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Evaluation of a Visual, Rapid, Membrane Enzyme Immunoassay for the Detection of Herpes Simplex Virus Antigen

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We evaluated a 12-min, direct, monoclonal antibody-based enzyme immunoassay (EIA) (SureCell; Kodak, Rochester, N.Y.) which aids in the detection of herpes simplex virus infection; the assay system is also approved for culture confirmation. The test was evaluated from direct clinical samples and compared with conventional culture methodology by using a single swab. A total of 265 specimens from 180 female cervical-urogenital sites, 62 male urogenital sites, 4 rectal sites, 3 skin sites, 6 oral sites, and 10 colposcopy sites were collected on Dacron or cotton swabs and placed in viral transport medium (VTM). Within 6 h of receipt, 0.2 ml of the vortexed VTM was inoculated into each of two replicate cell cultures. Cell monolayers were observed daily for ten days, and cytopathic effect was confirmed by using an indirect immunoperoxidase reagent. The procedure for the SureCell assay conformed to the manufacturer's recommendations. When conventional culture was compared with EIA results, the overall sensitivity, specificity, positive predictive value, negative predictive value, and agreement were 64.4, 98.9, 96.7, 84.4, and 87.2%, respectively. Variables affecting the EIA sensitivity are the stage of the lesion and conventional culture methodologies. A review of culture results for 32 EIA false-negative tests indicated that 15 were detected after 48 h of incubation. Cytopathic effect observed at 48-, 72-, and 96-h cutoffs altered the sensitivity for the EIA. To ensure detection of SureCell herpes simplex virus-negative specimens, it is recommended that an unused aliquot of VTM be tested in cell culture.

Rapid and accurate diagnosis of herpes simplex virus (HSV) infection is essential for proper patient management. The need for more rapid laboratory diagnosis of severe HSV infections has become apparent over the past several decades as their prevalence in both normal and immunocompromised populations and the availability of antiviral agents have steadily increased in the United States (4). Conventional cell culture methodologies require a minimum of one day for isolation and identification and as many as 7 to 10 days (5); rapid techniques can be completed in less than 4 h. A limitation of cell culture is its capability to determine viral presence only when viable organisms are isolated from specimens. In contrast, enzyme immunoassay (EIA) methodologies can detect both viable and nonviable viruses. Regardless of the approach to the laboratory diagnosis of HSV infections, it remains clear that the parameters of laboratory assays are influenced by the patient population, stage of the disease, nature of the lesion, specimen type, sampling technique, and whether the infection is recurrent or primary (3). Comparisons of published reports from different laboratories evaluating rapid diagnostic assays are difficult because of the many variables associated with the patients at risk and the tests available.

We evaluated a 12-min, direct, monoclonal antibody-based EIA (SureCell; Kodak, Rochester, N.Y.) for its capability to detect HSV antigen in urogenital specimens. Results were compared with those of the cell culture method used in our laboratory. Additionally, we examined the many variables associated with HSV cell culture and their effects on culture results.

A total of 265 specimens were collected from individuals presenting at various local hospital clinics, the regional sexually transmitted disease clinic, and the Erie County Medical Center, a tertiary care institution. Specimens were obtained from 180 female urogenital-cervical sites, 62 male urogenital sites, 10 colposcopy sites, 6 oral-throat sites, 4 rectal sites, and 3 skin sites. Of the total, 255 were collected from lesions of possible herpetic etiology (uncharacterized as to the stage of the lesion) during the course of a sexually transmitted disease examination, whereas 10 were collected from nonlesion sites during colposcopy examination.

Each specimen was collected with a single Dacron or cotton-tipped swab and was placed in 1.5 ml of viral transport medium (VTM) consisting of Hanks balanced salt solution (Whittaker Bioproducts, Inc., Walkersville, Md.) supplemented with bovine albumin (Armour Pharmaceuticals, Kankakee, Ill.), gentamicin (100 μg/ml), vancomycin (100 μg/ml), and amphotericin B (Fungizone) (5 μg/ml). Specimens were transported to the laboratory on ice within 24 h of collection and maintained at 4°C prior to testing. Within 6 h of receipt in the laboratory, cell cultures were inoculated. Following culture inoculation, the residual VTM and swab were maintained at 4°C. EIA testing was initiated within 24 h of the receipt of the specimen.

