JOURNAL OF CLINICAL MICROBIOLOGY, May 1984, p. 705–706
0095-1137/84/050705-02$02.00/0
Copyright © 1984, American Society for Microbiology
Vol. 19, No. 5

Comparison of Cultureset and Bartels Immunodiagnostics with Conventional Tissue Culture for Isolation and Identification of Herpes Simplex Virus

DAVID L. SEWELL,[1,2]* STEPHEN A. HORN,[1] AND PATRICIA W. DILBECK[1]
Pathologists Central Laboratory, Inc.,[1] and Veterans Administration Medical Center,[2] Portland, Oregon 97207

Received 5 December 1983/Accepted 23 January 1984

Two 48-h Vero cell systems were compared with viral culture in human fibroblastic cells for isolation and identification of herpes simplex virus from clinical specimens. Both 48-h systems had 79% sensitivity and >99% specificity as compared with the conventional tissue culture method.

The rapid detection of herpes simplex virus (HSV) in clinical specimens is an important goal for clinical virology laboratories since the results may be used to minimize the consequences of the infection and to effectively manage patients. The most effective method of diagnosis is virus isolation and identification in tissue culture(1). Although HSV-infected cell monolayers may develop a cytopathic effect (CPE) within 2 days, some cultures may require more than 5 days (1,5). Immunoperoxidase staining of cell monolayers is a rapid technique for the detection of viral antigen (1, 2, 4, 5). In two separate studies, we compared identification by systems that combined isolation of HSV in Vero cell monolayers with identification by indirect (Cultureset; Ortho Diagnostics Systems, Inc., Raritan, N.J.) and direct (Bartels Immunodiagnostics, Inc., Bellevue, Wash.) immunoperoxidase assays with a conventional cell culture system.

Totals of 235 specimens in the Cultureset (CS) system and 131 specimens in the Bartels Immunodiagnostics (BI) system were inoculated. Swabs from lesions were transported as recommended by each manufacturer and centrifuged at low speed for 10 min. The supernatant fluid (0.5 ml) was used to inoculate both the conventional tissue culture and the test systems.

With the conventional tissue culture system, the specimen supernatant was inoculated into one tube of human fibroblastic cells (Bartels Immunodiagnostics, Inc.) and incubated at 37°C. Cultures were fed with Eagle minimum essential medium containing 2% fetal bovine serum and antimicrobial agents (Bartels Immunodiagnostics, Inc.). The monolayer was observed for CPE at 48 h and daily for 1 week. No attempt was made to correlate the type of CPE observed with the virus isolated. CPE-positive tubes were passed to each Vero cell system, and the presence of HSV was confirmed by the immunoperoxidase assay. The results of these passages were not included in the comparative study.

Both the CS and BI systems employ a Leighton tube with the monolayer grown on a removable paddle. Cells maintained in minimum essential medium containing antibiotics were purchased weekly. Each system was used according to instructions of the manufacturer except that 0.5 ml was used as the inoculum. All specimens were fixed and stained at 48 h. In the CS kit, the monolayer was fixed with 10% Formalin for 30 min and stained by the indirect immunoperoxidase method. This method consisted of a primary antibody (rabbit anti-HSV), a linking reagent (sheep anti-rabbit immunoglobulins), and a labeling reagent (peroxidase-rabbit-antiperoxidase complex). In the BI system, the monolayer was fixed in acetone for 10 min and stained by the direct immunoperoxidase procedure (peroxidase-labeled HSV antibody). The evaluation of the two systems was performed over two different periods so that a direct comparison of CS and BI could not be made.

The sensitivity, specificity, and the predictive accuracy of positive and negative tests were determined by the method of Feinstein (3).

In the CS study (Table 1), HSV was isolated from 72 of 235 specimens by conventional tissue culture (30.6%) and 57 specimens by CS (24.3%). One false-positive reaction was observed with CS. The sensitivity and specificity of CS as compared with conventional tissue culture were 79.2 and 99.4%, respectively. The predictive values of positive and negative test results were 98.3 and 91.5%, respectively.

