JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1989, p. 700–704
0095-1137/89/040700-05$02.00/0

Vol. 27, No. 4

Diagnosis of Herpes Simplex Virus Infection in a Clinical Setting by a Direct Antigen Detection Enzyme Immunoassay Kit

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Received 14 October 1988/Accepted 4 January 1989

A commercial 4-h direct herpes simplex virus (HSV) antigen detection enzyme immunoassay (EIA) kit (Du Pont Herpchek) was evaluated by using 273 clinical specimens obtained in a hospital-based infectious disease practice. The EIA was compared with a standard culture method in which WI38 cells were inoculated within 20 min of sample collection. Cultures were observed for 2 weeks, and positive findings were confirmed by fluorescein-labeled monoclonal antibody (FA) staining. The values for the overall HSV detection rate were 40.7% by the standard culture method and 41.4% by EIA. In eight cases, the EIA was positive, while the culture method was negative; however, clinical data and confirmatory blocking EIA suggested that a true HSV infection was present. For six FA-confirmed, culture-positive samples, the direct EIA was negative; however, an EIA performed on the supernatants of these cultures was positive, suggesting that the failure of the EIA to detect these samples was not due to lack of strain specificity of the test. After confirmatory tests of standard culture and EIA discrepant results, the overall sensitivity of the test was 95.0% (113 of 119) and the specificity was 100% (154 of 154).

Herpes simplex virus (HSV) is the viral agent most commonly isolated by the clinical laboratory (32). It is responsible for a wide range of disease in a variety of settings (14). Standard cell culture (CC) techniques require a minimum of 18 to 24 h for viral isolation and up to 14 days for a definitively negative result. This delay may make the management of infant delivery of a woman with herpes or the treatment of a seriously ill immunocompromised patient less than optimal. Other aspects of CC viral isolation which make it inconvenient include the need for specialized laboratory space and highly trained personnel, and the need for rapid transport and inoculation of samples for optimal viral isolation yield (5, 32).

In order to circumvent some of these drawbacks of viral isolation, several immunologically based direct HSV antigen detection systems have been developed. These consist of direct immunofluorescence, immunoperoxidase staining, and enzyme immunoassays (EIAs) (2, 3, 12, 18, 20, 21, 23, 26). Although these methods offer advantages in terms of rapidity and ease of performance, clinical reports have failed to demonstrate adequate sensitivity and specificity to permit replacement of CC viral isolation (12, 18, 20, 21, 23, 26). Another approach combines spin-amplified CC with immunofluorescence or immunoperoxidase staining for a more rapid diagnosis (11, 13, 17, 19, 22, 25, 27). Although this latter approach offers some improvement over CC, it still requires CC expertise, is more expensive, and requires a minimum of 24 h. Owing to the continued need for improved rapid diagnosis as outlined above, we evaluated a new commercially available EIA kit and compared it with standard CC viral isolation techniques for the identification of HSV.

MATERIALS AND METHODS

Patient population. This institutionally approved study was conducted at the Infectious Disease and Sexually Transmitted Diseases clinic of the Sir Mortimer B. Davis-Jewish General Hospital (McGill University), Montreal, Canada, between March 1988 and July 1988. The clinic is staffed by three physicians who specialize in infectious diseases and microbiology (A.D., J.P., and J.M.). In all cases, a standard history was obtained and a physical exam was performed. Emphasis was placed on the sex and age of the patient, age of the lesion at presentation, and location and stage of the lesion (4). Additional information about reproductive history and status, and the use of systemic and topical acyclovir was also recorded. Duplicate swabs were simultaneously collected from patients with suspected lesions or from asymptomatic patients with previously proven HSV infections. One swab was used for CC, and the other was used for EIA.

CC. Samples for viral culture were obtained by using Culturettes (Marion Laboratories, Kansas City, Mo.) which were inoculated onto WI38 cells (Connaught Laboratories, Willowdale, Ontario, Canada) within 20 min of sampling and often immediately at the bedside (5, 8). Emphasis was placed on rapid CC inoculation to avoid loss of viral viability in transport (5, 6). Inoculated WI38 cells were maintained in M-199 medium with Earle salts supplemented with 2% fetal calf serum, L-glutamine, penicillin, streptomycin, and amphotericin B in glass tubes (16 by 175 mm). CC tubes were examined for any cytopathic effect (CPE) daily for 2 weeks. Positive cultures were confirmed and typed by immunofluorescence staining (FA) utilizing monoclonal reagents (Syva Diagnostics, Palo Alto, Calif. [29]).

