb-FA stain.

Overall discrepancies. There were 24 specimens in which all six cell-stain combinations did not agree. There was no one particular pattern that predominated. The most common pattern appearing (with 4 of the 24 discrepancies) was when all systems gave positive results except the SVC–MAb-FA-stained vials. Of the SVC-conventional culture discrepancies, 67% were detected between days 4 and 7 with the conventional culture. This is in contrast to the overall results, in which only 21% of cultures were detected after day 3. In addition, of the discrepant samples, eight of the MRC-5 and four of the PRK conventional cultures were positive in only one of the two tubes inoculated. In all other positive specimens in the study, both tubes of each cell line were positive. Therefore, this is further evidence that the discrepant samples probably contained less viable virus than the other positive specimens in the study.

DISCUSSION

The results of this study show that the sensitivity of the SVC technique was more dependent on the type of staining system used than on the cell line used, i.e., MRC-5 or PRK. The indirect poly-HRP stain gave a significantly higher yield of positive cultures than did the MAb-FA stain. This could be due to the greater sensitivity, in general, of indirect antibody staining versus direct staining (9), to the amplification effect of the enzyme tag of the poly-HRP stain (10), and/or to the greater number of antigenic sites that may be recognized with polyclonal sera versus a mixture of a few HSV epitopes in the form of a monoclonal "cocktail." This last factor could become even more evident when looking for expression of an early event for which antigenic determinants are limited.

Cell lines that have been reported as being used in an SVC assay for HSV detection include Vero (7), mink lung (8), HEp-2 (2), and MRC-5 (4). Gleaves et al. (4), examining a total of 119 specimens with 27 positive cultures by using a direct MAb-FA stain, reported a sensitivity of 100% when MRC-5 cells were compared by SVC with an MRC-5 conventional culture. The sensitivity we observed with 300 samples, comparing the same cell and stain system used by Gleaves et al., was lower, 88%, which might just be a reflection of our larger sample size or of the fact that two tubes of each cell type were included in our conventional culture rather than the one tube used in their conventional method. Therefore, in our study design, merely because more tubes were inoculated with sample, it might appear that conventional culture is more sensitive for specimens with a low level of infectious virus than are SVCs. Salmon et al. (8) detected 96% positive specimens when using mink lung cells in shell vials stained with the indirect poly-HRP stain compared with mink lung cells in a conventional culture. Pruneda and Almanza (7) reported an SVC method using Vero cells stained with an MAb-FA to be 94% sensitive compared with a 48-h Vero cell SVC evaluated for CPE. Darougar et al. (2), using an SVC assay with HEp-2 cells stained at 48 h by an indirect polyclonal fluorescent-antibody stain, detected 54 positive specimens compared with the 24 detected by an HEp-2 conventional culture when screening 2,100 cervical cultures from randomly selected women. In this last study, a major difference was the speed of centrifugation of 15,000 × *g*, which is well above the 700 × *g* and 3,500 × *g* used by us and other investigators (4, 7, 8).

In the study by Salmon et al. (8), a positive culture by the SVC method needed to exhibit CPE that was also specifically stained. In our study, many specimens in the SVC format did not exhibit CPE, yet cells were specifically stained, and this was true especially for cultures in which only a few cells were stained. Using only specific staining as a criterion, we had only one positive result, which was in the PRK–poly-HRP SVC format, that was not confirmed by tissue culture; thus, for the other three formats, there was 100% specificity. Therefore, on the basis of our results, we do not think CPE is a necessary criterion for defining a positive specimen when either of the stains in an SVC format reported in this study is used.

In summary, we found conventional culture with PRK cells to be more sensitive than either PRK or MRC-5 cells in an SVC method. Although the cell line used in the conventional culture made a significant difference in the number and time of detection of positive specimens, this was not the case with the SVC method. However, with SVC, the type of stain used made a significant difference in the number of positive specimens detected. Therefore, since it appeared even more sensitive than MRC-5 conventional culture and comparable to PRK conventional culture, we recommend that PRK cells in SVC stained by an indirect poly-HRP method be considered as a sensitive and rapid alternative to conventional cultures for detection of HSV.

LITERATURE CITED

1. **Callihan, D. R., and M. A. Menegus.** 1984. Rapid detection of herpes simplex virus in clinical specimens with human embryonic lung fibroblast and primary rabbit kidney cell cultures. J. Clin. Microbiol. **19**:563–565.
2. **Darougar, S., P. Walpita, U. Thaker, B. T. Goh, and E. M. C. Dunlop.** 1986. A rapid and sensitive culture test for the laboratory diagnosis of genital herpes in women. Genitourin. Med. **62**: 93–96.
3. **Fayram, S. L., S. L. Aarnaes, E. M. Peterson, and L. M. de la Maza.** 1986. Evaluation of five cell types for the isolation of herpes simplex virus. Diagn. Microbiol. Infect. Dis. **5**:127–133.
4. **Gleaves, C. A., D. J. Wilson, A. D. Wold, and T. F. Smith.** 1985. Detection and serotyping of herpes simplex virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16 h post-inoculation. J. Clin. Microbiol. **21**:29–32.
5. **Miller, M. J., and C. L. Howell.** 1983. Rapid detection and identification of herpes simplex virus in cell culture by a direct immunoperoxidase staining procedure. J. Clin. Microbiol. **18**: 550–553.
6. **Moore, D. F.** 1984. Comparison of human fibroblast cells and primary rabbit kidney cells for isolation of herpes simplex virus. J. Clin. Microbiol. **19**:548–549.
7. **Pruneda, R. C., and I. Almanza.** 1987. Centrifugation-shell vial technique for rapid detection of herpes simplex virus cytopathic effect in Vero cells. J. Clin. Microbiol. **25**:423–424.
8. **Salmon, V. C., R. B. Turner, M. J. Speranza, and J. C. Overall, Jr.** 1986. Rapid detection of herpes simplex virus in clinical specimens by centrifugation and immunoperoxidase staining. J. Clin. Microbiol. **23**:683–686.
9. **Schmidt, N. J.** 1982. Advances in the laboratory diagnosis of viral infections, p. 1–27. *In* L. M. de la Maza and E. M. Peterson (ed.), Medical virology I. Elsevier Biomedical Press, New York.
10. **Schmidt, N. J., J. Dennis, V. Devlin, D. Gallo, and J. Mills.** 1983. Comparison of direct immunofluorescence and direct immunoperoxidase procedures for detection of herpes simplex virus antigen in lesion specimens. J. Clin. Microbiol. **18**:445–448.