Detection of Herpes Simplex Virus in Conventional Tube Cell Cultures and in Shell Vials with a DNA Probe Kit and Monoclonal Antibodies

MARK J. ESPY AND THOMAS F. SMITH*

Section of Clinical Microbiology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

Received 3 April 1987/Accepted 13 October 1987

Specimens submitted for diagnosis of herpes simplex virus (HSV) infection were inoculated into shell vials and reacted with a commercial DNA probe kit (Pathogen; Enzo Biochem, Inc., New York, N.Y.) and an immunofluorescence assay at 16 h postinoculation. The results were compared with isolation of the virus in conventional tube cell cultures. Of 504 specimens, 108 (20.8%) were positive for HSV. Of the 105, 93 HSV-positive specimens (89%) were detected by all three assay systems. Maximum detection of HSV (100 of 105 [95%]) was obtained by probe or monoclonal antibody assay in shell vials, which had sensitivities of 98 and 97%, respectively, compared with viral recovery in conventional tube cell cultures (mean time for recognition of cytopathic effects, 2 days). Both shell vial assays were 99% specific. The DNA probe kit may be used as an alternative to a monoclonal antibody and fluorescence assay in shell vials as a diagnostic method for rapid laboratory detection of HSV infection.

Herpes simplex virus (HSV) causes local oral, genital, and dermal infections in addition to severe systemic involvement in neonates and immunocompromised patients. The clinical importance and prevalence of HSV infections is reflected by the frequency of detection of this virus in diagnostic laboratories (16). Availability of monoclonal antibodies has facilitated the development of rapid and sensitive tests for the detection of HSV infections (4, 6). Radiolabeled nucleic acid probes have been shown to be sensitive and specific for detection of both HSV and cytomegalovirus, but these reagents are not commercially available (1, 10, 14, 17, 18). More recently, the development and availability of some biotinylated probes has allowed introduction of these assays into clinical laboratories without the attendant disadvantage of working with radioactive reagents (2, 4, 5, 7, 8, 9, 11). We compared a biotinylated DNA probe kit and a monoclonal antibody and immunofluorescence assay for the detection of HSV from clinical specimens at 16 h postinoculation in shell vials with recovery of the virus from clinical specimens in conventional tube cell cultures.

MATERIALS AND METHODS

Cells. MRC-5 cells (Viromed Laboratories, Minneapolis, Minn.) were grown in Eagle minimal essential medium containing 10% fetal bovine serum, penicillin, streptomycin, and gentamicin (15).

HSV monoclonal antibody. Fluorescein isothiocyanate-labeled monoclonal antibodies specific for HSV-1 and HSV-2 were purchased from Syva Co., Palo Alto, Calif.

HSV DNA probe kit. HSV Pathogene kits were purchased from Enzo Biochem, Inc., New York, N.Y. (catalog no. EBP-875). The HSV DNA probe consisted of a mixture of three sequences, two (3.0- and 8.0-kilobase fragments) from the cleavage of HSV-1 with BamHI and one (16.0-kilobase fragment) from the cleavage of HSV-2 with BglII. The fragments were cloned into pBR322 and labeled with biotin. The resultant probe reacts with both serotypes of HSV.

Specimens. Specimens were obtained from genital (n = 360), oral (n = 45), dermal (n = 94), and ocular (n = 5) sites from patients suspected of having HSV infections and inoculated into a single conventional cell culture tube (16 by 25 mm) and three 1-dram (ca. 3.7-ml) shell vial cell cultures, each containing 12-ml medium. Specimens were incubated at 36°C for 16 h, cover slips from two shell vials were stained with monoclonal antibody and the remaining cover slip was reacted with the DNA HSV probe.

