JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1984, p. 490–493 Vol. 20, No. 3
0095-1137/84/090490-04$02.00/0
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Evaluation of a Commercial Enzyme-Linked Immunosorbent Assay for Detection of Herpes Simplex Virus Antigen

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Received 16 April 1984/Accepted 21 May 1984

A commercial enzyme-linked immunosorbent assay (ELISA) for detection of herpes simplex virus (HSV) antigen in clinical specimens and culture lysates was evaluated. A total of 1,155 specimens and an additional 335 cell culture lysates were tested by ELISA, and results were compared to those obtained by cell culture in primary rabbit kidney, human foreskin, and MRC-5. The sensitivity and specificity of the direct test were 52.5 and 96.9% and those of the culture lysate test were 98.7 and 96.9%, respectively. The sensitivity of the direct ELISA correlated with early cytopathic effect in cell culture and varied with specimen source, ranging from 100% with skin lesions to 40.9% with cervical swabs. Of 60 cervical specimens from asymptomatic individuals, 22 (36.6%) yielded false-positives, which may be due to noninfectious HSV. No reproducible cross-reactions were found with other viruses isolated. The Ortho HSV ELISA was found to be rapid, sensitive, and specific for detection of HSV from cell culture lysates, but it needs reevaluation for direct specimen testing, in particular for screening of asymptomatic obstetrical patients.

Detection of herpes simplex virus (HSV) antigen in clinical specimens with the enzyme-linked immunosorbent assay (ELISA) technique has been reported by several authors (2, 5, 8). This technique offers the advantages of increased speed of detection and decreased occurrence of culture false-negative results due to inadequate transport of specimens.

Morgan and Smith reported that the Ortho Diagnostics ELISA for HSV antigen detection was "rapid and reliable," with a sensitivity of 75% (15 of 20), and 100% specific as compared with cell culture in primary rabbit kidney and MRC-5 (M. A. Morgan and T. F. Smith, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C74, p. 249). The Kaiser Permanente Regional Virology Laboratory will receive over 20,000 specimens for HSV diagnosis in 1984 from a predominantly obstetrical population; therefore, the evaluation of the Ortho HSV ELISA test was undertaken to determine its sensitivity and specificity as compared with the current tissue culture method.

MATERIALS AND METHODS

The clinical specimens were collected from patients of Southern California Kaiser Permanente hospitals and clinics and transported on wet ice in styrofoam containers to the Regional Virology Laboratory. The specimens submitted were primarily obtained from obstetrical and immunocompromised patients. The majority of specimens received were cervical swabs (48.2%), vulvar swabs (23.2%), and vaginal swabs (7.7%); fewer than 20% of specimen requisitions indicated lesions present. The two types of transport media used, sucrose-phosphate-glutamate buffer (2 ml) and Transporter tubes (Bartels Immunodiagnostics, Bellevue, Wash.) of human fibroblasts in 2 ml of minimum essential medium, have been previously described (7).

Cell culture method. Patient specimens collected in 2 ml of transport medium were treated on arrival at the laboratory with 0.5 ml of antibiotic mixture. This mixture consisted of minimum essential medium containing 10% fetal bovine serum (Sterile Systems, Logan, Utah), vancomycin hydrochloride (1,000 μg/ml), gentamicin sulfate (100 μg/ml), and amphotericin B (250 μg/ml). Undiluted urine specimens (2 ml) were treated with 1 ml of antibiotic mixture to neutralize acidity and reduce toxicity. Treated specimens (0.3 ml) were inoculated into three cell culture tubes each: primary rabbit kidney (Ortho Diagnostics, Carpinteria, Calif.), MRC-5, and human foreskin (Bartels). Inoculated cell culture tubes were refed with minimum essential medium containing 2% fetal bovine serum, gentamicin sulfate (15 μg/ml), and amphotericin B (2.5 μg/ml) at 10 to 12 h and weekly thereafter. All inoculated culture tubes were checked daily for cytopathic effect (CPE). This was done for a total of 7 days for genital specimens and between 14 and 35 days for specimens from other sources.

