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Comparison of Standard Tissue Culture, Tissue Culture Plus Staining, and Direct Staining for Detection of Genital Herpes Simplex Virus Infection

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Genital herpes simplex virus infection in women was studied by using conventional tissue culture (TC) virus isolation compared with short-term (24-h) TC on Lab-Tek chamber slides followed by fluorescent-antibody (FA) staining. Three different staining techniques were used after TC: (i) staining with biotin-avidin (TC-BA/FA), (ii) direct FA (TC-FA), and (iii) indirect FA. The TC-BA/FA method showed complete correlation with the TC method. The TC-FA method showed no false-positive results but 31.5% false-negative results compared with the TC method. In contrast, the TC-indirect FA method showed 11.9% false-positive results and 11.7% false-negative results. The direct staining of specimens by the biotin-avidin technique (direct BA/FA) without prior tissue culture showed 37.7% false-positive results and 11.1% false-negative results. The TC-BA/FA technique thus was as sensitive as, but more rapid than, the TC method. The quality of fluorescence was far superior in TC-BA/FA staining as compared with TC-FA or TC-indirect FA procedures. The TC-BA/FA appears to be a valuable technique in laboratory diagnosis of genital herpes infections, especially in clinical situations requiring rapid detection of the virus.

A rapid and sensitive technique is needed to detect herpes simplex virus (HSV) infections in adults and infants, particularly at the time of delivery. Genital HSV infection of a mother at the time of delivery can be transmitted to the child. The risk of transmission of infection is 50% when the baby is delivered by the vaginal route (10) but can be decreased to 7% by cesarean section. Women with a history of genital herpes or women with sexual partner(s) with genital herpes are considered to be at high risk and should be checked weekly for herpes infection after the week 34 of gestation (3). If infection is present, the delivery should be by cesarean section to reduce the risk of transmission of the virus to the newborn. If the infection is transmitted to the newborn, the child should be cultured for HSV so that appropriate therapy can be used.

The objective of the present study was to compare the sensitivity and specificity of the standard technique of tissue culture observed for 7 days for cytopathic effect (TC-CPE) with the newly developed method of 24-h tissue culture followed by staining with biotin-linked anti-HSV antibody and fluorescein-avidin conjugate (TC-BA/FA) (11). In addition, we evaluated 24-h tissue culture followed by direct fluorescence (TC-FA) or indirect fluorescence (TC-IFA), as well as direct staining of smears of lesions using the biotin-avidin fluorescence method (direct BA/FA).

MATERIALS AND METHODS

Collection of specimens. Specimens were collected from patients by rubbing the lesions or genital area or both with cotton swabs. The swabs were placed in 3 ml of Eagle minimum essential medium containing streptomycin, penicillin, and mycostatin (henceforth referred to as collection medium), refrigerated before transport, and frozen at −70°C if not studied immediately (12).

HSV isolation by TC-CPE. Human foreskin fibroblast monolayers (Flow 7000, Flow Laboratories, McLean, Va.) were inoculated with 0.2-ml volumes of patient specimens. After absorption of the virus, fresh Eagle minimal essential medium with 2% fetal calf serum was added. The cultures were incubated in a 5% CO_2 incubator at 37°C for 7 days and evaluated for cytopathic effect daily.

HSV detection by TC-BA/FA, TC-FA, or TC-IFA. (i) Choice of cell line. The human foreskin fibroblast (Flow 7000) line was used in the detection of HSV since its relative adherence and sensitivity for detection of virus as determined by the immunofluorescence staining method were found to be optimum among several cell lines tested (11).

