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Comparison of the Detection of Herpes Simplex Virus in Direct Clinical Specimens with Herpes Simplex Virus-Specific DNA Probes and Monoclonal Antibodies

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A comparison of two commercially available kits for rapid herpes simplex virus (HSV) detection directly in patient specimens was performed. The immunofluorescence assay (IFA) utilized monoclonal antibodies to HSV, and the DNA probe assay utilized three HSV sequences cloned into pBR322. A sample of 243 specimens received in viral transport medium were inoculated into MRC-5 tissue cultures. The remainder of the specimen was centrifuged, and the cellular pellet was examined by IFA and DNA probes. One hundred and sixty-two (66.7%) specimens were considered satisfactory for IFA and DNA probe testing, based on a criterion of observing ≥2 intact cells per high-power field. Of the 162 specimens, 35 (21.6%) yielded HSV by culture. By IFA, the sensitivity of detecting HSV culture-positive specimens was 77.1%; specificity was 100%, positive predictive value was 100%, and negative predictive value was 93.3%. DNA probe sensitivity was 71.4%; specificity was 90.6%; positive predictive value was 67.6%; and negative predictive value was 92%. Forty-four (27.2%) of the 162 specimens exhibited nonspecific cytoplasmic staining with the DNA probe. IFA and DNA probe assays can be completed in 2 to 3 h, whereas the average time to culture positivity in this series was 2.2 days. Rapid HSV diagnosis can aid in timely and appropriate patient management.

The incidence of genital herpes simplex virus (HSV) infection has increased in the past 20 years. During the period from 1960 to 1979, there has been a ninefold increase in patient consultation for genital herpes in the United States (6). A major concern of patients with genital HSV infections is transmission of the virus to neonates at the time of delivery. HSV neonatal infections are acquired in 5 to 50% of vaginal deliveries to mothers with genital HSV (13). Approximately 1 in 7,500 live births or 500 cases of neonatal HSV infections occur in the United States annually (7). At present, the reduction of neonatal HSV infection is one of prevention. Weekly or biweekly viral cultures or cytologic monitoring of pregnant women with recurrent genital HSV, beginning between 32 to 36 weeks of gestation, may determine whether Caesarean section should be performed (2). However, viral cultures take a minimum of 2 days for positive results, while the Papanicolaou smear has a sensitivity of 26 to 75% (5, 13). Rapid and accurate diagnostic techniques for diagnosing HSV at the time of labor may be a better screen for those women at risk. In addition, rapid diagnostic tests for detecting HSV can direct immediate antiviral therapy to patients with disseminated HSV infections or HSV encephalitis.

The purpose of the present investigation is to evaluate two recently available kits for rapid HSV detection in direct specimens. The two kits are based on different detection methods. The first is an indirect immunofluorescence assay (IFA) for the detection of HSV antigen. Though IFA for HSV detection has been evaluated by several investigators (12, 14, 16, 17), this study utilizes monoclonal antibodies to HSV. The second method is the detection of HSV DNA by utilizing DNA probes. The two methods were compared with tissue culture isolation for HSV.

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MATERIALS AND METHODS

Patient specimens. Two hundred and forty-three specimens submitted to the Microbiology Division of the University of Connecticut School of Medicine for HSV cultures were processed for viral isolation, HSV antigen detection, and HSV DNA detection. Of these 243 specimens, 206 were from genital sites, 6 were from rectal sites, 13 were from oral sites, 5 were from eye specimens, 9 were from skin lesions, 1 was from a cyst site, 1 was from gastric aspirate, 1 was from a lung site, and 1 was from a liver site. Two hundred and ten specimens were from female patients, and 33 specimens were from males. The specimens were transported to the laboratory in viral Culturettes (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.). Samples were stored at 2 to 8°C before transport and were submitted to the laboratory within 48 h of collection.