ELISA test procedure. The Ortho HSV antigen ELISA system may be used to detect HSV antigen in direct specimen material or in inoculated cell cultures treated with Ortho cell lysis agent (culture-amplified test). All genital specimens submitted were tested by the direct test. In addition, all cell culture tubes with CPE detected during the first 7 days of incubation were treated with cell lysis agent at 1+ CPE and tested by ELISA. Cultures from specimens which were positive by direct ELISA but without CPE on day 5 after inoculation were scraped, pooled, lysed, and tested by ELISA. All specimens showing discrepant results between ELISA and culture were retested.

For the ELISA, Ortho microwells coated with rabbit anti-HSV capture antibody were inoculated with 200 μl of direct specimen, cell culture lysate, or controls in duplicate. The following controls were run with each test: substrate blank well, two or three wells of Ortho high positive control, three negative transport medium control wells, and a laboratory HSV antigen control (three wells).

The laboratory HSV antigen control was prepared by inoculating MRC-5 cell culture tubes with HSV-2 (ATCC MS strain), incubating the tubes at 35°C, and harvesting tubes with 3+ CPE. The tubes were sonicated, pooled, and diluted with minimum essential medium to yield a lower optical density (OD) than the Ortho high positive control. The laboratory HSV antigen control was stored in 1-ml volumes at −70°C.

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Microwells containing specimens, culture lysates, and controls with capture antibody were incubated at room temperature for 2 h. Wells were washed three times for 1 min each with Ortho phosphate-buffered saline-Tween Wash Buffer and a Skatron AS 8-channel Mini Microwash (Sterling, Va.).

Ortho detector antibody (peroxidase-conjugated rabbit anti-HSV; 200 μl) was added to all wells except the blank with a Costar Octapette (Van Nuys, Calif.), and tests were incubated for 2 h at room temperature. Microwells were washed five times as above. Fresh substrate (*O*-phenylenediamine-hydrogen peroxide) was prepared in citrate-phosphate buffer. This substrate (200 μl) was added to all wells, tests were incubated at room temperature for 30 min, and 50 μl of 2 N hydrochloric acid was added to all wells. The ODs of all wells were determined at 490 nm with a Dynatech Minireader II spectrophotometer after blanking to zero on the first substrate blank well. In accordance with the instructions of the manufacturer, the results were considered invalid when the negative control mean OD exceeded 0.10 (or 0.15 before 22 January 84) or the high positive control mean OD was less than 0.35. Results of any invalid test runs were discounted. The formula specified in the directions of the manufacturer for calculating the positive HSV ELISA cutoff value was as follows: (positive control mean OD − negative medium mean OD) × 0.15 + negative medium mean OD.

Specimens or culture lysates with a mean OD equal to or greater than this cutoff value for the test run were considered positive for HSV antigen.

Identification procedures. All cultures with CPE detected during the first week after inoculation were tested for HSV by ELISA with the culture-amplified method. Cultures with typical HSV CPE and negative HSV ELISA results from two of the three culture tubes were retested by ELISA after passage to a fresh culture tube and reincubation to 1+ CPE. These cultures were also confirmed by passage to cover slip foreskin monolayers in vials (Bartels) and staining with monoclonal fluorescein-conjugated HSV-1 and HSV-2 antisera (Syva Co., Palo Alto, Calif.) when CPE was detected.

Specimens that were negative by ELISA, HSV fluorescent antibody staining, or both, were identified by fluorescent antibody testing as follows: cytomegalovirus with mouse monoclonal antisera (Biotech, Rockville, Md.) and antimouse conjugate (Litton Bionetics, Kensington, Md.) (4); mumps virus with fluorescein conjugate (Flow Laboratories, Inc., McLean, Va.); and adenovirus with fluorescein conjugate (M. A. Bioproducts, Walkersville, Md.). The picornavirus isolates were referred to University of California Clinical Virology Laboratory, Los Angeles, Calif.