(ii) Preparation of monolayers with Flow 7000 cells and inoculation with clinical specimens. The cells were grown on eight-well Lab-Tek chamber-slides (Miles Laboratories, Inc., Naperville, Ill.) as described previously (11). Briefly, the chamber slides were first rinsed and incubated overnight with Eagle minimal essential medium containing 10% fetal calf serum to remove any toxic products which were occasionally seen in certain batches of these chambers. Approximately 30×10^3 to 40×10^3 cells were seeded in each well and were allowed to grow for 24 h. All specimens (100 to 200 μl) were inoculated at undiluted concentration in two different wells and studied after 24 h. Two wells of each chamber slide were either mock infected or infected with laboratory-purified HSV type 2 (HSV-2) (MS strain) preparations to give a negative and a positive control, respectively, on the same slide. At the end of incubation, the medium was aspirated, the slide was washed three times with phosphate-buffered saline (PBS), the plastic chamber was removed, and the slide was fixed in chilled (4°C) acetone for 10 min. The acetone was evaporated completely and slides were frozen at −70°C until stained.

(iii) Fluorescent-antibody staining techniques. Three different staining methods were studied after tissue culture of the genital specimens, and the results of each were compared with those of the TC-CPE method.

TC-BA/FA. The rabbit anti HSV-2 immunoglobulin G (IgG) (Dako-Accurate Chemicals and Scientific Corp., West-

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TABLE 1. Detection of HSV by the standard TC-CPE method at various times post-inoculation[a]

Day(s) postinoculation	No. of HSV-positive specimens detected (% positive)
1	15 (26.3)
2	38 (66.7)
3	48 (84.2)
4	53 (93.0)
5	56 (98.2)
5–8	57 (100)

[a] A total of 201 specimens were tested. Average time for detection of HSV was 2.4 days.

bury, N.Y.) was linked with biotin as described previously (11). The biotin-linked antibody was then titrated and used at a1:20 to 1:40 dilution. Fluorescein-avidin conjugate was obtained from Vector Laboratories, Burlingame, Calif. A 100-μl volume of properly diluted biotin-linked antibody was added to each well and incubated for 1.5 h at 37°C in a moist chamber. At the end of incubation the slides were washed with PBS three times, blot dried, and incubated with properly diluted fluorescein-avidin conjugate at 37°C for 0.5 h in a dark, moist chamber. The slides were then washed in PBS three times and counterstained with Evans blue. The soft plastic bonding material was peeled off with forceps when slides were still moist. The slides were blotted, air dried, and mounted with buffered glycerol (PBS-glycerol [1:9]) and viewed under a fluorescence microscope.

TC-FA. Rabbit anti-HSV-2 IgG antibody fluorescein conjugate was prepared as described previously (13). The slides were rinsed with PBS and incubated with properly diluted fluorescein-conjugated rabbit HSV IgG antibody (1:20) for 1.5 h at 37°C. At the end of incubation, the slides were washed three times with PBS, counterstained and mounted as described above, and viewed under a fluorescence microscope.

TC-IFA. Rabbit anti-HSV-2 IgG and sheep antirabbit IgG fluorescein conjugate (Progressive Labs, Inc.) were used. The slides were rinsed with PBS and incubated with rabbit anti-herpes antibody (1:40) for 1.5 h. They were rinsed with PBS three times and incubated with sheep anti-rabbit antibody (1:50) for 1.5 h. At the end of incubation, the slides were washed three times in PBS, counterstained with Evans blue, and viewed under a fluorescence microscope.

Direct BA/FA. Scrapings of lesions were taken by nonabrasive metal spatulas on alcohol-moistened slides and then dried at room temperature. The slides were either stained immediately or saved at −20°C until stained. The staining was performed by using biotin-labeled anti-HSV-antibody and fluorescein-avidin as described in the TC-BA/FA section.

RESULTS

The clinical genital herpes specimens were studied by five techniques. The virus isolations were carried out on each specimen by the standard tissue culture method (TC-CPE) and observed for the appearance of cytopathic effects for 7 days. For the purpose of this analysis, the TC-CPE technique was considered 100% accurate and the sensitivity and specificity of each method were compared with those of this method. Isolates showing the presence of virus were also inoculated on primary rabbit kidney monolayer cultures to confirm the presence of HSV. The detection of HSV by TC-CPE at various postinoculation periods is given in Table 1. Only 26% of the HSV-positive specimens were detected as positive by the TC-CPE method in 24 h. Three different staining techniques were used after 24-h tissue culture on slide chambers: TC-BA/FA, TC-FA, and TC-IFA. The results of each procedure were compared with those from the TC-CPE method and are given in Table 2.

