JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1988, p. 1772–1774
0095-1137/88/091772-03$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 26, No. 9

Comparison of Diploid Fibroblast and Rabbit Kidney Tissue Cultures and a Diploid Fibroblast Microtiter Plate System for the Isolation of Herpes Simplex Virus

ANDRIA LANGENBERG, REGINA ZBANYSZEK, JOAN DRAGAVON, RHODA ASHLEY, AND LAWRENCE COREY*

Departments of Laboratory Medicine, Medicine, and Microbiology, University of Washington, Seattle, Washington 98105

Received 28 March 1988/Accepted 7 June 1988

We evaluated the relative sensitivities of two cell systems (rabbit kidney [RK] and human diploid fibroblast [DF; human embryonic tonsil]) in standard tube cultures versus DF cells in a 48-well microtiter plate system for the detection of both symptomatic and asymptomatic herpes simplex virus (HSV) infection. At least one system isolated HSV in 111 of 809 specimens (13.7%). HSV was isolated in RK tube cultures from 110 specimens (99%), in DF tube cultures from 91 specimens (82%), and in DF microtiter plates from 95 specimens (86%). The frequency of HSV isolation varied with the anatomic site and the presence or absence of a herpetic lesion. The sensitivities of the three culture systems remained similar whether the specimens were obtained from lesions or whether the specimens were taken to determine if asymptomatic excretion of HSV was present. While RK tube cultures were more sensitive than DF tube cultures, the DF microtiter plate system was as sensitive as DF tube cultures and its use is supported as a cheaper and less labor-intensive method for the detection of HSV.

Herpes simplex virus (HSV) can be isolated with various sensitivities and efficiencies from cell lines of human and nonhuman origin. Both primary and nonprimary cell lines support HSV replication. In general, continuous and semicontinuous cell lines are more convenient and economical than primary cell cultures. While some studies have shown little difference in isolation rates between primary rabbit kidney (RK) or guinea pig cells and continuous cell lines (6–8, 10), others have demonstrated increased sensitivity, especially in samples with low-inoculum titers (1–5, 9, 12). To evaluate this concern, as well as to address whether a microtiter system with its reduced reagent costs would be cost effective, we compared the isolation rate of HSV in standard tube cultures with that in a microtiter plate system. Specimens were obtained from a group of patients with documented genital herpes. Diploid fibroblast (DF; human embryonic tonsil) and RK cells were used for the tube cultures, and DF cells were used for the microtiter system. Hereafter, these three systems are designated DF, RK, and DFMT, respectively.

MATERIALS AND METHODS

Clinical population. Between February and June 1986, we obtained 848 specimens from patients who had a history of recurrent genital ulcers. Genital exams were performed, external lesions typical of HSV were sampled, and lesion types (vesicles, pustules, or ulcers) were recorded. In addition, the penile skin in men and the vulva, urethra, cervix, and rectum in women were cultured independent of visible evidence of lesions as part of the routine protocol for the detection of asymptomatic shedding from concomitant sites. Thus, cultures were obtained both when no genital lesions were present and when lesions were present in clinically noninvolved sites. Nongenital lesions suspicious for HSV were also cultured; 758 genital and 90 nongenital specimens were submitted.

* Corresponding author.

For sampling, the lesion was cleansed with sterile saline and mucus, if present, was removed. Vesicular or pustular lesions were opened with a 25-gauge needle, and fluid was collected with a Dacroswab and placed in viral transport medium (veal infusion broth [25 g of Difco veal infusion dissolved in 1,000 ml of deionized water, autoclaved at 121°C for 15 min, with 20 ml of sterile 25% gelatin added] supplemented with 1,000 U of penicillin G, 25 U of amphotericin B, 10 μg of gentamicin, and 0.06 μg of cefoxitin per ml). Ulcers were sampled by rubbing the base of the lesion with a swab, which was transported in viral transport medium. Cultures for the detection of asymptomatic shedding were obtained by swabbing the respective genital anatomic site with a swab premoistened with sterile saline. Cultures were processed without knowledge of the clinical presentation of the patient.

