NOTES

Sandwich Enzyme Immunoassay and Latex Agglutination Test for Herpes Simplex Virus Keratitis

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We determined the in vitro sensitivities and the diagnostic efficacies of a commercially available latex agglutination test and an enzyme-linked immunosorbent assay using a rabbit model of herpes simplex virus type 1 dendritic keratitis. In contrast to the latex agglutination test, the HERPCHEK enzyme-linked immunosorbent assay offers an extremely sensitive and reliable means of laboratory diagnosis of experimental herpes simplex virus keratitis and is more rapid than viral isolation in tissue culture.

Recent advances in immunologic technology have led to the development of new assays for the rapid and specific diagnosis of herpes simplex virus (HSV) infections of the ocular surface (5). Rapid identification of HSV antigens can resolve clinical diagnostic dilemmas and directly influence the choice of medical therapy.

Previous commercially available indirect enzyme-linked immunosorbent assays (ELISAs) for the detection of HSV antigens have not proven to be as sensitive as tissue cultures in the diagnosis of nonocular herpetic infections (1, 4, 7, 8). However, because of its exquisite sensitivity, the HERPCHEK ELISA is the first assay to have been granted U.S. Food and Drug Administration approval for the detection of HSV in lieu of tissue culture (3). The HERPCHEK assay has recently demonstrated a 100% correlation with the diagnosis of active clinical dendritic keratitis (3). In a study of oral and genital lesions, HERPCHEK showed a sensitivity and specificity of 97.5 and 98.6%, respectively (2).

In this study, the diagnostic efficacy of a commercially available latex agglutination (LA) test (Virogen Herpes Slide Test; Wampole Laboratories, Cranbury, N.J.) and an ELISA (HERPCHEK; Du Pont Co., Wilmington, Del.) was evaluated in vitro as well as in vivo by using a rabbit model of HSV type 1 (HSV-1) dendritic keratitis.

Primary stromal (fibroblastic) cultures were established from human and rabbit corneal buttons by using standard techniques. Primary epithelial cultures were established from rabbit corneal buttons, as previously described (7). Confluent Vero cell monolayers (4×10^6 to 5×10^6 cells per 75-cm² flask) were infected with HSV McKrae at a multiplicity of infection of 10 PFU per cell. Cell monolayers were maintained at 37°C in Eagle minimal essential medium containing 20% fetal bovine serum and antibiotics. At 24 and 48 h postinfection, the cell monolayers were trypsinized and suspended in minimal essential medium. Cell suspension concentration was determined with a hemacytometer, and serial dilutions were made in minimal essential medium. Samples of each dilution (100 and 50 μ l) were tested by the HERPCHEK and LA methods, respectively. The Du Pont HERPCHEK Direct HSV Antigen Test has been described previously (3). The Virogen Herpes Slide Test, a 30-min LA

slide test for the detection of HSV antigens, has been described previously (6).

New Zealand female albino rabbits weighing 2.0 to 3.0 kg were inoculated with HSV-1 McKrae in minimal essential medium (10^4 PFU/20 μ l). A 20- μ l drop of virus suspension was applied to each eye after corneal scarification in an interlocking crosshatch pattern with a sterile no. 10 Bard-Parker scalpel blade. After viral inoculation, the eyes were closed and gently massaged for 10 to 20 s. Rabbit eyes were stained with fluorescein and examined with a slit-lamp biomicroscope preinoculation and on postinoculation days 1, 3, 5, 7, 9, and 11.

Each cornea was simultaneously swabbed with sterile Dacron and HERPTRAN swabs. The swabs, held adjacent to each other, were brushed lightly across the cornea. The Dacron swabs were placed immediately into sterile 15-ml tubes containing 0.5 ml of culture medium. The inoculated fluid was either processed by LA or plated on Vero cells and incubated in 5% CO₂ at 37°C. The cells were examined daily for 7 days for the appearance of the cytopathic effect characteristic of HSV-1. The HERPTRAN swabs were placed in HERPTRAN medium and stored at -20°C. The specimens were then processed by the HERPCHEK assay, as described above.

The lower limits of the LA assay (giving a 1+ result) were 250 to 300 human stromal keratocytes when tested 48 h after synchronous HSV infection. At 48 h postinfection, more than 840 rabbit corneal epithelial cells and 900 rabbit stromal keratocytes were required to produce a 1+ result. From each cell line, 3×10^3 HSV-infected cells were required to produce a 4+ result. Freeze-thawing the cells prior to testing marginally enhanced the sensitivity of the assay. All concentrations of uninfected cells gave negative results.

HSV-infected cells were serially diluted in HERPTRAN medium and tested by the HERPCHEK assay. The lower limits of sensitivity of HERPCHEK that gave a positive result were 0.8 and 1.5 HSV-infected human stromal keratocytes tested 24 and 48 h, respectively, after synchronous infection. The minimum number of HSV-infected rabbit stromal keratocytes detected was fewer than 24 and 5.3 cells when tested 24 and 48 h postinfection, respectively. Fewer than 4 and 1.6 HSV-infected rabbit epithelial cells were detected at 24 and 48 h postinoculation, respectively. All concentrations of uninfected human stromal keratocytes, rabbit stromal keratocytes, and rabbit epithelial cells produced a negative result.