RESULTS

A total of 1,155 specimens was tested by direct HSV ELISA in parallel with culture. An additional 335 cell cultures inoculated with specimens were lysed after detection of CPE or after 5 days of incubation. These culture lysates were tested by the culture-amplified method. The results of both direct and culture-amplified ELISAs are shown in Table 1.

The sensitivity and specificity of the direct HSV ELISA were 52.5 and 96.9%, respectively, and those of the culture-amplified ELISA were 98.7 and 96.9%, respectively, as compared with HSV CPE in cell culture.

The sensitivity and specificity of the direct HSV ELISA varied considerably with specimen source and type (Table 2). One hundred percent (15 of 15) of positive results were detected from buttock lesion specimens, whereas asymptomatic cervical specimens yielded 22 false-positives (positive by ELISA, negative by culture) with only 13 of 38 specimens positive by culture detected by ELISA, a sensitivity of 40.9%. However, 3 of the 22 false-positives were specimens from women with previous positive HSV cervical cultures within 10 days, and one additional specimen was positive by culture-amplified ELISA although no CPE was detected in 5 days. If these four cases are considered true-positives, the sensitivity and specificity of the direct HSV ELISA would be 53.2 and 97.4%, respectively.

The sensitivity of the direct HSV ELISA correlated with early detection of HSV CPE in cell culture (Table 3). Specimens that were positive by direct ELISA included 95 of 133 (71.4%) specimens positive on day 1 of culture and 55 of 116 (47.4%) of specimens that were positive on day 2 of culture, but only 5 of 46 (10.9%) specimens that were positive on days 3 to 7 of culture.

The results of the culture-amplified HSV ELISA showed it to be more sensitive and specific than the direct ELISA (Table 1). Of 335 culture lysates tested, only 1 culture was negative for CPE for 5 days; this patient had previous HSV-

TABLE 1. Culture and ELISA results

ELISA result	No. of specimens with typical HSV CPE in culture		% Sensitivity	% Specificity	% Efficiency	% Predictive value	
	Positive	Negative				Positive	Negative
Direct			52.5	96.9	85.6	85.6	85.6
Positive	155	26[a]					
Negative	140	834					
Culture lysates			98.7	96.9	98.5	99.7	88.6
Positive	299[b]	1[c]					
Negative	4[d]	31[e]					

[a] Twenty-five specimens were negative by culture-amplified ELISA and culture on day 5, with the same results upon repetition; 1 specimen was positive by culture-amplified ELISA and negative by culture on day 5, with the same results upon repetition; 8 specimens were nonreproducibly positive (negative upon repetition) by ELISA and negative by culture; 1 specimen was positive by ELISA and negative by culture for 28 days, with the same results upon repetition; and three patients were positive by previous cultures (within 10 days before present study).

[b] Four cultures were first negative by ELISA but then positive by ELISA from passage or other cell culture tube.

[c] Specimen was positive by direct and culture-amplified ELISA, but it exhibited no CPE in 5 days.

[d] Cell culture identified as HSV with monoclonal fluorescent-antibody test.

[e] Cell cultures exhibited toxicity or other viruses.

TABLE 2. Source of specimens for positive direct ELISA and HSV culture results

Specimen source	No. (%) of specimens with the following result:		
	Positive by direct ELISA only	Positve by direct ELISA and culture	Negative by direct ELISA and postive by culture
Labia-vulva	1 (0.8)	55 (41.9)	75 (57.3)
Cervix	22[a] (36.7)	13 (21.7)	25 (41.6)
Penis-scrotum	0	30 (65.2)	16 (34.8)
"Genital"	1 (3.8)	17 (65.4)	8 (30.8)
Vagina	0	16 (64.0)	9 (36.0)
Buttocks	0	15 (100)	0
Rectal-anal	0	2 (66.7)	1 (33.3)
Chin	0	0	1 (100.0)
Knee	0	0	1 (100.0)
Urine[b]	1 (100)	0	0
Liver tissue[c]	0	1 (100)	0

[a] Three patients had previous positive HSV cultures within 10 days.
[b] Urine specimen showed no CPE for 28 days; same results when repeated.
[c] Disseminated neonatal HSV infection.

positive genital cultures within 20 days. Four of the 335 specimens were negative by ELISA from the first cell culture tube sampled but were positive by ELISA from the passaged cell culture tube.