HSV culture. Patient specimens (0.2 ml) were inoculated in duplicate into two cell cultures, MRC-5 and A549 cells (Whittaker Bioproducts). Cell cultures were maintained with minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) containing 5% fetal bovine serum (Whittaker Bioproducts), gentamicin (100 μg/ml), vancomycin (100 μg/ml), and amphotericin B (5 μg/ml). Following incubation at 35 to 37°C, cell cultures were examined daily for up to 10 days for the presence of cytopathic effect (CPE). CPE was semiquantitated from 1+ to 4+ (1+ = 25% of the cell monolayer affected, 2+ = 50%, 3+ = 75%, and 4+ = 100%) and was confirmed by using an HSV-specific indirect immunoperoxidase stain (Ortho Diagnostics, Raritan, N.J.). Pos-

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TABLE 1. Parameters of Kodak SureCell HSV EIA for various types of specimens and sources

Specimen/source	No. tested	%				
		Sensitivity	Specificity	Positive predictive value	Negative predictive value	Agreement
Female						
Cervical/vesicle	20	66.7	100.0	100.0	87.5	90.0
Urogenital/vesicle	160	56.8	98.3	92.6	85.7	86.9
Colposcopy	10	NA[a]	100.0	NA[a]	100.0	100.0
Male: urogenital/vesicle	62	71.9	100.0	100.0	76.9	85.5
Total	252	63.4[b]	98.8	96.3	84.8	87.3
Rectal/vesicle	4	100.0	100.0	100.0	100.0	100.0
Skin/vesicle	3	100.0	100.0	100.0	100.0	100.0
Oral-throat/vesicle	6	50.0	100.0	100.0	50.0	66.7
Total	13	75.0[c]	100.0	100.0	71.4	84.6
Overall total	265	64.4[d]	98.9	96.7	84.4	87.2

[a] NA, Not applicable (no specimens in this category).
[b] Confidence interval = 57.5 to 69.3%.
[c] Confidence interval = 51.0 to 99.0%.
[d] Confidence interval = 58.5 to 70.3%.

itive cultures were defined as one or more of the cell cultures exhibiting HSV-specific CPE. Isolates that were false negatives on the basis of EIA results were subsequently serotyped.

EIA studies. The Kodak SureCell herpes (HSV) system is approved to detect HSV type 1 (HSV-1) and HSV-2 from genital, rectal, oral, and dermal lesions (i) directly from swabs, (ii) from swabs in VTM, or (iii) as a culture confirmation assay. All specimens collected were cultured first and were then tested by EIA directly from swabs previously placed in VTM and vortexed. HSV-specific glycoproteins, if present, are extracted from the swab by placing it in the Kodak extraction tube previously filled with 0.5 ml of inoculated VTM and 0.5 ml of buffered extraction solution. The swab is rotated and squeezed for 1 min to release HSV-1 or HSV-2 antigen, after which a filter tip is attached to the tube and the solution is filtered (in equal aliquots) into the three test wells. The test cell consists of three wells (1 to 3), each with a filter membrane and absorbant pads; the wells contain one negative control membrane (no. 1), one test membrane for the patient specimen (no. 2), and one positive control membrane containing inactivated HSV-1 and HSV-2 antigens (no. 3). The solution is drained through the test wells, allowing any HSV antigen present to bind to the membrane at the bottom of the well. Following a rinse, hydrogen peroxide is added to each well; this is followed by another rinse. Antibody conjugate is then added to each well (HSV nonspecific for the negative control well and HSV-1 and HSV-2 specific for the positive control and test wells), and after 5 min at room temperature the wells are rinsed and leuco dye is added.