Cell cultures. DF tubes were prepared from flask cultures at passages 11 through 28, and RK tubes were prepared weekly according to the method of Hsiung, using 1- to 3-day-old New Zealand rabbits, and were used at passage 1 or 2 (5, 6). For DF and RK tube cultures, 1.2 ml of growth medium (Eagle minimal essential medium with 10% fetal calf serum) containing 1×10^6 cells per ml was added to each tube and then incubated at 37°C with 5% CO_2. When the cells were confluent (3 to 4 days), the medium was replaced with Eagle minimal essential medium with 2% fetal calf serum. For preparation of the DF monolayer in the microtiter plate system, 0.8 ml of a suspension of 10^6 cells per ml of Eagle minimal essential medium containing 10% fetal calf serum was added to each well of a 48-well microtiter plate (48 11.3-mm wells, catalog no. 3548; Costar), and the plate was incubated at 37°C in 5% CO_2. Plates were prepared in parallel with the DF tubes. Confluence generally occurred at 4 days after inoculation.

Virus isolation. For tube cultures, 0.2 ml of the specimen in the viral transport medium was inoculated into duplicate tubes of DF (human embryonic tonsil strain) and RK cells within 24 h of collection (11). After inoculation, the cultures were examined at 2- to 3-day intervals for cytopathic effect.

TABLE 1. Comparison of DF in tube cultures and microtiter plates with RK in tube cultures for isolation of HSV

Culture result for:			No. of specimens			
DF	RK	DFMT	Total (n = 809)	Symptomatic genital (n = 184)[a]	Asymptomatic genital (n = 537)[b]	Nongenital (n = 88)
+	+	+	83	51	28	4
+	+	−	8	4	3	1
+	−	−	0	0	0	0
−	+	+	11	7	3	1
+	−	+	0	0	0	0
−	+	−	8	4	4	0
−	−	+	1	1	0	0
−	−	−	698	117	499	82

[a] Specimens from lesions.
[b] Specimens from genital sites without lesions.

For specimen inoculation of microtiter plates, 0.1 ml of the specimen was added to duplicate wells of the prepared DF monolayer in the microtiter plates, plate sealers (catalog no. 7688-24; Abbott Laboratories) were applied, and each plate was examined with an inverted light microscope at a magnification of ×40 on alternating days for cytopathic effect.

All three culture systems were inoculated in parallel with portions of each specimen. Cells exhibiting typical HSV cytopathic effect were removed for HSV typing. In the microtiter plate system, this typing was done by puncturing the cover slip overlaying the specific well to avoid droplet contamination of the surrounding wells. These cells were then washed with phosphate-buffered saline, transferred to glass slides, allowed to air dry, fixed for 10 min in cold acetone, and stained by direct immunofluorescence using fluorescein-labeled mouse monoclonal antibodies against either HSV type 1- or HSV type 2-specific antigens (Syva Microtrak; Syva Corp.).

RESULTS

A total of 848 specimens were evaluated in the three cell culture systems. Thirty-four specimens were toxic in one or more cell systems (12 in RK, 9 in DF, and 13 in DFMT) and were excluded from analysis. Five specimens (0.6%) were excluded because of bacterial or fungal contamination in the microtiter plate system. These five specimens were negative in the tube culture systems. Of the remaining 809 (721 genital and 88 nongenital) specimens, 111 (13.7%) were positive for HSV. The RK tube cultures detected 110 (99%), the DF tube cultures detected 91 (82%), and the DF microtiter plate detected 95 (86%) of the positive specimens (Table 1). Eight cultures were positive in the RK system only, one culture was positive in the DFMT system only, and none was positive in the DF system only. Concordance among the three isolation methods was demonstrated in 781 of 809 specimens (96.5%); 83 of 111 positive specimens (75%) were positive in all three culture systems, and 698 negative specimens were negative in all three culture systems. The positive and negative predictive values for the RK, DF, and DFMT systems were 100 and 99.9%, 100 and 97%, and 100 and 98%, respectively.

