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Rapid Detection of Herpes Simplex Virus in Clinical Specimens by Use of a Capture Biotin-Streptavidin Enzyme-Linked Immunosorbent Assay

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A sensitive enzyme-linked immunosorbent capture assay with biotin and streptavidin (capture B/SA ELISA) was developed to detect herpes simplex virus (HSV) antigen. Rabbit anti-HSV antibody (immunoglobulin G fraction) was coated on flat-bottom, irradiated, 96-well polystyrene microtiter plates and served to capture HSV antigen. Clinical specimens from patients with genital herpes were added. Biotin-linked rabbit anti-HSV immunoglobulin G was used as the second antibody. The antigen-antibody complex was detected with alkaline phosphatase-conjugated streptavidin, which linked to the biotin. With clinical specimens, the test had a sensitivity of 95.6% and a specificity of 91.4% when compared with the tissue culture method. The presence of HSV antigen in specimens devoid of infectivity was confirmed by blocking the reaction with unlabeled rabbit and human antibody to HSV. The level of antigen detected by the capture B/SA ELISA did not necessarily correlate with the infectivity titer of the specimens. HSV antigens could be detected by the capture B/SA ELISA when the virus infectivity was destroyed at 37°C, by UV irradiation, or by Triton X-100 treatment, but not when hypochlorite treatment was used. Greater sensitivity was obtained when HSV-1- and HSV-2-specific antibody reagents were used simultaneously in each test. The capture B/SA ELISA provides a relatively rapid method (4.5 h) which is quite sensitive and specific when compared with other non-tissue culture, direct assay methods.

Genital herpes is one of the most common venereal diseases in the United States. It is caused by herpes simplex virus (HSV), particularly HSV type 2 (HSV-2) (11). These infections can be primary or recurrent and pose a great risk to the neonate when a pregnant mother has active genital herpes at the time of delivery (5). If HSV infection is present at term, the delivery should be performed by cesarean section to reduce the risk of transmission of the virus to the newborn (5). The constraints of time and risk to the newborn emphasize the need for a sensitive and rapid test which can give reliable information about the presence of HSV.

Some of the methods currently available to detect HSV include conventional tissue culture techniques (15), short-term tissue culture followed by staining (12, 13) or electron microscopy (9), and direct staining of tissue smears taken from the lesions of the patient (3, 7, 17, 18). The tissue culture method is generally considered standard, but it requires up to 7 days for completion. We have reported that short-term tissue culture followed by staining is as sensitive as and more rapid (24 h) than tissue culture alone (12, 13). Direct staining of tissue smears is rapid, but not as sensitive or specific as tissue culture (13), and electron microscopy is rapid, but less available and less sensitive than tissue culture (9).

The objective of this study was to develop a more rapid, sensitive, specific technique to detect HSV without the use of tissue culture. The present report describes a method in which specific antibody was used to capture HSV antigen. This was then detected with a second antibody linked with biotin and streptavidin-enzyme conjugate in the immunosorbent assay.

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MATERIALS AND METHODS

Preparation of stock virus. HSV-1 and -2 were grown in monolayer cultures of owl monkey kidney cells maintained on complete Eagle minimum essential medium (EMEM; Microbiological Associates, Bethesda, Md.) containing 2 mM glutamine (GIBCO Laboratories, Madison, Wis.), penicillin (100 U/ml), and streptomycin (100 μg/ml) with 2% fetal calf serum. The culture supernatants were harvested at 48 h and concentrated 10-fold with an Amicon fiberglass concentrator (Amicon Corp., Lexington, Mass.). The virus was further purified by discontinuous density gradient centrifugation with 20 and 60% sucrose in Tris buffer (20 mM; pH 7.8) with EDTA (1 mM) and NaCl (0.15 M). The virus was collected from the interphase of 20 and 60% sucrose and stored frozen in small portions at −70°C until used.

Collection of clinical specimens. Clinical specimens were collected from patients by rubbing lesions or genital areas or both with sterile cotton swabs. The swabs were transferred to 3 ml of Hanks balanced salt solution containing streptomycin, penicillin, acromycin, mycostatin, and gentamicin (henceforth referred to as collection medium), refrigerated before and during transport, and frozen at −70°C if not studied immediately (15).