Staining procedures. (i) Monoclonal antibody and indirect immunofluorescence. Cells on two cover slips were fixed for 10 min in cold acetone and then stained in shell vials with 150 μl of HSV-1 and HSV-2 reagent. Shell vials were incubated at 36°C in a moist chamber for 30 min, and then the monolayers were washed twice in phosphate-buffered saline. The cover slips were removed from the vials, mounted (cell side down) on glass slides, and examined with a fluorescence microscope at ×200 and ×400 magnifications (6).

(ii) DNA probe and peroxidase staining. Cells on cover slips were fixed in cold acetone for 5 min in shell vials. The acetone was aspirated, the cover slip was removed from the shell vial and mounted (cell side up) on a glass slide with a drop of clear nail polish as an adhesive. Biotinylated probe solution (1 drop, 0.025 ml) was added to the cell monolayer, and the slide was placed in a moist chamber and incubated in a water bath at 90°C for 30 min. The moist chamber was then placed in a 36°C incubator for 1 h to allow hybridization with the HSV probe. After the slide was washed with a solution of 0.015 M sodium citrate and 0.15 M sodium chloride (SSC), 0.1% Triton X100, and phosphate-buffered saline (for 2, 2, and 1 min, respectively), the cells were reacted with an avidin-horseradish peroxidase solution at 36°C for 30 min. The slide was then washed as previously described and then reacted with hydrogen peroxide and aminoethylcarbazole in acetate buffer for 5 min at room temperature. Cover slips were then washed in distilled water, counterstained for 1 min with fast green, and examined at ×200 and ×400 magnifi-
RESULTS

HSV was detected in 105 (20.8%; 71 genital, 10 oral, 23 dermal, and 1 ocular) of 504 specimens. Of the 105 HSV-positive specimens, 93 (89%) were detected by cytopathic effects in conventional tube cell cultures, by nucleic acid hybridization with a DNA probe in shell vial assays, and by monoclonal antibody and fluorescence (Table 1). Of the 100 HSV strains detected in shell vials, 61 were HSV-2 and 39 were HSV-1. Of the 12 instances in which HSV was not detected by at least one test, the specimen was obtained from genital sites (10 of 12; 83%; 2 oral). Compared with conventional tube cell cultures, the sensitivities of nucleic acid hybridization and the monoclonal antibody and fluorescence assay in shell vials were 98 and 97%, respectively; both assays yielded specificities of 99%. Maximum detection of HSV (100 of 105; 95%) was obtained by both assays performed in shell vials, whereas 97 isolates (92%) were recovered in conventional tube cell cultures.

Positive reactions of the DNA probe assay were easily detected in monolayers on cover slips by the red-colored precipitate deposited almost exclusively in the nuclei of HSV-infected cells at 16 h postinoculation (Fig. 1). The probe did not react with other members of the herpesvirus group, cytomegalovirus, and varicella-zoster virus that were similarly inoculated and stained in shell vials with the HSV probe assay.

DISCUSSION

Two previous studies have been reported that evaluated this DNA probe kit with clinical specimens. In the first report, HSV genome was detected by in situ hybridization in 16 (94%) of 17 stored human brain specimens that were previously positive for HSV by cell culture isolation (4). Further, the utility of in situ hybridization for detecting HSV in frozen tissue in this study was indicated by positive findings in six specimens that had negative immunofluorescence results after repeated testing. Fung et al. compared the utility of the commercial DNA probe with those of indirect immunofluorescence and cell culture for detecting HSV from genital, oral, ocular, and dermal sites (5). In their procedure, cells that remained in the specimen extract after cell culture inoculation were sedimented, suspended, and deposited on glass slides in preparation for nucleic acid hybridization and indirect immunofluorescence testing. Importantly, of the 243 specimens processed in their study, only 162 (67%) were considered satisfactory for DNA probe and immunofluorescence testing on the basis of observation of ≥2 intact cells per high-power field on the slide preparations. Relative to cell culture recovery of HSV, the sensitivity and specificity of the DNA probe assay for 162 acceptable specimens were 71 and 91%, respectively, but only 42% (25 of 59) compared with total viral recovery from the initial 243 specimens. These investigators expressed additional concerns regarding accurate interpretation of results with the hybridization procedure in that 27% of the acceptable specimens in their study exhibited nonspecific cytoplasmic staining with the DNA probe, consistent with the finding of Forghani et al., who observed staining in squamous or blood cells in normal tissue preparations (4).