Viral isolation. Upon receipt in the laboratory, each Culturette swab was placed in 2 ml of viral transport medium containing Hanks balanced salt solution, 0.5% fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and amphotericin B (1 μg/ml). The inoculated viral transport medium was mixed vigorously on a vortex apparatus, and 0.5 ml was inoculated into one tube of MRC-5 cells (Ortho Diagnostic Systems, Carpinteria, Calif.) with 1 ml of maintenance medium consisting of Eagle minimal essential medium with Earle balanced salt solution, nonessential amino acids, and sodium pyruvate (M.A. Bioproducts, Walkersville, Md.) supplemented with 2 mM L-glutamine, penicillin (100 IU/ml), streptomycin (100 μg/ml), amphotericin B (1 μg/ml), gentamicin (50 μg/ml), and 2% fetal bovine serum. MRC-5 tubes inoculated with oral and rectal specimens were washed one to two times with 1 ml of maintenance medium within 4 to 6 h of inoculation, before incubation. Tissue specimens were homogenized in Hanks balanced salt solution to make a 10 to 20% suspension before

inoculation of tissue cultures. Tissue cultures were incubated at 35°C for 2 weeks with daily examination for cytopathic effect (CPE).

Typing of HSV isolates were done only upon physician request. HSV was typed with the MICRO TRAK HSV type 1 (HSV-1) and HSV type 2 (HSV-2) typing reagents (Syva Co., Palo Alto, Calif.). Cells of CPE-positive cultures were scraped, centrifuged at 183 × *g* for 10 min, washed with 1 ml of phosphate-buffered saline (PBS) (pH 7.2), and centrifuged again at 183 × *g* for 10 min. The pellet was applied onto two areas of a slide, air dried, and fixed in acetone for 5 min. Each area of the slide was stained with either HSV-1 or HSV-2 reagent for 30 min at 37°C. The slide was rinsed with distilled water, mounted with glycerol, and read for fluorescent staining.

HSV antigen detection. After tissue culture inoculation, the remaining specimen in viral transport medium was centrifuged at 1,146 × *g* for 10 min. The cellular pellet was examined for HSV antigen with the Virgo Antigen Detection System (Electro-Nucleonics, Inc., Columbia, Md.). The Virgo HSV reagents consist of two mouse monoclonal antibodies, the HSV type-common antibody which reacts with both HSV-1 and HSV-2 and the HSV-2-specific antibody which reacts only with HSV-2. Both monoclonal antibodies are of the immunoglobulin M (IgM) class and are intended for the detection and typing of HSV in direct clinical smears and from tissue culture isolates.

Each cellular pellet was suspended in a minimal volume of viral transport medium and applied onto a three-well slide. The slide was air dired, fixed in acetone for 10 min, and stored at −70°C before IFA staining. IFA staining was performed by adding 20 μl of HSV type-common antibody to well 1, PBS to well 2, and HSV-2-specific antibody to well 3, followed by a 30-min incubation at 37°C in a moist chamber. The slide was rinsed with IFA buffer (PBS [pH 7.4]) provided with the kit, and then 20 μl of fluorescein-labeled goat anti-mouse immunoglobulin was applied and incubated for 30 min at 37°C in a moist chamber. The slide was again rinsed with IFA buffer, mounted with buffered glycerol, and examined for apple-green fluorescent cells over a red background at ×400 magnification with a Leitz Laborlux microscope with epifluorescence.

Specimens were considered satisfactory for IFA evaluation if an average of two or more intact cells per field were observed. The entire well was examined. Specimens were graded 1+ to 4+, depending on the quantity of cells observed per high-power field (HPF), i.e., 1+ for specimens containing two cells per HPF, 2+ when less than 50% of the field was covered with cells, 3+ when 50 to 75% of the field contained cells, and 4+ when 75 to 100% of the field contained cells. Satisfactory specimens not staining with either monoclonal antibody were considered negative. Satisfactory specimens staining only with the HSV type-common antibody and not with the HSV-2-specific antibody or PBS were considered to contain HSV antigen and to be of HSV-1. Satisfactory specimens staining with both monoclonal antibodies but not with PBS were considered to contain HSV antigen and to be of HSV-2.