The 111 positive specimens were obtained from 90 patients (56 women and 34 men). Eight women had two positive samples, and three women had three positive samples. Four men had two positive samples, and one man had three positive samples. The percentage of positive specimens varied greatly by genital anatomic site, with 7.6% of cervical and 33% of perianal specimens yielding HSV in at least one cell culture system. Overall, 36.4% of specimens from patients with genital lesions and 7% of genital specimens taken at times when no lesions were detected were positive for HSV (Table 2).

Of the 111 positive specimens, 16 isolates were HSV type 1 and 95 were HSV type 2. The RK system detected 16 of 16 HSV type 1 isolates (100%) and 94 of 95 HSV type 2 isolates (99%), whereas the DF system detected 14 of 16 (88%) and 77 of 95 (81%) and the DFMT system detected 13 of 16 (81%) and 82 of 95 (86%), respectively.

Sensitivities for samples from genital lesions were 66 of 67 (99%), 55 of 66 (83%), and 59 of 66 (89%) for the RK, DF, and DFMT systems, respectively, while the sensitivities for

TABLE 2. Isolation of HSV from genital and nongenital sites

Anatomic site	No. of positive specimens/total (%)		
	All	Lesions present	No lesions present
Penis	29/160 (18)	28/94 (29.8)	1/66 (1.5)
Vulva	36/186 (19.5)	25/52 (48)	11/134 (8.2)
Cervix	12/158 (7.6)	2/2 (100)	10/156 (6.4)
Urethra	7/91 (7.7)		7/91 (7.7)
Perianal area	14/43 (33)	12/30 (40)	2/13 (15.4)
Rectum	7/76 (9)		7/76 (9)
Buttocks	0/7 (0)	0/6 (0)	0/1 (0)
Subtotals			
Genital specimens	105/721 (14.6)	67/184 (36.4)	38/537 (7)
Nongenital specimens	6/88 (6.8)	6/88 (6.8)	
Total (all specimens)	111/809 (13.7)	73/272 (26.7)	38/537 (7)

samples from asymptomatic shedding sites were 38 of 38 (100%), 31 of 38 (82%), and 31 of 38 (82%) for the three systems, respectively. Collectively, both DF systems detected 63 of 66 symptomatic sources (95%) and 34 of 38 asymptomatic sources (89%) (Table 2).

DISCUSSION

Our study indicates that specimen source, anatomic site, and the type of cell culture system used influence the sensitivity and the rate of HSV isolation. Moreover, even in a population with a history of genital herpes, the isolation rate will be much higher in symptomatic than asymptomatic individuals. In our study, isolation rates were 36.4% from specimens with genital lesions and only 7% from those with no evidence of lesions. This distinction is important in evaluating any HSV detection system. Our evaluation indicated that an RK cell system was a more sensitive means of isolating HSV than DF systems, particularly in detecting asymptomatic HSV shedding, and that a DFMT system performed as well or with slightly enhanced sensitivity compared with standard DF tube cultures.

The microtiter system was initiated for cost effectiveness, ease of method, and the fact that it required less space to process large numbers of specimens, especially in studies involving asymptomatic excretion of HSV. Neither medium changes nor capping of individual tubes was required by the plate system. Microtiter plates have the additional advantage of being rapidly screened for cytopathic effect by use of a mechanical stage inverted microscope. The microtiter system required an average of 4.25 h less technician time per 500 cultures than did tube cultures. In addition, disposable glass tubes represent approximately 2.5 times the cost of the plate system in our laboratory. It should be noted that in developing the microtiter system, cross-contamination between specimens and fungal contamination of the plates may occur. We experienced this problem early in the establishment of this system in approximately 1.5% of specimens and with five specimens (0.6%) inoculated in all three cell systems, as reported in this article. This problem was eliminated after completion of this study by changing the plate model from a 48-well system to a 24-well system with deeper wells (catalog no. 3424, no. 24, 16-mm wells; Costar). With the 24-well system, 98 known positive HSV cultures were inoculated in duplicate such that each inoculated well was surrounded by uninoculated control wells. These plates were checked daily for 10 days. No cases of cross-contamination or bacterial or fungal contamination were noted.