Tissue culture determination of HSV in clinical specimens. Human foreskin fibroblast (Flow 7000; Flow Laboratories, Inc., McLean, Va.) monolayer tube cultures were inoculated with 0.2-ml volumes of the clinical specimens. The speci-

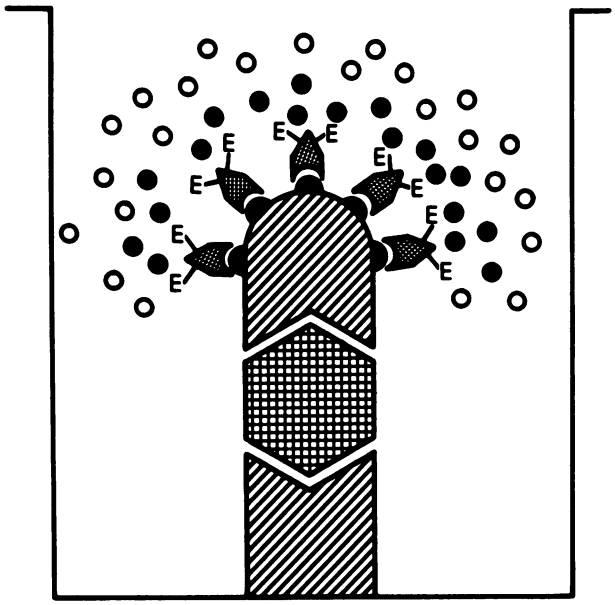

FIG. 1. Antigen capture B/SA ELISA for HSV. Symbols: ▨, antibody; ●, antigen; ⌒, biotin; ▩, streptavidin; ○, substrate; ●, product. E, Enzyme.

mens were absorbed on monolayers for 1 h, after which fresh, complete EMEM with 2% fetal calf serum was added (1.5 ml). The cultures were incubated at 37°C and evaluated for cytopathic effects up to 7 days. Specimens with positive cytopathic effects were further passaged on Flow 7000 and rabbit kidney cell monolayers and confirmed for the presence of virus. The negative cultures were discarded after 7 days.

Determination of infectivity of HSV. The infectivity of HSV in stock virus preparations and in clinical specimens was determined as described previously (12). In brief, Flow 7000 monolayer cultures were infected with 0.2-ml volumes of serial 10-fold dilutions of the virus preparations and observed for cytopathic effects. The endpoints were determined at 7 days and calculated as 50% tissue culture infective doses ($TCID_{50}$) by the method of Reed and Muench (16).

Capture B/SA ELISA for detection of HSV antigen. The schematic diagram of the antigen capture biotin-streptavidin (B/SA) enzyme-linked immunosorbent assay (ELISA) is shown in Fig. 1.

(i) Choice of plastic. Several 96-well microtiter plates were tested initially to choose the best solid-phase support. They included Immulon I and Immulon II plates (Dynatech Laboratories, Inc., Alexandria, Va.), polyvinyl chloride plates (Dynatech), and Costar polystyrene plates (Costar, Cambridge, Mass.). All the plates were used without treatment.

(ii) Preparation of biotin-linked rabbit anti-HSV antibody. The method used for linking biotin to antibody was previously described by Nerurkar et al. (12). In brief, rabbit anti-HSV-1 or -2 immunoglobulin G antibodies (Accurate Chemical & Scientific Corp., Westbury, N.Y.) was linked with biotin as follows. The antibody solution was exhaustively dialyzed to remove any traces of $(NH_4)_2SO_4$. It was then dialyzed against 0.1 M $NaHCO_3$ (pH 8.2 to 8.6), adjusted to a concentration of 1 mg of protein per ml, and centrifuged at 1,000 rpm at 4°C. It was then mixed with freshly prepared biotin succinimide ester (1 mg/ml; Vector Laboratories, Burlingame, Calif.) in dimethyl sulfoxide, kept at room temperature for 4 h, and extensively dialyzed to remove unconjugated biotin ester and dimethyl sulfoxide. The biotin-linked antibodies could be stored without loss of activity for over a year.

(iii) Preparation of streptavidin-enzyme conjugates. Streptavidin was obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md. Purified alkaline phosphatase prepared from bovine calf intestine was obtained from Sigma Chemical Co., St. Louis, Mo. The streptavidin-alkaline phosphatase conjugates were prepared by a one-step glutaraldehyde conjugation procedure as described previously (20). During initial trials, egg avidin-enzyme conjugates (Vector) were compared with streptavidin-enzyme conjugates prepared by us. The streptavidin conjugates were found to be much superior to the egg avidin-enzyme conjugates and hence were used in all experiments described in this report.