We evaluated the HSV probe in a study design different from those of these two previous investigations. In our study, HSV in clinical specimens was amplified by inoculation and centrifugation in shell vial cell cultures for 16 h, after which the monolayers on cover slips were stained with monoclonal antibody or reacted with the DNA probe. In this system, deposition of precipitated stain subsequent to reaction with the probe kit components was clearly evident in intact HSV-infected cells in contrast to a background of normal cells in a monolayer that were not stained. Interpretation of results was clear-cut. In addition, all 504 specimens received in our laboratory during this study were acceptable for processing and not dependent on the number of intact cells remaining after centrifugation of specimen extracts as for the direct detection assay (5).

Recovery of HSV in conventional tube cell cultures did not significantly differ from detection of HSV by nucleic acid hybridization or a monoclonal antibody and fluorescence assay in shell vials; nevertheless, both assay procedures in shell vials detected three more positive specimens than did the conventional tube cell cultures that required 2 or more days for 68 (70%) of 97 of the isolates to be recognized by characteristic cytopathic effects. Eight specimens that did not yield HSV in conventional tube cell cultures were positive in shell vials by either the fluorescence (monoclonal antibody) or hybridization (DNA probe) technique. The medical charts of only two of these patients were available for review; both had clinical evidence of HSV infection. Nevertheless, the high individual specificity of each method evaluated in this study was indicated by the agreement in results of 93 (93%) of 100 of the total positive specimens that agreed with a previous report from our laboratory (6).

Specimen sources (genital, oral, dermal, and ocular) represent a cross section of sites that yield over 99% of the HSV routinely detected in clinical laboratories. Therefore, from a standpoint of the amount of virus present in each sample and the ability to detect low-titered samples, our study with 504 specimens should be representative of the usual workload in most laboratories. Conceivably, tissue specimens—particularly from the brain—may not have the quantity of virus present compared with, for example, genital sites. On the other hand, during one 8-year period in our laboratory (1974 to 1982), 1,141 HSV isolates were detected, but only 7 (0.6%) were from tissue sources.

The simplest and probably least expensive method for the diagnosis of HSV infections in the laboratory is isolation of the virus in conventional tube cell cultures. On the other hand, the clinical need for more rapid diagnostic procedures has provided impetus for the development of sensitive and specific tests that provide results for all specimens within 16 h after receipt in the laboratory and can be performed at costs comparable to those of the slower conventional meth-

<table>
<thead>
<tr>
<th>No. of specimens positive for HSV</th>
<th>Detection by:</th>
<th>Conventional tube cell cultures</th>
<th>Shell vials</th>
<th>DNA probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE 1. Detection of HSV in conventional tube cell cultures and in shell vials by DNA probe and monoclonal antibody
ods. Both the DNA probe and monoclonal antibody assays after shell vial amplification fulfill this goal (7). Although the reagent expense was greater for the monoclonal antibody reagents that for the DNA probe ($13.75 versus $7.25 per test), the actual time required for the hybridization procedure was 19 min, compared with 9.2 min for the immunofluorescence assay. Of the 97 HSV isolates recognized by cytopathic effects in conventional tube cell cultures, only 29 (30%) of 97 could be reported to the attending physician 24 h after receipt into the laboratory, in agreement with the experience of other investigators (3, 12, 13).

Our results indicate that the commercially available biotinylated probe for HSV is sensitive and specific for the diagnosis of the virus in shell vials at 16 h postinoculation and may be used as an alternative to monoclonal antibodies and a fluorescence assay.

LITERATURE CITED