In summary, our study indicates that, as others have reported (2, 5, 10, 12), standard tissue cultures using RK cells demonstrated an enhanced sensitivity for HSV. This system should be considered in laboratory situations requiring maximum sensitivity, such as screening for asymptomatic genital shedding of HSV. The narrower spectrum of the RK system, however, may argue against its routine use, since RK cells are not permissive for cytomegalovirus, adenoviruses, or enteroviruses, any of which might be isolated from genital sites. The genital anatomic site or presence of lesions influenced relative isolation rates, but these were consistent within each culture system. The microtiter plate system utilizing DF cells for HSV is a technically easy, rapid, and cost-effective method, and it is an alternative to the same DF cell line used in standard tube cultures.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant AI-20381 from the National Institutes of Health.

We gratefully acknowledge the technical support of Olga Addae and Nancy Coomer in preparing the manuscript.

LITERATURE CITED

1. **Beckwith, D. G., D. C. Halstead, and C. H. Beckwith.** 1987. Comparison of the Ortho cultureset (Vero cells) and Difco cellmatics system (mink lung and primary rabbit kidney cells) for the detection of herpes simplex virus from clinical specimens. Diagn. Microbiol. Infect. Dis. **6**:145–149.
2. **Buthod, L. M., N. L. Schindler, and H. W. Rogers.** 1987. Determination of cell line suitability for rapid isolation of herpes simplex virus. J. Virol. Methods **18**:37–46.
3. **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.
4. **Chang, R. S., D. Arnold, Y. Y. Chang, I. Pan, and G. W. Jordan.** 1986. Relative sensitivity of cell culture systems in the detection of herpes simplex viruses. Diagn. Microbiol. Infect. Dis. **5**:135–141.
5. **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.
6. **Hsiung, G. D.** 1982. Diagnostic virology, p. 247–249, 276. Yale University Press, New Haven, Conn.
7. **Landry, M. L., D. R. Mayo, and G. D. Hsiung.** 1982. Comparison of guinea pig embryo cells, rabbit kidney cells, and human embryonic fibroblast cell strains for isolation of herpes simplex virus. J. Clin. Microbiol. **15**:842–847.
8. **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.
9. **Salmon, V. C., L. R. Stanberry, and J. C. Overall, Jr.** 1984. More rapid isolation of herpes simplex virus in a continuous line of mink lung cells than in vero or human fibroblast cells. Diagn. Microbiol. Infect. Dis. **2**:317–324.
10. **Smith, C. A., and S. J. Rubin.** 1986. Comparison of commercial sources of primary rabbit kidney and MRC-5 cell cultures for herpes simplex virus isolation. Diagn. Microbiol. Infect. Dis. **4**: 147–152.
11. **Wentworth, B., P. Bonin, K. K. Holmes, and E. R. Alexander.** 1973. Isolation of viruses, bacteria, and other organisms from venereal disease clinic patients: methodology and problems associated with multiple isolations. Health Lab. Sci. **10**:75–81.
12. **Zhao, L., M. L. Landry, E. S. Balkovic, and G. D. Hsiung.** 1987. Impact of cell culture sensitivity and virus concentration on rapid detection of herpes simplex virus by cytopathic effects and immunoperoxidase staining. J. Clin. Microbiol. **25**:1401–1405.