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FIG. 1. Comparison of HERPCHEK and tissue culture methods in detecting HSV from ocular swabs of rabbits infected with experimental HSV keratitis over an 11-day time course.

Thirty-two rabbit eyes infected with the McKrae strain of HSV-1 were examined daily by biomicroscopy for twelve days. All corneas showed signs of acute HSV infection between days 4 and 6. Most corneas had classic dendrites, and the remaining corneas had other forms of ulcerative epithelial disease. Most of these epithelial lesions had resolved by days 9 to 11. There was no attrition of rabbits due to encephalitis prior to day 7. The attrition rates on days 9 and 11 were 6 and 13%, respectively.

At 5 days postinoculation, eight rabbit corneas with dendrites characteristic of acute HSV infection were swabbed, and the swabs were placed in culture medium. None of the culture medium samples tested positive by LA. In contrast, all of the samples tested positive by tissue culture.

Rabbits were swabbed for viral isolation in tissue culture and for the HERPCHEK assay preinoculation and on post-inoculation days 1, 3, 5, 7, 9, and 11. The results of the tissue culture can be seen in Fig. 1. Of the infected eyes, 38% were positive by tissue culture viral isolation 1 day after infection. Peak recovery (100%) of the virus was seen on days 3 and 5. Viral isolation had dropped to 21% on postinoculation day 11.

The HERPCHEK ELISA gave results similar to those obtained by viral isolation in tissue culture (Fig. 1). At 1 day postinoculation, however, only 13% of infected eyes gave a positive reading. All animals tested positive on days 3 and 5, in complete concord with viral recovery in tissue culture. By day 11, virus detection had declined to 33% in a fashion similar to the decline with tissue culture. All animals sacrificed but not inoculated with HSV tested negative by the HERPCHEK assay and by tissue culture at all times.

We demonstrated that HERPCHEK has a sensitivity similar to that of tissue culture in detecting HSV directly from corneal lesions over an 11-day time course. The detergents in the HERPTRAN medium serve to break up infected cells and viral particles. This may account for the enhanced detection of even fractions of infected cells in some cases and demonstrates the exquisite sensitivity (compared with that of LA) of HERPCHEK in detecting low HSV loads. Unlike viral isolation in tissue culture, the HERPCHEK assay does not require viable infectious particles in order to yield a positive result.

HERPCHEK is a rapid 4-h assay that has been approved by the Food and Drug Administration for detection of HSV in lieu of tissue culture. It is considerably more rapid than tissue culture and has comparable HSV detection capabilities. Although the assay is not as rapid as LA, we demonstrated that HERPCHEK has a much higher sensitivity in detecting HSV antigens both in vitro and in vivo. Disadvantages of HERPCHEK include its lack of specificity in detecting HSV-1 versus HSV-2, which can be clinically useful information on rare occasions, and the requirement of special equipment (an ELISA reader) and technical expertise. In addition,

the lack of a live viral isolate precludes epidemiologic studies such as HSV DNA restriction endonuclease mapping or determination of antiviral susceptibilities. Finally, HERPCHEK cannot detect a wide range of viral pathogens but detects only HSV and otherwise yields negative results.

Our results demonstrate that the LA assay lacks the sensitivity to detect HSV antigens in experimental HSV dendritic keratitis. No culture-positive corneal lesion gave a positive result by LA. Parallel to this, in vitro studies revealed that hundreds of infected cells were necessary to produce a positive LA reading. LA is a rapid 30-min test which does not require expensive equipment or special technical expertise. Like the HERPCHEK assay, LA does not distinguish between HSV-1 and HSV-2 and therefore requires additional forms of testing to determine type.

We compared all of our results with results of viral isolation in tissue culture, which has historically been considered the gold standard for HSV detection. Advantages of tissue culture include antiviral susceptibility determination, HSV DNA restriction endonuclease mapping, and high sensitivity. Theoretically, one complete virus particle is sufficient for a positive result. Finally, tissue culture represents a more "open-minded" laboratory method which can be utilized to detect a variety of viral pathogens which are not predetermined. Disadvantages of viral recovery in tissue culture include the length of time for isolation and the requirement of special materials, equipment, and expertise. Additionally, tissue culture requires complete, enveloped, infectious virus as opposed to HSV antigens alone.

We conclude that the HERPCHEK assay provides a rapid and reliable means of diagnosis of experimental (and clinical [3]) dendritic keratitis in contrast to the LA assay, which is of suboptimal sensitivity. The HERPCHEK ELISA offers sensitivity equal to that of viral isolation in tissue culture while providing a result in a fraction of the time.

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