According to the manufacturer's specifications, the results are visually interpreted as follows. After the addition of leuco dye and 5 min at room temperature, (i) the negative control membrane (no. 1) should remain white—the presence of uniform pink color indicates an invalid result; (ii) the sample membrane (no. 2) should change color as a function of the amount of HSV antigen present in the sample—uniform pink color more intense than in the negative control is interpreted as a positive HSV result; and (iii) the positive control membrane (no. 3) containing HSV-1 and HSV-2 antigens should develop a uniform red-pink color independent of the presence or absence of HSV antigen in the test sample. In our study, a patient result could not be interpreted unless positive and negative control reactions occurred as expected. The results of the EIA were visually interpreted by two investigators.

Evaluation. The rate of infection as determined by cell culture within this population was 34.0% (90 of 265) during the study period. This was comparable to the isolation rates determined in other studies (5, 8, 10).

The sensitivity and specificity of the Kodak SureCell HSV EIA for urogenital specimens were 63.4 and 98.8%, respectively, compared with values for confirmed HSV-associated CPE in cell culture examined daily for 7 to 10 days (Table 1). Likewise, the sensitivity and specificity for nonurogenital specimens were 75.0 and 100.0%, respectively. The overall sensitivity and specificity of the assay were 64.4 and 98.9%. The reproducibility of the EIA results was 100.0% on the basis of the visual interpretations of the two investigators. The statistical precision of the sensitivity was evaluated by using 95% confidence intervals (7). The confidence limits for the overall population studied (265 specimens) were between 58.5 and 70.3%. For the urogenital specimens, the confidence limits were between 57.5 and 69.3%, and they were 51.0 and 99.0% for the nonurogenital specimens (Table 1). This indicates that the sample size for the overall study was adequate, as was the urogenital specimen size, and that the parameters expressed in Table 1 are valid; however, the sample size of the nonurogenital specimens was inadequate on the basis of the wide range of confidence limits.

The assay parameters varied with the type of specimen submitted and the source from which it was collected (Table 1). Of the 62 male urogenital specimens, 71.9% of the culture-positive specimens (23 of 32) were positive by EIA, compared with 58% of the female urogenital specimens (29 of 50).

The sensitivity of the EIA varied with respect to culture methodology. As the length of time needed to confirm HSV-associated CPE in cell culture increased, the sensitivity of the EIA decreased; 73.2% of culture positives detected within 48 h (41 of 56) were EIA positive, compared with 64.4% (58 of 90) detected by culture in 6 days. However, of the 32 false-negative specimens (positive culture and nega-

tive EIA), 22 (68.8%) demonstrated CPE in all four of the culture tubes, 3 (9.4%) demonstrated CPE in three of the four tubes, 5 (15.6%) demonstrated CPE in two of the four cultures, and 2 (6.3%) revealed CPE in one of the four tubes. Nineteen of the 32 false-negative EIA results had CPE scores of $\leq 1+$ in one or more of the cell cultures. This semiquantitative analysis of the cultures indicated that a low virus titer of the specimen was associated with false-negative results.

Serotyping of the false-negative specimens was performed to determine whether any trends in type existed. Thirty-one of the 32 false-negative specimens were serotyped as HSV-2; one specimen was typed as HSV-1. Since we were unaware of the distribution of HSV-1 and HSV-2 in our population, the significance of these findings cannot be evaluated.

The efficiency of the two cell lines (A549 and MRC-5) was observed for the 32 false-negative EIA results. Twenty-two of the 32 false negatives demonstrated CPE in all four culture tubes. However, of the 10 specimens (20 A549 culture tubes and 20 MRC-5 culture tubes) that resulted in CPE in less than four tubes, 14 A549 tubes exhibited HSV-associated CPE, whereas only 7 MRC-5 tubes resulted in definitive CPE. An analysis of the CPE patterns of the 58 true positives indicated that results with A549 and MRC-5 cell lines were identical in sensitivity and CPE detection time.

Cell culture efficiency for HSV was affected by variables in the methodology used. Of the 90 specimens that were culture positive following incubation for 7 to 10 days, only 40.0% (36 of 90) demonstrated CPE within 24 h; however, 100.0% (90 of 90) exhibited HSV-associated CPE by the sixth day of incubation.

