Detection of Herpes Simplex Virus by the Kodak SureCell Herpes Test

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Received 5 February 1990/Accepted 12 June 1990

The Kodak SureCell Herpes Test Kit (SC-HSV) was evaluated in a high-risk sexually transmitted disease clinic population. A total of 97 lesion specimens (from 94 patients) were tested for the presence of herpes simplex virus by SC-HSV and by spin-amplified tissue culture confirmed by an enzyme immunoassay. The overall sensitivity of SC-HSV compared with that of spin-amplified tissue culture–enzyme immunoassay was 81.1%; the specificity was 100%. For vesicular lesions only, SC-HSV sensitivity was 100%; for nonvesicular lesions, sensitivity was 75.6%. SC-HSV was rapid (<15 min) and very easy to perform.

Herpes simplex virus (HSV) causes an infection characterized by a localized primary lesion, latency, and a variable degree of localized recurrence. Genital HSV infection, which can be caused by either HSV type 2 (HSV-2) or HSV type 1 (HSV-1), is a disease of major public health importance. In the United States, HSV-1 antibodies can be detected in over 50% of the adult population (5), and HSV-2 antibody prevalence rates have been found to be nearly as high (2). Isolation in tissue culture is currently accepted as the most sensitive and specific method for the diagnosis of HSV infection. However, even spin-amplified tissue culture (SATC) is at best a 1- to 2-day procedure (12, 13, 15). Quicker, if somewhat less sensitive, results can be obtained by various nonculture techniques. These include the cytological examination of lesion cells by Wright-Giemsa stain (Tzanck preparation) (3, 17), the use of fluorescein-conjugated HSV antibodies in a direct immunofluorescence test (14), immunoperoxidase staining (6, 14), direct enzyme immunoassay (EIA) (7, 10), and DNA hybridization (4, 8).

We evaluated the SureCell Herpes Test Kit (SC-HSV) (Eastman Kodak Co., Rochester, N.Y.) for the detection of HSV infection. This test is a monoclonal antibody-based filtration type EIA which can be performed directly on clinical specimens. Results are available in less than 15 min, the specimen does not have to contain viable virus, and no instrumentation is required. In this study, direct SC-HSV was compared with SATC confirmed by another EIA (Ortho HSV Antigen EIA Test; Ortho Diagnostics, Raritan, N.J.). This combination has been shown to be equal in sensitivity and specificity to conventional tissue culture techniques (9, 10, 16).

A total of 94 patients (54 males and 40 females) who were found to have possible herpetic lesions during the course of a routine sexually transmitted disease exam were included in the study. These lesions were primarily genital, but some were oral or dermal. Dual cotton-tipped wood swabs, one for direct SC-HSV and one for SATC-EIA, were used for specimen collection by holding the two swabs together and vigorously rubbing the base of the lesion. After sampling, the swab for SATC testing was placed in 1.5 ml of Earle minimal essential medium with 10% fetal bovine serum, vancomycin (40 μg/ml), gentamicin (10 μg/ml), and nystatin (25 U/ml). The SC-HSV swab was placed in an empty tube. SC-HSV specimens were refrigerated until tested (within 24 h). SATC specimens were also refrigerated until cultured (within 24 h). (Seven SATC specimens were frozen at −70°C until inoculation.)

For SATC-EIA testing, one flat-bottomed tube each of MRC-5 human embryonic lung cells and primary rabbit kidney cells were inoculated with 0.25 ml of specimen. After being centrifuged for 30 min at 2,500 × g, the cultures were incubated at 36°C for 3 to 6 days. The Ortho HSV Antigen EIA Test was then performed. Briefly, cell lysis reagent was added to each tube, and after 5 min of incubation, the two tubes for each specimen were combined. Then, 0.2 ml of this mixture was placed into a microwell coated with antibodies for both HSV-1 and HSV-2. After incubation for 1 h at 36°C, the microwells were rinsed and peroxidase-conjugated HSV antibodies were added. After incubation for 1 h at 36°C and subsequent rinsing, 0.2 ml of an ortho-phenylenediamine–hydrogen peroxide mixture was added to each microwell. After 30 min of incubation at room temperature, sulfuric acid was added to stop the reaction and the A₅₉₀ of each microwell was read. Any specimen yielding an optical density value greater than the cutoff value (negative control mean plus 0.15) was considered positive for HSV.