EIA. The Herpchek direct HSV antigen test, a 4-h microtiter plate-based EIA (Du Pont Co., North Billerica, Mass.), was evaluated according to the instructions of the manufacturer. Samples were collected using the Herptran collection and transport pack included with the kit. The pack consists of sterile cotton swabs and EIA transport medium (ETM) containing an antigen extraction agent. The swab containing the specimen was placed directly into ETM, and for the purposes of this study, stored at −80°C until tested. For determining the presence of HSV antigen, samples contained in ETM were added to strips of microwells coated with purified rabbit anti-HSV serum. Samples were run in

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duplicate, except when the sample volume was insufficient. After a 2-h incubation and washing, biotinylated, monoclonal, HSV-specific detector antibody was added. The test does not distinguish between the two HSV types. Following a 30-min incubation period and washing, streptavidin-horseradish peroxidase was added and after a further incubation of 15 min, *o*-phenylenediamine substrate was applied.

After 1 h, the optical density (OD) of each well was determined. A cutoff value was calculated by adding 0.09 to the mean of the OD values of three negative controls. The net OD values reported in this paper were determined by subtracting the cutoff value from the sample OD. In this way, OD values from different runs are normalized so that 0 and all positive numbers represent EIA-positive samples.

Additional testing of discrepant samples. The CC results of samples were not known to those carrying out EIA testing. When CC and EIA results were not in agreement, two additional tests were performed. If the EIA was positive, but CC was negative, an antibody blocking test was done. This consisted of incubating a 100-μl portion of the sample (contained in ETM) with 10 μl of high-titer anti-HSV human serum (Du Pont) for 30 min at room temperature. A second 100-μl portion of the sample was incubated with 10 μl of HSV antibody-negative human serum (Du Pont) for the same time period, and both portions of the sample were then run in the standard EIA. Reduction of 50% or more in the sample OD value by the HSV antiserum compared with that of the negative serum was regarded as confirmation of the presence of HSV antigen in the sample (24). In addition, clinical information was reviewed to determine the likelihood of the presence of an HSV infection at the time of sampling.

For CC-positive, EIA-negative samples, the CC isolate was tested by EIA in order to exclude the possibility that the EIA failed to detect certain clinical HSV strains. A positive result would eliminate this as the likely reason for the initial failure of the EIA.

Statistical analysis. Sensitivity and specificity were calculated by standard methods (9). Statistical analysis of EIA versus CC was performed by the McNemar test for the significance of changes and the distribution of crusted lesions was analyzed by the chi-square test (30).

RESULTS

A total of 273 samples were obtained from 176 women and 97 men. Of these, 235 were from genital lesions, 23 were from orofacial lesions, and 15 were from other anatomical sites. Of the 235 genital samples, 39 were from asymptomatic individuals currently without lesions but with proven recurrent HSV in the past. The median age of the male population was 32, with a range from 18 to 82 years, whereas the median age of the female group was 29, with a range from 16 to 88 years.

CC results. The overall viral isolation rate was 40.7% (111 of 273). Of the positive samples, 61.3% were from women. Thus, of the 97 male samples, 43 (44.3%) were CC positive, whereas 68 (38.6%) of the female samples were CC positive.

Of 235 genital samples, 96 were positive by CC. Of the 23 orofacial samples, 11 were positive, as were 4 of 15 samples from other sites. Of all vesicular, ulcerated, and crusted lesions studied, 68.8, 51.6, and 19.0%, respectively, were positive by CC. An additional sample from an asymptomatic woman was also positive. Of the CC-positive samples, 28 (25.2%) yielded CPE after the first 24 h. However, 27 (24.3%), 24 (21.6%), 15 (13.5%), and 17 (15.3%) of the CC-positive samples exhibited CPE 2, 3, 4 and ≥5 days after inoculation of CC. Thus in 74.8% of the samples, the time to identification was greater than 24 h compared with the 4 to 5 h required to obtain an EIA result.