HSV DNA detection. The cellular pellet was also examined for HSV DNA detection with the Herpes Simplex Virus Patho-Gene Kit (Enzo Biochem, Inc., New York, N.Y.). The HSV DNA probe consists of a mixture of three sequences, two (3.0- and 8.0-kilobase fragments) from the cleavage of HSV-1 genome with *Bam*HI and one (16.0-kilobase fragments) from HSV-2 genome cleaved with *Bgl*II (11, 19). The fragments were cloned into pBR322 and labeled with biotin. The HSV DNA probe is shipped in hybridization solution consisting of 50% formamide, 10% dextran sulfate, and 2 mg of carrier DNA per ml in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). At present, the Patho-Gene kits are intended for research use only.

The hybridization and detection protocols used were those provided by the manufacturer. The cellular pellet was applied to a glass slide, air dried, and fixed for 5 min in Carnoy B fixative consisting of 60% ethanol, 30% chloroform, and 10% acetic acid. Slides were stored at −70°C before hybridization. Hybridization was performed by applying 20 μl (240 ng) of the HSV DNA probe mixture to the specimen fixed on the slide, covering the slide with a glass cover slip, and placing the slide in a moist chamber. The chamber was incubated first in an 80°C water bath for 30 min to denature double-stranded DNA and then at 37°C for 1 h for hybridization. The cover slip was removed, and the slide was washed with 2× SSC for 5 min, followed by washes of 0.1× SSC for 10 min at 65°C, 2× SSC for 5 min, 0.1% Triton X-100 for 2 min, and PBS for 5 min to remove unhybridized DNA probe. Twenty microliters of the streptavidin-horseradish peroxidase detection complex was added to the slide and incubated at 37°C for 30 min. The slide was again washed in 2× SSC for 5 min, 0.1% Triton X-100 for 2 min, and PBS for 10 min to remove unbound streptavidin. Unless stated, all washes were done at room temperature. Biotin-streptavidin binding was detected by adding 20 μl of a freshly prepared 0.05% diaminobenzidine tetrahydrochloride–0.02% hydrogen peroxide substrate solution incubated at room temperature for 5 min. The slide was rinsed with distilled water, counterstained with cobalt chloride for 30 s, and rinsed again in distilled water. The slide was examined at ×400 magnification for rusty brown color over a blue background.

Specimens were similarly considered satisfactory for hybridization evaluation if two or more intact cells per field were observed. Satisfactory specimens that had no brown color were considered negative for HSV DNA. Satisfactory specimens which stained brown at confined nuclear regions of the cell were considered positive for HSV DNA. In some specimens, only diffuse brown staining over the entire cell or over cytoplasmic areas of the cell were observed. These specimens were categorized as having nonspecific background staining and no HSV DNA.

RESULTS

Of the 243 specimens examined, 59 (24.3%) contained HSV by cell culture isolation. However, only 162 specimens (66.7%), were considered satisfactory for HSV antigen and HSV DNA detection by using the criterion of observing two or more intact cells per HPF. Based on the 1+ to 4+ criteria of quantitating cells per HPF (see Materials and Methods), 23 specimens were graded as 1+, 26 as 2+, 20 as 3+, and 95 as 4+. Of the 162 satisfactory specimens, 147 were from genital sites, 3 were from rectal sites, 5 were from oral sites, 4 were from skin sites, 1 was from a cyst site, 1 was from a lung site, and 1 was from a liver site. None of the five eye specimens were satisfactory for HSV antigen or HSV DNA detection. Of the 162 specimens, 149 were from females and 13 were from males.

Of the 162 acceptable specimens, 35 (21.6%) were culture positive for HSV. The mean number of days to CPE detection was 2.2, with a range of 1 to 6 days. Sixteen specimens demonstrated CPE by day 1 of incubation, 26 demonstrated CPE by 2 days, and 28 (74.3%) demonstrated CPE by 3 days.