Thirty-one cell cultures with toxicity or early CPE due to other viruses were tested by the culture-amplified ELISA. The following viruses were isolated: cytomegalovirus (13), picornavirus (3), varicella-zoster virus (2), mumps virus (1), and adenovirus (1). None of these isolates gave a reproducible cross-reaction. One of 13 cytomegalovirus isolates yielded a low positive value on one ELISA run, as did a cell culture of varicella-zoster virus, but these results were negative when repeated.

Nine specimens yielded nonreproducible false-positive results by direct ELISA. The OD values of these nonreproducible tests ranged from 15.75 to 27.73% of the Ortho high positive control, and the results of repeated tests were clearly negative (less than 9.9% of the high positive control) when a cutoff value of 15% was used. These specimens were retested because no CPE was detected in cell cultures in 5 days, and the culture-amplified tests were negative. Of 20 specimens repeated, only these 9 gave nonreproducible results.

The variability of the controls over the 10-week evaluation period is shown in chronological order in Table 4. The range, mean, and standard deviation for all three controls decreased with increasing experience in the ELISA technique. The range for the laboratory HSV antigen control in December (lot no. 123043) included all values obtained for two subsequent lots used in January and February 1984.

DISCUSSION

With the increasing numbers of adult genital HSV infections and consequently increased neonatal HSV infections transmitted from vaginal deliveries in the presence of active viral shedding, the rapid and accurate diagnosis of genital HSV infection in near-term obstetrical patients is becoming the primary function of the Kaiser Permanente Regional Virology Laboratory.

Culture is still the standard method for diagnosis of HSV. Forty-five percent of isolates were detected in 18 to 24 h, and 84.4% were detected in 48 h, but detection of 100% of isolates by culture required 7 days. The other limitation of this method of HSV detection is that only infectious virus is isolated in culture, so that stringent specimen transport conditions are necessary to avoid loss of infectivity.

A rapid and accurate method of detecting HSV antigen is desired. Although the direct HSV ELISA was 100% sensitive and specific for skin lesions, it was insensitive for cervical specimens from asymptomatic patients, which comprised more than 40% of the specimens submitted to the Kaiser Permanente Laboratory. Unfortunately, the majority of neonatal HSV infections are from asymptomatic women (1). Women with a history of HSV infection and obvious genital lesions at delivery would undoubtedly be delivered by cesarean section without laboratory testing.

The low sensitivity of the direct ELISA can be attributed to several causes. (i) Fewer than 60% of specimens submitted were from symptomatic patients, as submission of "typical herpetic vesicles" is discouraged as unnecessary. (ii) The volume of 2.5 ml of transport medium and antibiotics diluted the amount of virus on the swab specimens, as evidenced by the fact that direct ELISA results correlated with detection of early CPE. (iii) Technical problems also contributed to a lack of sensitivity. The test was run under actual laboratory conditions over two shifts, 6 days per week, so it was performed by four different people. Technical problems were evident on several runs, as reflected by high negative control values and nonreproducible results. According to the manufacturer, the nonreproducible positive values can be considered to be due to inaccurate OD readings from condensation developing on the bottom of the wells in contact with a warm spectrophotometer. Technical problems decreased as experience was gained with the microwasher, as reflected in the decreasing range and standard deviation of the control values over time. However, a lower sensitivity of 50.8%, as compared with our sensitivity of 52.5%, was reported by "Medical School Laboratory D," which conducted a clinical trial of the Ortho HSV ELISA (Ortho Diagnostics HSV ELISA instruction manual).