TABLE 1. Results of EIA versus CC

Sample	Sensitivity of EIA (no. of EIA positives/no. of total positives[a])	Sensitivity of CC (no. of CC positives/no. of total positives)
All (total)	95.0 (113/119)	93.3 (111/119)
Site		
Genital	95.2 (99/104)	92.3 (96/104)
Orofacial	90.9 (10/11)	100 (11/11)
Other	100 (4/4)	100 (4/4)
Lesion		
Vesicle	100 (33/33)	100 (33/33)
Ulcer	91.3 (63/69)	95.7 (66/69)
Crust	100 (16/16)	68.7 (11/16)
No lesion	100 (1/1)	100 (1/1)

[a] Total positives are the sum of all confirmed positives by either CC or EIA.

All positive cultures were confirmed by standard direct FA (Syva). There were 26 HSV type 1 cultures and 84 type 2 cultures and in one case, immunofluorescence was not performed.

Comparison of CC results and EIA. Of the 111 CC-positive samples, 105 were also positive by EIA. However, there were an additional eight CC-negative, EIA-positive samples. Confirmatory testing and clinical information suggested that these eight CC-negative, EIA-positive samples probably represented true positives for a total of 119 EIA- or CC-positive samples (see below).

A total of 113 samples were positive by EIA, and of these, 46 (40.7%) were from men and 67 (59.3%) were from women. Of the 113 samples, 99 (42.1%) of the genital samples were EIA positive, whereas 10 (43.5%) orofacial samples and four (26.7%) samples from other sites were EIA positive. Of the 113 EIA-positive samples, typing was performed on 104 isolates. Of these 104 isolates, 25 were type 1 and 79 were type 2; in the remaining 9 isolates, no typing was performed. In eight of the isolates that were not typed, no isolates were available for typing as they did not grow in CC, and in one case, the isolate was lost. This yields EIA sensitivities of 96.2% for type 1 and 94.0% for type 2. Of the 273 samples obtained in the study, concordance of EIA and CC was noted in 259 samples. The overall sensitivity and specificity of the EIA compared with CC were found to be 94.6 and 95.1%, respectively. For genital samples, the sensitivity and specificity compared with CC were 94.8 and 94.2%, respectively. For orofacial samples, the sensitivity and specificity compared with CC were 90.9 and 92.3%, respectively.

Table 1 illustrates the sensitivity of EIA and CC with respect to the total number of samples confirmed to be positive by either of the tests used. EIA was positive in 68.8, 49.2, and 27.6% of vesicular, ulcerated, or crusted lesions, respectively. It was also positive in one (2.6%) asymptomatic patient. No statistical difference was demonstrated between the sensitivity of EIA or CC when the data was analyzed by sex, site, or lesion stage. No correlation was noted between EIA sensitivity and days until CPE appeared. No significant difference in sensitivity of EIA and CC was noted when data were analyzed by the age of lesion.

The OD values for the majority of samples fell at both extremes of the scale. Of the samples, 2.9% had OD values that were within ±0.03 around the cutoff value. Thus,

TABLE 2. Clinical and laboratory data on patients in whom discrepant CC and EIA results were noted