(iv) Performance of capture B/SA ELISA. All tests were run against anti-HSV-1 and -2 antibody reagents simultaneously. The capture antibodies were identical to the detecting antibodies except that the latter were linked with biotin. The microtiter plates were coated with various concentrations of rabbit anti-HSV-1 and -2 immunoglobulin G preparations. An antibody dilution of 1:250 was found to be sufficient to capture the levels of viral antigen present in the clinical specimens. This dilution of antibody was used in all the subsequent experiments. Microtiter plates were sensitized with 300 μl of antibody diluted in sodium carbonate-bicarbonate buffer (1.59 g of Na_2CO_3, 2.93 g of $NaHCO_3$, 0.2 g of sodium azide per liter [pH 9.6]) in a humid chamber at 4°C overnight (20). The plates were washed three times with phosphate-buffered saline containing 0.05% Tween 20 (Sigma), 0.1% bovine serum albumin (Miles Laboratories, Inc., Elkhart, Ind.), and 0.02% sodium azide (Sigma), henceforth referred to as ELISA wash. Stock virus or clinical specimens were then added at least in duplicate (100 μl per well), and the plates were incubated at 37°C in a 5% CO_2 incubator. The optimum time of incubation was determined to be 2 h in the initial experiments. Unbound material was removed by washing the plates three times with ELISA wash. Pretitrated biotin-linked antibody diluted in phosphate-buffered saline containing 0.05% Tween 20, 1.0% bovine serum albumin, and 0.02% sodium azide (100 μl per well) was added to the plates. After incubation for 1 h at 37°C in a humid chamber, the plates were washed three times with ELISA wash. Pretitrated streptavidin-alkaline phosphatase conjugate (100 μl per well) was then added, followed by incubation for 0.5 h at 37°C in a humid chamber. The optimum time of incubation on the last two steps was evaluated in the initial experiments. The unbound conjugate was removed by washing thoroughly, and 100 μl of substrate (*para*-nitrophenyl phos-

TABLE 1. Evaluation of various plastic plates for the HSV capture B/SA ELISA

HSV $TCID_{50}$ per ml	A_{405} (mean ± SD)[a]			
	Polyvinyl chloride	Immulon I	Costar	Immulon II
10^6	>1.500	0.698 ± 0.211	>1.500	>1.500
10^5	0.947 ± 0.058	0.204 ± 0.033	>1.500	>1.500
10^4	0.125 ± 0.013	0.077 ± 0.086	0.290 ± 0.027	0.357 ± 0.019
10^3	0.019 ± 0.012	0.042 ± 0.024	0.033 ± 0.022	0.027 ± 0.021

[a] A_{405}, Absorbance at 405 nm.

TABLE 2. Sensitivity of the antigen capture B/SA ELISA for gradient-purified HSV detection

$TCID_{50}$ per ml	A_{405} (mean ± SD)[a]	
	HSV-1	HSV-2
10^6	>1.500	>1.500
5×10^5	>1.500	1.092 ± 0.142
10^5	>1.500	0.303 ± 0.023
5×10^4	>1.500	0.162 ± 0.051[b]
10^4	0.490 ± 0.021	0.111 ± 0.014
5×10^3	0.233 ± 0.027[b]	0.089 ± 0.033
10^3	0.079 ± 0.022	0.057 ± 0.008
5×10^2	0.039 ± 0.033	0.038 ± 0.036
Control	0.037 ± 0.020	0.043 ± 0.015

[a] A_{405}, Absorbance at 405 nm.
[b] Lowest dilution of virus which was considered positive in the ELISA.

phate; Sigma) per well was added to each plate (1 mg of substrate per ml of diethanolamine buffer [0.5 mM; pH 9.8] containing 100 mg of $MgCl_2 \cdot 6H_2O$ and 200 mg of sodium azide per liter). The plates were incubated for 30 min in a humid chamber at 37°C, and the reaction was stopped by adding 50 μl of 3 M NaOH to each well. The absorbance measurements were made at 405 nm with a Dynatech ELISA reader model MR580.

Several replicate readings of viral collection medium and control buffer were taken in each experiment and served as negative controls. Known dilutions of stock virus served as a positive control.

The fluorogenic substrate methylumbelliferyl phosphate was evaluated in the assay (21). It did not improve the sensitivity of detection of HSV antigen in clinical specimens or in purified virus preparations.

(v) Evaluation of results. The absorbance reading on at least duplicates of specimens and four to eight replicates of viral control medium were averaged, and standard deviation (SD) of the control average in a given experiment was calculated. The specimens which had an average absorbance value equal to or above the predicted limit of absorbance value for a positive were considered positive. The predicted limit of absorbance value for a positive was determined by using the 95% prediction bound formula $\bar{x} + (SD_{\bar{x}} \cdot C_{f \cdot 95\%} \cdot \sqrt{(n+1)/n})$, where $\bar{x}$ and $SD_{\bar{x}}$ are the average and the standard deviation, respectively, of n observations of control buffer absorbance values and $C_{f \cdot 95\%}$ is the t value at 95% probability for $f (= n - 1)$ degrees of freedom.