Table 1 shows the correlation between HSV isolation and

HSV antigen detection. Of the 35 HSV-containing specimens, 27 demonstrated HSV antigen by the IFA technique. Of the 27 IFA-positive specimens, 12 also reacted with the HSV-2-specific antibody. In no case was a specimen positive only with the HSV-2-specific antibody and not with the HSV type-common antibody. Also, no specimen demonstrated nonspecific staining in the PBS control well. With each IFA run, HSV-1 and HSV-2 tissue culture control slides were also stained. The immunofluorescence patterns of HSV-2-infected cells were speckled and patchy staining in the nucleus and diffuse staining in the cytoplasm with either monoclonal antibodies. HSV-1 infected cells staining similarly with the type-common monoclonal antibody. One hundred and twenty-seven specimens were negative for HSV by both culture and IFA. None of the HSV culture-negative specimens stained with the HSV IFA reagents. However, eight HSV culture-positive specimens failed to demonstrate HSV antigen by IFA. Thus, the sensitivity of detection of HSV-containing specimens was 77.1% by IFA, with a specificity of 100%, positive predictive value of 100%, and negative predictive value of 93.3% (Table 2). Neither HSV monoclonal antibodies stained tissue culture cells infected with Epstein-Barr virus, varicella-zoster virus, or cytomegalovirus (CMV).

Since HSV typing of culture isolates was done only on physician request, 11 of the 35 HSV isolates were typed. The direct typing of one specimen, however, could not be compared with the typing of its corresponding culture isolate, since both HSV monoclonal antibodies failed to react with the direct clinical specimen. In the other 10 cases, 6 were correctly typed in direct clinical specimens. One was typed correctly as HSV-1. In the five specimens which reacted with both HSV type-common antibody and HSV-2-specific antibody, the latter antibody stained with notably less intensity than did the type-common antibody. The four specimens whose direct typing failed to correspond to typing of their corresponding HSV isolates were HSV-2.

Table 1 shows the correlation between HSV isolation and HSV DNA detection. Of the 35 HSV-containing specimens, 25 demonstrated HSV DNA by the nucleic acid hybridization technique (Fig. 1A and B). Of the 10 specimens which did not hybridize with the DNA probe, 4 also did not react with the IFA monoclonal antibodies (Table 3). On the other hand, six specimens were DNA probe positive, IFA negative, and four were DNA probe negative, IFA positive (Table 3). These differences may be due to sampling differences of the specimens. Additionally, 12 other specimens from which HSV was not isolated also demonstrated nuclear brown staining with the DNA probes. Interestingly, two of these specimens may have been true-positive HSV DNA detection results, since both patients had HSV isolated from an additional specimen taken from the same site within 3 days of the other specimen. Both patients also had HSV isolated from these sites at least 1 to 4 weeks before the HSV DNA-positive, HSV culture-negative specimen. No HSV DNA was detected in 115 HSV culture-negative specimens. However, 10 specimens from which HSV was isolated did not hybridize with the HSV DNA probes. Based on HSV culture results, the HSV DNA probes had a sensitivity of detecting HSV in direct clinical specimens of 71.4%, with a specificity of 90.6%, positive predictive value of 67.6%, and negative predictive value of 92.0% (Table 2). The HSV DNA probe did not hybridize with tissue culture cells infected with Epstein-Barr virus, varicella-zoster virus, or CMV.