For SC-HSV testing, the dry swab was placed in a tube with 1.0 ml of extraction buffer. This tube was part of the test kit and was made of flexible plastic such that it could be compressed around the swab tip. The swab was then rotated and squeezed for 1 min to release any HSV antigen present. A filter tip was attached to the top of the tube, and the specimen solution was squeezed through the filter in equal portions into each of the three test wells (negative control, positive control [which contains both HSV-1 and HSV-2 antigens], and sample wells) of the SC-HSV test cell. The specimen drained through the test wells, and any HSV antigen was bound to the filter membrane at the bottom of each well. After a rinsing step, hydrogen peroxide was added to each well. After another rinsing step, antibody conjugate was added to each well. (That added to the negative control well was non-HSV specific, while a monoclonal antibody cospecific for both HSV-1 and HSV-2 was added to the positive control and sample wells.) After 5 min at room temperature, the wells were rinsed and a leuco dye was added. Five minutes later, the level of red or pink color formed in each well was determined. Any red or pink color...
produced in the sample well above the level of color observed in the negative control well indicated the presence of HSV. Since SC-HSV was completed before SATC-EIA was done, the test was, in effect, blinded.

Of the 97 specimens tested, 53 were positive by SATC-EIA. SC-HSV found 43 of these positive, for a sensitivity of 81.15%. SC-HSV was in complete agreement with SATC-EIA in finding 44 specimens negative, for a specificity of 100%. Table 1 shows how the sensitivity of SC-HSV varied depending on the type of lesion and whether the patient reported having similar genital lesions previously. That SC-HSV was found to be more sensitive for vesicular lesions and for first-episode genital HSV infections is not surprising. It has been shown that the ability to detect HSV (antigen) varies over the course of the disease, and viral titers have been found to be highest in the vesicular stages of the disease (6, 8, 11). Also, first episodes of genital herpes often have higher viral titers and a longer duration of virus shedding (1, 6). It appears that this decrease in viral antigen had more of an effect on SC-HSV than on SATC-EIA. It is possible that below a certain level of HSV antigen SC-HSV was unable to detect infection, whereas with SATC-EIA that same level of virus could replicate to a detectable level.

Table 1 also shows the differences in the sensitivities of SC-HSV with penile and female genital lesions. This difference can be largely attributed to the fact that the penile group included 11 vesicular lesions, all of which were positive, while the female genital group included only 1 positive vesicular lesion. If only the nonvesicular lesions in these two groups are considered, the difference in sensitivities is only 7.4%.

For the 10 specimens that were SATC-EIA positive and SC-HSV negative, SC-HSV was performed on the remainder of the original SATC swab specimens in an attempt to determine if the false-negativity of the first (direct) SC-HSV may have been due to sample variation. However, in only one case was the second SC-HSV positive. While some increased dilution had occurred in these SATC specimens prior to SC-HSV testing, it was minimal. Therefore, it appeared that in 9 of 10 of the specimens that were false-negatives, the HSV antigen level was below the level detectable by SC-HSV. The lower sensitivity of SC-HSV seemed not to be caused by sampling variation but more likely by lower viral titers.

No specimens were SC-HSV positive and SATC-EIA negative. It is possible that under less optimal conditions of viral specimen transport, storage, and/or processing, in which virus might have become nonviable before SATC inoculation, such specimens would have been more likely to occur.

SC-HSV was found to be quick (<15 min), very easy to learn and to perform, and highly specific for the detection of active HSV infection. The sensitivity of the test was very good for vesicular lesions but somewhat lower for other lesions. As the manufacturer recommends, all negative SC-HSV results should be followed-up by tissue culturing. However, in those settings in which HSV culturing is not readily available or in which it is clinically advantageous to give a patient at least a preliminary result at the current visit (e.g., in a sexually transmitted disease clinic), SC-HSV could be very helpful in the diagnosis and management of HSV infection.

**LITERATURE CITED**