Staining of smears taken directly from lesions was carried out by the direct BA/FA method, and the results were compared with those of the TC-CPE method (Table 2). The ability to obtain good fluorescence staining with direct smears was dependent on careful collection and fixation. Extended drying or prolonged storage of unstained smears reduced the fluorescence and distorted the cells on the smears. When this occurred, the antigen-positive preparations could not be identified with certainty from the artifacts or debris which frequently stained intensely. Since it was necessary to rely on personnel in the local clinics for the cultures and smears, it was not possible to completely control the collection and fixation of the smears.

There was perfect correlation between the results of TC-CPE and TC-BA/FA. The comparison between TC-CPE and TC-FA showed 31.5% false-negative results but no false-positive results. The comparison between TC-CPE and TC-IFA showed 11.7% false-negative results and 11.9% false-positive results. The direct BA/FA showed 37.7% false-positive and 11.1% false-negative results.

This indicated that the TC-BA/FA method was as sensitive and specific as the TC-CPE method and was significantly more rapid. In contrast, TC-FA and TC-IFA were not as sensitive or specific as TC-BA/FA and were not methods of choice. The quality of fluorescence with BA/FA was considerably superior to that with the FA or IFA techniques. It was much brighter than either FA or IFA and did not show the nonspecific staining seen with IFA. Direct BA/FA gave a high frequency of false-positive reactions.

TABLE 2. Detection of HSV by standard TC-CPE method and by other methods[a]

No. of specimens tested for HSV by TC-CPE		Second testing method	No. of specimens tested for HSV by TC-CPE followed by second method			
Positive	Negative		Positive	Negative	False-positive (%)	False-negative (%)
73	175	TC-BA/FA	73	175	0 (0)	0 (0)
19	29	TC-FA	13	35	0 (0)	6 (31.5)
17	42	TC-IFA	20	39	5 (11.9)	2 (11.7)
36	61	Direct BA/FA	55	42	23 (37.7)	4 (11.1)

[a] Details of testing methods are given in the text.

DISCUSSION

Tissue culture has been recognized to be the most sensitive technique available for the detection of HSV (2, 4, 9, 12). This method is slow, however, taking up to 7 days for the detection of small amounts of virus. In clinical situations, this may be too long to obtain information of value in making a decision concerning method of delivery or initiation of therapy. Only 26% of the positive specimens could be detected at 24 h by the TC-CPE method. The average time required for detection of an HSV-positive specimen by the TC-CPE method was 2.4 days, which was comparable to that in another published report (7). By combining viral culture with the sensitive biotin-avidin fluorescence technique, we have found that HSV can be rapidly and accurately detected in clinical samples. The 24-h TC-BA/FA method was as sensitive and specific as the 7-day standard TC-CPE method. TC-FA and TC-IFA were neither as sensitive nor as specific as the TC-BA/FA method. The TC in combination with staining has been used in the past for HSV (5–8, 11) and for other viruses (16, 18).

Other rapid methods have been reported, including direct FA (14, 15) and direct immunoperoxidase (4, 14). Papanicolaou or crystal violet staining (1) of scrapings of HSV lesions has been tried, but all of these methods are less sensitive for detecting HSV than is TC-CPE isolation (17). The sensitivity of these methods varies and is much lower than that of the TC method. We have also tried the direct BA/FA method, but we encountered a high rate of false-positive results. Observations in this study show poor correlation between TC and direct BA/FA. The outcome of direct staining technique depends upon the number of intact cells showing the presence of viral antigens and morphological changes in some instances.

The use of 24-h tissue culture followed by biotin-avidin fluorescence staining has the advantages of sensitivity, specificity, and increased speed. The test is easily adaptable for routine laboratory use.

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