Culture remains the "gold standard" for diagnosis of HSV; yet, many factors affect HSV isolation in cell culture. It has been reported in some patient populations that only 80.0% of HSV infections are detected by culture from a single specimen (4). This observation and the advancing numbers of adult urogenital, neonatal, and other severe HSV-associated infections indicates the need for a test with the capability to offer sensitive and specific results in hours rather than days. Such a test, available to an obstetrical population, would influence the numbers of cesarean deliveries (12).

In this study, 40% of isolates were detected within 24 h, whereas 62.2% were detected within 48 h. Six days of incubation was required for the detection of 100.0% of the isolates. The literature indicates that differences in cell sensitivity for HSV are detected only with low virus inocula (2, 3, 14). Time required for the first detection of CPE and the overall rate of isolation appear to be less affected at higher titer concentrations. These differences were unnoticed in this study, in part because of the unavailability of inoculum titers for each specimen. The only trend we were able to observe was that in a subgroup of false-negative EIA specimens, a greater number (14 of 20 compared with 7 of 20) had viruses detected by the A549 cell line than by the MRC-5 line. From these data, we determined that the use of one cell line (in duplicate) could modify the sensitivity of the EIA in this type of study. If the MRC-5 line had been used exclusively, the overall sensitivity would have increased from 64.4 to 67.4% (58 of 86); the sensitivity would have increased to 65.2% (58 of 89) had only the A549 line been used for culture.

Our overall isolation rate of HSV by using cell culture (34.0%) was comparable to those of previous reports of similar patient populations (5, 8, 10). This offers an indication that our methodology provided appropriate results. Assuming cell culture to be the "gold standard" for HSV detection, the overall sensitivity and specificity of the Kodak SureCell HSV EIA compared with those of culture were 64.4 and 98.9%, respectively. For urogenital specimens alone, the parameters were slightly altered to 63.4 and 98.8%. Theoretically, several explanations could be made for both false-positive and false-negative EIA results. Reasons for those cases in which the EIA was positive and the culture was negative could be (i) improper transport of the specimen to the laboratory, (ii) patients receiving antiviral chemotherapy resulting in nonviable viruses, and (iii) the poor sensitivity of our culture system. Similarly, explanations for EIA-negative and culture-positive specimens include (i) poor sample collection technique, (ii) patients with recurrent HSV infection having circulating antibodies that interfere with antigen detection, (iii) the excellent sensitivity of our culture system, and (iv) interference of the EIA by specimen-associated compounds.

Finally, it becomes apparent that the parameters of any assay, when compared with those of another methodology, may differ in relation to the variables of that test. We observed a change in EIA sensitivity from 77.6 to 64.4% when the length of time for cell culture was increased from 24 h to 7 to 10 days. Other published reports in the literature have indicated that commercial EIAs have sensitivities ranging from 52.5 to 96.1% (5, 9–11, 13). However, the protocols used in these studies differed so much that appropriate comparisons are difficult. Recently, Dorian et al., using a culture methodology different from the one we use (spin-amplified tissue culture confirmed by enzyme immunoassay) reported a sensitivity and specificity of 100.0 and 100.0%, respectively, for vesicular lesions tested with the Kodak SureCell and a sensitivity and specificity of 75.6 and 100.0%, respectively, for nonvesicular lesions (6). This demonstrates the variability associated with the evaluation of assays assessed by different conventional protocols.

The results of our study demonstrate that the Kodak SureCell HSV EIA is not as sensitive as conventional cell culture, but its parameters compare favorably with those of other commercially available immunoassays. The use of this assay, as with all methodologies for HSV detection, is highly dependent on specimen quality, history of the infection, patient population, and the methods used to evaluate it. We feel the Kodak SureCell HSV EIA is a fast and easy assay to perform and may be applicable to the clinical diagnosis of HSV infection; however, to ensure the validity of SureCell HSV-negative results, it is recommended that an unused aliquot of VTM be tested in cell culture.

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