TABLE 1. Comparison of tissue culture isolation of HSV, IFA, and hybridization with DNA probes

Tissue culture	No. of specimens	No. of specimens reacting with:			
		IFA		DNA probes	
		Positive	Negative	Positive	Negative
Positive	35	27	8	25	10
Negative	127	0	127	12	115

TABLE 2. Comparison of the significance of IFA and DNA probes in relation to tissue culture isolation for HSV

Method	Results (%) with the following parameters			
	Sensitivity	Specificity	Predictive value	
			Positive	Negative
IFA	77.1	100	100	94
DNA probes	71.4	90.6	67.6	92

Of the 162 satisfactory specimens, 44 (27.2%) stained nonspecifically with the HSV DNA probe. The nonspecificity appeared as diffuse brown staining throughout the cell or only in the cytoplasmic region of the cell (Fig. 1C and D). Except for the one specimen each from oral, rectal, and cyst sites, all other nonspecific specimens were from genital sites, which contain an enormous amount of microbial flora. The majority of cells staining diffusely with the probes exhibited bacterial organisms adhering to their cell surfaces. Hypothetically, the hybridization of the HSV DNA probes with some bacterial organisms may occur. The HSV DNA probes were cloned by using plasmid vector pBR322. This plasmid was constructed from fragments of several natural plasmids, including pSC101 which has a tetracycline resistance gene and the Tn*A* segment of an R-factor which carries an ampicillin resistance gene (3, 4). It may be that bacteria which naturally carry plasmids to either or both antibiotic resistance gene(s) will hybridize with HSV DNA probe containing the pBR322 plasmid. We attempted to examine this possibility by examining whether such nonspecific-staining specimens will also react with the Enzo Biochem CMV DNA probes. The CMV probe reagent is a mixture of two CMV sequences also cloned into the *Bam*HI site of pBR322. It does not cross-hybridize with HSV-1 or HSV-2. When duplicate samples of three specimens staining nonspecifically with HSV DNA probes were examined by using the CMV DNA probed under identical hybridization conditions, there was little or no nonspecific staining observed. Thus, the nonspecific staining may not be just hybridization of HSV DNA probes to bacterial organisms carrying plasmid sequences similar to pBR322. Four of the nonspecific specimens were graded as false-positive HSV DNA staining by HSV culture results in that they also exhibited nuclear staining with the probe. One additional specimen which was considered to have nonspecific staining by the probes was culture positive for HSV and HSV antigen.

The percentage of cells in each specimen hybridizing with HSV DNA probes was compared with those reacting with the HSV common antibody (Table 3). The percentage of cells containing either HSV DNA or HSV antigen appears to be approximately the same. There was no direct correlation between specimens with a high percentage of HSV-positive