Discrepancy and patient no.	Age (yr)	Sex[a]	Age (days) of lesion at time of sampling	Site	Stage of lesion	Treatment	Net OD	FA type	EIA on CC supernatant	Confirmatory blocking EIA	Clinical data
CC positive, EIA negative											
1	37	M	>7	Genital	Ulcer	None	−0.024	2	+	NA[b]	NA
2	50	M	5	Genital	Ulcer	None	−0.1	2	+	NA	NA
3	40	F	3	Genital	Ulcer	None	−0.1	2	+	NA	NA
4	21	F	3	Genital	Ulcer	None	−0.041	2	+	NA	NA
5	24	F	9	Genital	Ulcer	None	−0.092	2	+	NA	NA
6	30	M	6	Orafacial	Ulcer	None	−0.07	1	+	NA	NA
CC negative, EIA positive											
1	24	M	3	Genital	Crust	None	0.177	NA	NA	Confirmed	Known recurrent HSV with clinical episode suggestive of HSV
2	48	F	7	Genital	Ulcer	Acyclovir	2.601	NA	NA	Confirmed	Known recurrent HSV with clinical episode suggestive of HSV
3	34	F	>7	Genital	Crust	None	0.139	NA	NA	Confirmed	Clinical episode suggestive of HSV
4	26	M	4	Genital	Crust	None	0.757	NA	NA	Confirmed	Clinical episode suggestive of HSV; sexual partner HSV positive
5	34	M	>7	Genital	Crust	None	3.009	NA	NA	Confirmed	Known recurrent HSV with clinical episode suggestive of HSV
6	35	M	2	Genital	Crust	None	0.23	NA	NA	Confirmed	Clinical episode suggestive of HSV
7	31	M	4	Genital	Crust	None	0.245	NA	NA	Confirmed	Known recurrent HSV with clinical episode suggestive of HSV
8	48	M	3	Genital	Crust	None	2.988	NA	NA	Confirmed	Known recurrent HSV with clinical episode suggestive of HSV

[a] M, Male; F, female.
[b] NA, Not applicable.

EIA-positive and -negative results were clearly distinguished. Discrepant results were not clustered near the EIA cutoff value. Specifically, 78.1% of positive, vesicular lesions and 60.3% of positive, ulcerated lesions had OD values of ≥2.0 compared with only 31.2% of positive, crusted lesions.

Discrepant results. Table 2 presents a detailed analysis of the 14 discrepant samples. There were six cases which were CC positive but EIA negative. In all of these cases, the culture supernatant was positive on testing by EIA, suggesting that the failure of the direct assay is due to a reason other than lack of recognition of the particular clinical HSV strain. All six CC-positive EIA-negative samples were typed by FA; five were type 2, and one was type 1.

Another group of discrepant results consisted of eight CC-negative, EIA-positive samples. Table 2 shows the relevant clinical information and results of the blocking confirmatory EIA tests. Except for the one patient on acyclovir, all samples in this category were obtained from crusted lesions. This was significantly different from the total CC-positive sample population ($P < 0.05$). In six of these eight cases, the patient or the sexual partner had a previous culture-proven HSV infection. In the remaining two cases, the clinical history and physical exam strongly suggested HSV infection as the diagnosis (Table 2).

Effect of acyclovir therapy. Eleven patients in the study received acyclovir treatment. Four patients, three on systemic and one on topical acyclovir, were positive for HSV by both EIA and CC. One patient on systemic therapy was EIA positive only. Six patients, four on systemic and two on topical therapy, were HSV negative by both tests.

DISCUSSION

Our overall HSV isolation rate of 40.7% and specific rates of 68.8, 51.6, and 19.0% for vesicular, ulcerated, and crusted lesions, respectively, compare favorably with previous reports on similar patient populations, indicating that our viral culture system had appropriate sensitivity (3, 12, 15, 18, 20, 23). If the "gold standard" is CC isolation, the overall sensitivity and specificity of the EIA, irrespective of the confirmatory procedure, are 94.6% (105 of 111) and 95.1%, respectively. Taking the confirmatory tests into account, we had a sensitivity of 95.0% (113 of 119) and a specificity of 100% (154 of 154) (Table 3). Exclusion of the patients receiving acyclovir therapy did not alter the sensitivity and specificity of this EIA.

Theoretical explanations to account for CC-positive, EIA-negative results include the following: (i) an inadequate swab sample obtained for EIA, (ii) failure of the monoclonal antibody to detect all HSV strains, (iii) interference in the assay by patient-derived HSV antibody present in blood or

TABLE 3. Performance of direct EIA for HSV diagnosis[a]

EIA result for HSV	No. of samples by CC showing indicated reaction for HSV		No. of samples by CC or blocking EIA showing indicated reaction for HSV	
	Positive	Negative	Positive	Negative
Positive	105	8	113	0
Negative	6	154	6	154

[a] EIA was compared with CC and CC or blocking EIA. When compared with CC, the sensitivity and specificity of EIA were 94.6 (105/111) and 95.1 % (154/162), respectively. When compared with CC and a confirmatory blocking test, the sensitivity and specificity were 95.0 (113/119) and 100% (154/154).

secretions in the sample, and (iv) greater CC sensitivity. The EIA procedure missed six CC-positive lesions. The culture supernatants of these CC-positive and direct EIA-negative samples were all EIA positive, demonstrating that EIA did detect these HSV strains. No visible blood was present in these discrepant samples. No attempt was made to determine the presence of antibody either in serum or in secretions of these patients. Therefore, of the above explanations, the first is the most likely, although the third possibility cannot be excluded.