In the BI study (Table 1), HSV was isolated from 57 of 131 specimens by conventional tissue culture (43.5%) and 45 specimens by BI (34.4%). The sensitivity and specificity were 78.9 and 100%, respectively. The predictive values of positive and negative tests were 100 and 86%, respectively.

The sensitivity of each system decreased as the time to CPE development increased (Table 2). When results from both studies were combined, 52/129 (40%) culture-positive specimens demonstrated CPE at 2 days as compared with 102 (79%) cultures positive by the immunoperoxidase systems. After 4 days, 86% of the cultures were positive by CPE. The average time to detection of a positive culture by CPE was 3.4 days in the CS study and 2.9 days in the BI study. The lower number of positive cultures (30.6 versus 43.5%) in the CS study as well as the longer detection time suggested that the specimens received during the two study

TABLE 1. Comparison of the commercial systems and tissue culture method for isolation and identification of HSV

Commercial test system	HSV isolation	
	No. positive	No. negative
CS		
Positive	57	1
Negative	15	162[a]
BI		
Positive	45	0
Negative	12	74[b]

[a] Four cultures demonstrated CPE but were negative for HSV.
[b] Six cultures demonstrated CPE but were negative for HSV.

* Corresponding autho:

periods were significantly different. These differences may be related to the type of specimen submitted and the stage of the lesion at the time of sampling (1, 4). This information was not available for comparison.

Two other recent studies (2, 4) have compared CS with conventional tissue culture. Fayram et al. (2) used three tubes each of diploid fibroblasts (MRC-5) and cynomologous monkey kidney cells and a total inoculum of 1.5 ml, whereas Hayden et al. (4) used two tubes of human fibroblasts (MRC-5) and a total inoculum of 0.4 ml. The latter study compared 24-h CS results with those of the conventional system. The percentage of positive specimens for HSV in these studies varied from 21 to 35% and probably reflects variation in the type of specimen submitted to the laboratory. The sensitivity of the CS system varied from a low of 65% (2) to 79% in the present study. The specificity of the CS method was similar in all of the studies. Comparison of multiple studies (2, 4, 5) suggests that the sensitivity of an immunoperoxidase system for the detection of viral antigens in cell monolayers does not greatly improve when the monolayer is incubated longer than 24 h. This observation needs to be evaluated in another study.

The staining of a cell monolayer by either an indirect or direct immunoperoxidase assay yields very similar results. The direct method is much simpler to perform and costs less than the indirect procedure. However, both systems are significantly less sensitive than isolation in a conventional tissue culture method and therefore cannot be used as the only method for HSV detection.

TABLE 2. Detection time of positive cultures by conventional tissue culture and commercial systems

Commercial test system	No. of specimens positive for CPE on postinoculation day:				Avg time (days) to detection
	2	3	4	≥5	
CS					3.4
Positive	24	12	15	6	
Negative	1	4	3	7	
BI					2.9
Positive	27	14	3	1	
Negative	0	2	6	4	

LITERATURE CITED

1. **Corey, L., J. Dragavon, and D. Benjamin.** 1982. Immunoperoxidase staining in the clinical virology laboratory, p. 246–253. *In* R. C. Tilton (ed.), Rapid methods and automation in microbiology. American Society for Microbiology, Washington, D.C.
2. **Fayram, S. L., S. Aarnaes, and L. M. De La Maza.** 1983. Comparison of Cultureset to a conventional tissue culture–fluorescent-antibody technique for isolation and identification of herpes simplex virus. J. Clin. Microbiol. **18**:215–216.
3. **Feinstein, A. R.** 1977. Clinical biostatistics, p. 214–226. C. V. Mosby Co., St. Louis, Mo.
4. **Hayden, F. G., A. S. Sorensen, and J. A. Bateman.** 1983. Comparison of the Immulok Cultureset kit and virus isolation for detection of herpes simplex virus in clinical specimens. J. Clin. Microbiol. **18**:222–224.
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.