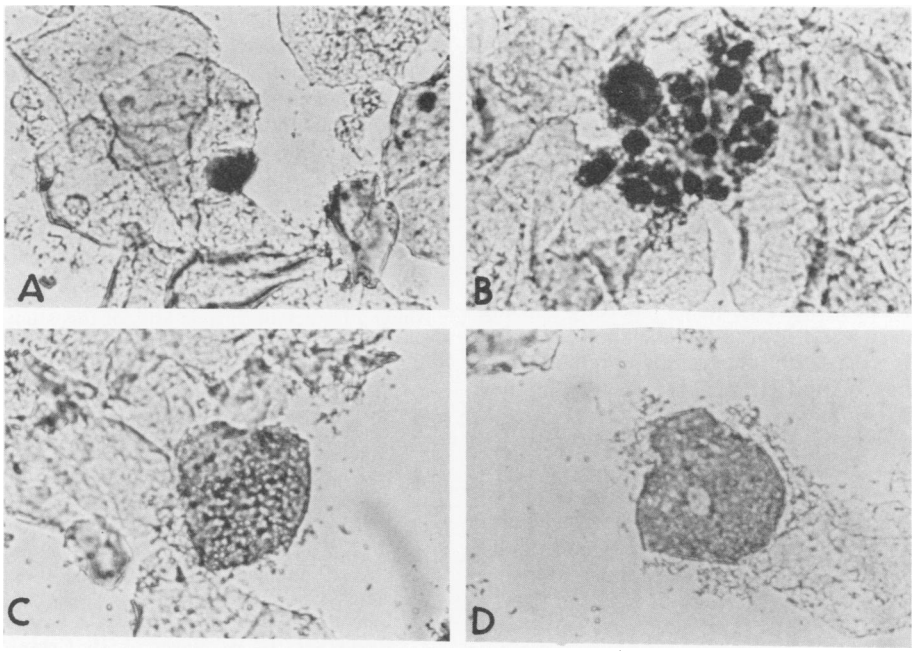

FIG. 1. Direct clinical specimens hybridizing with HSV DNA probes. (A) Nuclear staining; (B) multinuclear staining; (C) nonspecific staining throughout the cell; (D) nonspecific staining over cytoplasmic area of the cell.

cells by IFA or DNA probes and earlier CPE detection by culture.

DISCUSSION

The determination of HSV-containing specimens by HSV DNA or HSV antigen detection requires that the specimens contain sufficient numbers of cells for evaluation. Even after centrifugation, the cellular pellet applied onto glass slides did not contain the required average of two or more intact cells per HPF in 34.3% of the specimens examined. This criterion was selected since it was that used by the Electro-Nucleonics kit for adequacy in interpreting IFA slides with monoclonal antibody reagents. The Enzo Biochem kit, however, did not suggest a minimum number of cells for acceptability. Since both HSV DNA and HSV antigen detection procedures used in this study require microscopic examination, intact cells are necessary for the demonstration of specimen reactivity with antibody and DNA probe reagents. Though it is not statistically possible to determine which type of specimens collected will be more likely to be satisfactory for IFA and nucleic acid examination, it does appear that specimens from normally sterile sites may be inadequate for this processing technique. All five eye specimens were inadequate, probably due to the scarcity of cells obtained by scraping the eye. All specimens for HSV detection were collected with viral Culturettes containing Hanks balanced salt solution. A previous report (C. J. Huntoon, R. F. House, Jr., and T. F. Smith, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C104, p. 292) indicated that Hanks was superior in the Culturette system for the recovery of a number of viruses, including HSV. In vitro experiments indicated that HSV survived for 48 h in Culturettes containing any one of the three buffer transport media. We feel, then, that the Culturette system was adequate and maybe even optimal for the study.

TABLE 3. Comparison of the percentages of cells in direct clinical specimens reacting with IFA and DNA probe reagents

Cells hybridized with DNA probes (%)	No. of specimens with following % of cells stained in IFA				
	0	1	2–5	10	≥20
0	4	4		1	1
1	1	4		3	
2–5	2	7	2	1	
10		1		3	
≥20	1				

Though the cellular pellet method was used for IFA determination, Electro-Nucleonics recommends that a smear be made directly from patient lesions, exudates, etc. This difference in preparing IFA slides, however, may not explain the inadequacy of IFA interpretation in 34.3% of the specimens examined. It has been observed that, with IFA slides prepared directly from lesions, 23% were still unacceptable for evaluation with the Electro-Nucleonics kit (9).

However, the specimen collected may differ with the stage of the lesion. A vesicular lesion can be sampled by rupturing the lesion with a sterile needle, and the vesicular fluid can be collected for HSV culture examination. On the other hand, ulcerative lesions are sampled by rubbing the base of the ulcer with a swab. Thus, in vesicular lesions, fewer cells may be collected compared with samples from ulcerative lesions. Mean HSV titers from vesicles have generally been higher than those from ulcers and crusts (18). Even when smears of vesicular or ulcerative lesions were made from swabbing the base of the lesions, positive HSV IFA and culture results were higher for vesicles than for ulcers (12). During the vesicular stage of HSV disease, the isolation rate was 90% and the IFA value was 71%. For the ulcerative stage, isolation was 72% and the IFA value was 38% (12).