In eight cases, the EIA was positive, and the CC was negative. All of these were confirmed positive by a blocking EIA, and furthermore, the clinical data supported the high likelihood of the presence of a herpetic infection. Several explanations for the discrepancy are possible (12). If we accept that these EIA positives are true positives, then CC may have failed because of either (i) an inadequate swab sample obtained for CC, (ii) lack of sensitivity of our CC system, (iii) loss of viral infectivity in transport, (iv) shedding of nonviable antigen in late-appearing lesions in which antibody may be present, (v) shedding of nonviable antigen late in the disease, (vi) partial genome expression of CC without demonstrable CPE, and (vii) patients receiving antiviral therapy, especially topical acyclovir, which may result in shedding of defective virus.

Of the eight CC-negative, EIA-positive results, five occurred in patients with late-presenting lesions, and in five of the eight, the lesion was at a healing crusted stage. In one of these eight cases, acyclovir was used by the patient.

Antigen detection in the face of culture negativity is not surprising, since we cannot expect CC to be 100% sensitive. This situation is similar to the problems encountered in the evaluation of direct antigen tests for other infectious agents (28, 31). To arbitrate discrepant results, one could consider either performance of blocking confirmatory immunoassays (33) (as done in this study) or the use of a second direct detection target, such as a different epitope or nucleic acid.

Blocking tests have traditionally been used to confirm the specificity of antigen detection immunoassays for such agents as rotavirus and human immunodeficiency virus type 1, as well as for HSV (2, 3, 12, 16, 21, 33). In the present EIA, three possible choices for blocking reagent exist, namely, HSV-specific monoclonal antibody, HSV hyperimmune animal antiserum, or human HSV immune antiserum. Since monoclonal antibodies are epitope specific, only a monoclonal antibody with identical specificity to the EIA detector antibody can be used to block the reaction. The utilization of such an antibody as a blocking reagent must always lead to a positive blocking reaction and would not provide a valid proof of specificity. If hyperimmune animal serum were used as a blocking reagent, the possibility of a cross-reaction with contaminants in the original immunogen (such as cell components) may lead to nonspecific blocking activity. Used with appropriate controls, human immune antiserum is an appropriate choice for blocking reagent. We found that a pool of HSV antibody-negative human sera did not interfere with the detection of HSV antigen in this EIA, whereas a pool of HSV antibody-positive human sera prevented the detector monoclonal antibody from binding to HSV antigen control, leading to a positive blocking reaction. In three of the situations shown in Table 2, recurrent HSV was not proven by CC. However, in one of the three cases, the sexual partner of the patient did have CC-proven HSV infection, and in all three cases, HSV was considered the sole diagnosis by the clinician on the basis of the clinical history and physical exam. Crusted lesions reportedly have a low probability of being detected by CC (18). It is expected that an antigen detection assay may be more sensitive than CC for such lesions.

Management of genital HSV infection in the parturient has recently come under review, and the effectiveness of weekly antenatal culture in the prevention of this entity has been questioned as a result of the necessary delay in the reporting of CC results (1, 10, 24). However, as noted in a recent editorial (10), the availability of a rapid test with appropriate performance characteristics should lead to a reevaluation of the management of the delivery in an HSV patient. We feel that this EIA is a great step in that direction. Overall, it approaches an acceptable level of sensitivity so as to replace CC when compared with very sensitive CC techniques; however, more clinical data must be generated before the applicability of this or any other rapid diagnostic test in labor and delivery can be ascertained. This test will certainly prove to be useful in the management of HSV in patients with sexually transmitted disease and immunocompromised patients and has previously been demonstrated to be useful as a confirmatory test of the clinical diagnosis of ocular HSV (7).

ACKNOWLEDGMENT

This study was supported by a grant from the Du Pont Company.

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