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Evaluation of a Commercial Enzyme-Linked Immunosorbent Assay for the Detection of Herpes Simplex Virus

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A total of 136 specimens were tested for the presence of herpes simplex virus by routine tissue culture and a commercial enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Inc.). Forty-six (33.8%) of the specimens were positive by tissue culture. The sensitivity and specificity of the commercial system were 69.6 and 93.3%, respectively. The commercial system was rapid and moderately specific but lacked the sensitivity necessary for direct specimen testing.

Genital herpes simplex virus (HSV) infections cause major health problems related to the transmission of HSV between sexual partners and between mothers and infants during delivery. Antiviral therapy and delivery of the infant by cesarean section can be used to minimize the risks associated with HSV infections (5, 10). Current recommendations for the management of pregnant women with genital HSV infections include weekly virus cultures during the last month of gestation (1). These procedures are useful only when a rapid and accurate diagnostic test is available to identify persons with symptomatic or asymptomatic HSV infections.

The conventional tissue culture method is the most sensitive system for the detection of HSV in clinical specimens (2). Recently, immunoperoxidase staining of inoculated monolayers of conventional tissue culture cells has been used to decrease the time required to detect a positive culture (3, 8). This method is both rapid and specific, but its sensitivity varies from 65 to 79%, so that it cannot replace the conventional tissue culture. Enzyme-linked immunosorbent assays (ELISAs) are currently being evaluated for the direct detection of HSV antigen in clinical specimens (6, 7, 9). This approach is rapid and can eliminate problems associated with loss of viability during transportation of specimens. This report evaluates a commercial ELISA system (Ortho Diagnostic Systems, Inc.) for the direct detection of HSV in clinical specimens.

Clinical specimens were collected on swabs from patients with suspected HSV infections and from pregnant women with a history of genital herpes. The specimens were transported at 4°C in 2.0 ml of commercial virus transport medium (Bartels Immunodiagnostics, Inc.) When received in the laboratory, the specimens were vortexed briefly and centrifuged at a low speed for 10 min.

The specimen transport medium (0.5 ml) was added to one tube each of Vero and human fibroblast cells and incubated at 37°C. The specimens were stored at 4°C for up to 24 h before the presence of HSV was tested by the ELISA method. Vero cells were fixed at 48 h in acetone for 10 min and stained by a direct immunoperoxidase procedure (Bartels Immunodiagnostics, Inc.). The cell monolayer was examined for infected cells by light microscopy. The human fibroblast cells were examined for cytopathic effect at 48 h and daily thereafter for 1 week. Tubes positive for cytopathic effect were passed onto Vero cells for confirmation of HSV by the immunoperoxidase assay.

The ELISA was performed by the addition of specimen (0.2 ml) to microtiter wells coated with rabbit HSV antibody. The wells were incubated at room temperature for 2 h. After being washed, peroxidase-labeled HSV antibody (0.2 ml) was added and incubated at room temperature for 2 h. Excess conjugate was removed by washing, and *o*-phenylenediamine (0.2 ml) was added. The trays were incubated at room temperature for 30 min. The reaction was stopped by the addition of 2 N HCl (0.05 ml). The optical density at 490 nm was determined on a Dynatech Minireader II. A substrate blank, two negative transport medium controls, and two positive controls were included in each run. The specimen was considered positive when the result was greater than or equal to the mean optical density of the negative controls plus 0.1 optical density unit.

A total of 136 specimens were examined by ELISA and conventional virus culture. Forty-six specimens (33.8%) were positive by tissue culture. Of these, 32 were positive by ELISA and 14 were negative. The sensitivity of the ELISA was 69.6%. Six specimens were positive by ELISA but were isolation negative. The specificity of the assay was 93.3%. One of the six patients with a false-positive result had a positive HSV culture 10 days earlier, suggesting that the ELISA may have detected noninfectious virus. The predictive value of positive and negative ELISA results were 84.2 and 85.7%, respectively (4).

Twenty-seven specimens (58.7%) were positive for cytopathic effect at 48 h, compared with 22 specimens (47.8%) positive by ELISA (Table 1). As the time to development of cytopathic effect increased, the sensitivity of the ELISA decreased, indicating that it may be less efficient than tissue culture for the detection of low titers of HSV.

The direct detection of HSV in clinical specimens by a commercial or research ELISA is a rapid method (4 to 6 h) for the diagnosis of herpes simplex virus infections. The test was only moderately sensitive, detecting 53 to 78% of specimens positive by culture (7, 9). The sensitivity varied with the volume of transport medium and the type of specimen. However, the sensitivity of the system developed by Ortho Diagnostics Systems was comparable to that of culture when both the specimen and the inoculated culture tubes were assayed for the presence of HSV antigens (7).

A major problem associated with the immunological detection of HSV antigen in clinical specimens is the variability of false-positive results observed by different investigators

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TABLE 1. Detection time of positive cultures by conventional tissue culture and antigen ELISA

ELISA result	No. (%) of specimens positive for CPE on following day p.i.[a]:			
	2	3	4	≥5
Positive	22 (47.8)	5 (10.9)	4 (8.7)	1 (2.1)
Negative	5 (10.9)	3 (6.5)	5 (10.9)	1 (2.1)

[a] CPE, Cytopathic effect; p.i., postinoculation.

(6, 7, 9). More detailed studies are required to determine the variables that affect test specificity (e.g., source of specimen, stage of infection, transportation conditions, type of tissue cells used) and the clinical implications of a specimen that contains noninfectious virus particles. Until these problems are resolved, it is unlikely that major patient management decisions (i.e., cesarean section) would be based solely on the results of an ELISA performed directly on the clinical specimens.

LITERATURE CITED

1. **Corey, L., H. G. Adams, Z. A. Brown, and K. K. Holmes.** 1983. Genital herpes simplex virus infections: clinical manifestations, course, and complications. Ann. Intern. Med. **98**:958–972.
2. **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.
3. **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.
4. **Feinstein, A. R.** 1977. Clinical biostatistics, p. 214–226. C. V. Mosby Co., St. Louis, Mo.
5. **Kibrick, S.** 1980. Herpes simplex infection at term. J. Am. Med. Assoc. **243**:157–160.
6. **Land, S.-A., I. J. Skurrie, and G. L. Gilbert.** 1984. Rapid diagnosis of herpes simplex virus infections by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **19**:865–869.
7. **Morgan, M. A., and T. F. Smith.** 1984. Evaluation of an enzyme-linked immunosorbent assay for the detection of herpes simplex virus antigen. J. Clin. Microbiol. **19**:730–732.
8. **Sewell, D. L., S. A. Horn, and P. W. Dilbeck.** 1984. Comparison of Cultureset and Bartels Immunodiagnostics with conventional tissue culture for isolation and identification of herpes simplex virus. J. Clin. Microbiol. **19**:705–706.
9. **Warford, A. L., R. A. Levy, and K. A. Rekrut.** 1984. Evaluation of a commercial enzyme-linked immunosorbent assay for detection of herpes simplex virus antigen. J. Clin. Microbiol. **20**:490–493.
10. **Whitley, R. J., A. J. Nahmias, S.-J. Soong, G. G. Galasso, C. L. Fleming, and C. A. Alford.** 1980. Vidarabine therapy of neonatal herpes simplex virus infection. Pediatrics **66**:495–501.