Our data show that commercially available HSV DNA probe and HSV monoclonal antibodies have comparable capabilities for detecting specimens containing HSV. Both had similar sensitivities of HSV detection. Results with IFA and the DNA probe were 77.1 and 71.4%, respectively. Both

detected approximately the same percentage of HSV-containing cells directly in HSV culture-positive specimens. The specificity of IFA is higher than that of DNA probes, 100 versus 90.6%. The positive predictive value of IFA was 100% compared with only 67.6% for DNA probes. Negative predictive values were similar, with 93.3% for IFA and 92% for DNA probes.

DNA probes detect the presence of complementary sequences of DNA. The detection of HSV gene sequences in host cells does not necessarily denote the presence of viable HSV. This may be especially true for the herpesviruses due to their ability to persist in infected hosts, thereby causing periodic recurrent disease. This property is called latency.

The sensitivity of IFA with monoclonal antibodies is comparable to studies of anti-HSV polyclonal antibodies with direct clinical specimens. Sensitivities of IFA with polyclonal antibodies have ranged from 62 to 86% (12, 16, 17). The specificity of the Electro-Nucleonics HSV type-common antibody reagent is also comparable to if not slightly better than polyclonal antisera. Specificity of polyclonal antisera to HSV culture isolation have ranged from 89 to 99.6% (12, 16, 17). However, the advantage of using monoclonal antibodies ensures a continuous supply of serological reagents with known sensitivity and specificity.

Although the Electro-Nucleonic HSV antibodies can be used for typing HSV from culture isolates and in direct clinical specimens, we did not try to thoroughly evaluate this aspect of its application. It did appear, however, that the HSV-2-specific monoclonal antibody stained with less intensity than did the HSV type-common monoclonal antibody. This could explain our failure to correctly type four of the nine specimens containing HSV-2 directly in clinical specimens. Comparison of the IFA monoclonal antibodies, restriction endonuclease analysis of viral DNA, and viral sensitivity to *E*-5-(2-bromovinyl)-2-deoxyuridine showed 100% typing agreement of HSV culture isolates (D. H. Zimmerman, F. K. Mundon, R. P. Stone, J. J. Docherty, and S. P. O'Neill, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C72, p. 248). Only 72% were typable on direct patient specimens (Zimmerman et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984). Thus, typing ability of IFA reagents for direct specimens and culture isolates is not the same. In this laboratory, typing of HSV isolates was done only on physician request. In this series, only 11 of the 35 isolates were requested for typing. As differences in antiviral drug susceptibilities are being observed between HSV-1 and HSV-2 (8), the typing of HSV may become more routine. In addition, there appears to be an association between HSV-2 and cervical cancer (1). Recurrence also appears to be more common with HSV-2 infection, with a frequency of 88% compared with 55% in patients with HSV-1 primary infection (7).

At present, biotinylated or ^{32}P-labeled DNA probes are able to detect 1 pg of purified HSV DNA, two to eight infected cells, or 10^4 PFU of HSV-1 (15). The main advantage of the biotin system is the ability to use a nonradioactive method of probe detection, based on the very high affinity reaction of biotin and streptavidin (10). With the development of other innovative indicator systems, nucleic acid hybridization may yield an even higher detection sensitivity.

IFA and DNA probes, then, can detect approximately 70 to 80% of HSV culture-positive specimens which contain enough cells for evaluation. Both methods, however, were unable to evaluate the presence of HSV in 33.3% of the specimens examined, due to their lack of having $\geq$2 intact cells per HPF. Both techniques are quite specific. The time required to complete either of these procedures is 2 to 3 h, compared with the average 2.2 days for HSV CPE detection. The rapid results obtained from IFA and DNA probes can direct appropriate and immediate antiviral therapy in cases of HSV encephalitis and disseminated disease. In pregnant women, rapid HSV diagnosis can also aid in clinical management at delivery. At this time, both methods should be considered only as an adjunct to viral culture isolation.