More perplexing than the lack of sensitivity with the cervical specimens was the number of false-positive results by direct ELISA from the cervix. At least a portion of these are likely to be noninfectious HSV antigen, since only women with a history of HSV are tested and three of the women had recent positive HSV cultures. None of the false-positive specimens was in transit for more than 18 h, and all were presumably kept at 4°C. Is the lack of infectivity due to poor transport in every case, so that cesarean section is indicated on the basis of a positive direct ELISA result only? The rate of morbidity and mortality associated with cesarean section is higher than the Kaiser Permanente rate of neonatal

TABLE 3. Number of days to detection of CPE for HSV-positive cultures found positive or negative by direct ELISA

Days to detection	No. (%) of specimens with the following: result:	
	Positive by direct ELISA	Negative by direct ELISA
1	95 (71.4)	38 (28.6)
2	55 (47.4)	61 (52.6)
3	3 (10.3)	26 (89.7)
4	1 (8.3)	11 (91.7)
5	1 (25.0)	3 (75.0)
7	0	1 (100.0)

TABLE 4. Interassay variability of the OD of three controls on the HSV ELISA[a]

Lot no. (*n*)	OD					
	Negative transport		High positive		Laboratory HSV	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
123043 (23)	0.009–0.157	0.083 ± 0.037	0.390–1.134	0.762 ± 0.186	0.200–0.672	0.436 ± 0.118
014020 (20)	0.005–0.107	0.051 ± 0.028	0.420–0.910	0.656 ± 0.127	0.233–0.585	0.409 ± 0.088
0140091 (9)	0.003–0.071	0.037 ± 0.017	0.340–0.660	0.500 ± 0.080	0.214–0.418	0.316 ± 0.051

[a] Laboratory HSV antigen control (lot no. 014091; *n* = 10) OD values: range, 0.114 to 0.466; mean, 0.29 ± 0.088.

HSV infection (8 of 100,000 live births) and is six times as high as the vaginal deliver rate (6). Unnecessary cesarean sections are costly both for the patient and the health maintenance organization. Corey et al. have suggested that asymptomatic shedding may be detected more frequently from the vulva than from the cervix (3). In the future, collection of parallel specimens from both cervix and vulva and testing with ELISA and culture may assist in the evaluation of false-positive cervical samples.

One urine specimen also yielded a reproducible false-positive result. Of 32 urine specimens tested, 10 yielded cytomegalovirus or picornavirus isolates without cross-reaction or interference.

The culture-amplified ELISA used to identify CPE-positive HSV isolates in cell culture gave excellent results with a 99.7% positive predictive value. The culture-amplified ELISA also offers a convenient method of testing large numbers of cell cultures with CPE rapidly and accurately. The test is simple to perform and does not require special leighton tubes or scraping of the monolayer but merely the addition of a drop of cell lysis agent before ELISA.

Since the culture-amplified test OD values exceeded the positive control value in 99% (299 of 303) of the cases, a higher cutoff value would not diminish the sensitivity of the culture-amplified test and would eliminate the (2 of 335) nonreproducible borderline false-positive results. Therefore, we are adopting a higher cutoff value of 20% rather than 15% of the high positive control. This higher cutoff should increase the specificity of both the direct and the amplified tests. This will also lower the sensitivity of the direct test, which we can endeavor to overcome with a reduced volume of transport medium and greater technical precision.

The results of this trial indicated that the Ortho HSV ELISA cannot replace culture for HSV detection in clinical specimens. The Ortho HSV ELISA was found to detect 52.5% of HSV-positive specimens in 5 h. When direct ELISA specificity is assured, HSV culture could be reserved for specimens that are negative by ELISA. The Ortho HSV ELISA was found to be a rapid, sensitive, and specific method for confirmation of HSV isolated in cell culture.

We thank Rosemary Gooch, Robert Pimentel, Terisa Carter, Cathy Strong, and Ben Salva for technical assistance and Jean Gabriel and Ken Starobin for help in manuscript prepaıation.

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