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LITERATURE CITED

1. **Aurelian, L.** 1983. Herpesviruses and cervical cancer, p. 79–123. *In* L. A. Phillips (d.), Viruses associated with human cancer. Marcel Dekker, Inc., New York.
2. **Binkin, N. J., J. P. Koplan, and W. Cates, Jr.** 1984. Preventing neonatal herpes. J. Am. Med. Assoc. **251**:2816–2821.
3. **Bolivar, F., R. L. Rodriguez, M. C. Betlach, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. I. Ampicillin-resistant derivatives of the plasmid pMB9. Gene **2**:75–93.
4. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. V. Betlach, H. L. Heynecker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
5. **Brown, S. T., H. W. Jaffee, A. Zaidi, R. Rilker, K. L. Hermann, H. C. Lylerla, D. F. Jove, and J. W. Budell.** 1979. Sensitivity and specificity of diagnostic tests for genital infection with *Herpesvirus hominis*. Sex. Transm. Dis. **6**:10–13.
6. **Centers for Disease Control.** 1982. Genital herpes infections—United States, 1966–1979. Morbid. Mortal. Weekly Rep. **31**:137–139.
7. **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.
8. **DeClercq, E., J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence, and D. Shuger.** 1980. Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. J. Infect. Dis. **141**:563–574.
9. **Electro-Nucleonics, Inc.** 1984. Herpes simplex virus identification and typing test, p. 15. Electro-Nucleonics, Inc., Columbia, Md.
10. **Leary, J. J., D. J. Brigati, and D. C. Ward.** 1983. Rapid and sensitive colorimetric method for visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose: Bio-blots. Proc. Natl. Acad. Sci. USA **80**:4045–4049.
11. **Locker, H., and N. Frenkel.** 1979. *Bam*I, *Kpn*I, and *Sal*I restriction enzyme maps of the DNAs of herpes simplex virus strains Justin and F: occurrence of heterogeneities in defined regions of the viral DNA. J. Virol. **32**:429–441.
12. **Moseley, R. C., L. Corey, D. Benjamin, C. Winter, and M. L. Remington.** 1981. Comparison of viral isolation, direct immunofluorescence, and indirect immunoperoxidase techniques for detection of genital herpes simplex virus infection. J. Clin. Microbiol. **13**:913–918.
13. **Nahmias, A. J., and M. Visintine.** 1976. Herpes simplex virus, p. 156–190. *In* J. S. Remington and J. D. Kelin (ed.), Infectious disease of the fetus and newborn. The W. B. Saunders Co., Philadelphia.
14. **Nerurkar, L. S., M. Namba, and J. L. Sever.** 1984. Comparison of standard tissue culture, tissue culture plus staining, and direct staining for detection of genital herpes simplex virus infection. J. Clin. Microbiol. **19**:631–633.
15. **Richman, D., N. Schmidt, S. Plotkin, R. Yolken, M. Cherensky, K. McIntosh, and M. Mattheis.** 1984. Summary of a workshop on new and useful methods in rapid viral diagnosis. J. Infect. Dis. **150**:941–951.
16. **Schmidt, N. J., J. Dennis, V. Devlin, D. Gallo, and J. Mills.** 1983.

Comparison of direct immunofluorescence and direct immunoperoxidase procedures for detection of herpes simplex virus antigen in lesion specimens. J. Clin. Microbiol. **18**:445–448.

17. **Schmidt, N. J., D. Gallo, V. Devlin, J. D. Woodie, and R. W. Emmons.** 1980. Direct immunofluorescence staining for detection of herpes simplex and varicella-zoster virus antigens in vesicular lesions and certain tissue specimens. J. Clin. Microbiol. **12**:651–655.
18. **Spruance, S. L., J. C. Overall, Jr., E. R. Kern, G. G. Kreuger, V. Pliam, and W. Miller.** 1977. The natural history of recurrent herpes simplex labialis. N. Engl. J. Med. **197**:69–75.
19. **Swain, M. A., and D. A. Galloway.** 1983. Nucleotide sequence of the herpes simplex virus type 2 thymidine kinase gene. J. Virol. **46**:1045–1050.