Inactivation of stock HSV. Inactivation of stock HSV in distilled water, in EMEM at 37°C, and in water containing hypochlorite was performed as previously described (14). Inactivation by UV irradiation and by Triton X-100 (final concentration, 1%) was carried out for 3 min at room temperature.

Specificity of HSV detection. Specificity of the assay was tested with cytomegalovirus, *Candida albicans*, and *Staphylococcus aureus*. Since *C. albicans* and *S. aureus* are frequently seen in genital infections, mixing experiments were performed to determine whether they interfered with HSV detection. The clinical specimens which showed contaminations by tissue culture were also studied by the capture B/SA ELISA.

Inhibition of HSV antigen detection. The presence of HSV antigen in clinical specimens and stock virus preparations was confirmed by blocking the detection with rabbit and human HSV-1- and -2-specific antisera. Preimmune sera or sera without HSV-specific antibodies were used in the negative control. In these experiments, the specimens or stock virus preparations were allowed to react with antibody-coated plates for 2 h and were washed three times with ELISA wash. The unlabeled rabbit or human anti-HSV serum or their respective preimmune or negative control sera were then added, and the incubation was carried out for 1 h at 37°C in a humid atmosphere. The plates were washed three times with ELISA wash, and the remaining steps, e.g., from those involving a reaction with biotin-linked antibody to those showing the development of color in the alkaline phosphatase reaction, were followed as discussed above. This methodology ensured the capture of antigen but blocked only the detection. All the reactions were carried out in duplicate. The inhibition by either rabbit or human serum was considered positive when it was more than that achieved by the respective preimmune or negative sera.

RESULTS

The evaluation of various plastic microtiter plates for the capture B/SA ELISA is given in Table 1. Costar and Immulon II polystyrene plates, which are UV irradiated, gave the best results when the complete HSV capture reaction was carried out with different amounts of purified HSV-1. Immulon II plates showed slightly but not significantly higher absorbance values. Polyvinyl chloride plates were rated between irradiated polystyrene plates and unirradiated Immulon I polystyrene plates. Immulon I plates were not acceptable because of suboptimal binding. All experiments described in this report were performed with Costar plates.

The optimum time of incubation for antigen capture was determined. The antigen binding was generally linear up to 2 h. Incubation beyond 2 h did not give a significant increase in absorbance readings and did not improve sensitivity of detection of virus but did increase the background readings.

TABLE 3. Inhibition of HSV antigen detection[a] in the capture B/SA ELISA with rabbit and human HSV antisera

Specimen[b]	% Inhibition of A_{405}[c] by antisera				
	Rabbit		Human		
	Preimmune	Hyperimmune[d]	Negative	Low positive[e]	High positive[f]
HSV-1					
101A	21.5	82.2	2.0	77.0	82.5
196A	2.3	85.0	0.0	69.0	82.4
392A	7.9	78.1	0.0	62.8	74.9
276	9.3	79.3	0.0	68.0	72.9
478	7.1	81.2	12.0	74.6	78.0
846	0.0	83.7	6.6	60.9	80.7
HSV-2					
39A	23.4	89.9	0.0	60.8	69.5
50A	17.5	93.0	0.0	65.3	76.5
57A	14.7	89.6	0.0	45.6	61.7
61A	16.4	92.9	0.0	55.5	64.8
235	14.1	93.3	0.0	55.3	67.1
456	2.4	98.9	0.0	46.9	68.6

[a] Inhibition of HSV antigen detection in a given specimen was carried out with antiserum for the type of virus indicated.
[b] All the specimens in this table were tissue culture positive as well as HSV antigen positive. The specimens were typed by restriction enzyme analysis.
[c] A_{405}, Absorbance at 405 nm.
[d] Titer of >1:512.
[e] Titer of 1:8.
[f] Titer of 1:256.

TABLE 4. Inhibition of HSV antigen detection in the capture B/SA ELISA with rabbit and human HSV antisera

Specimen[a]	% Inhibition of A_{405}[b] by anti-HSV antisera	
	Rabbit[c]	Human[d]
105-A	81.0	QNS[e]
123-A-1	82.1	QNS
133-A	89.0	QNS
170-A	72.0	QNS
193-A	82.1	QNS
367-A	78.5	QNS
390-A	50.0	77.5
420-A	52.6	76.5
526-A	86.8	46.7
555-A	90.4	66.5
557-A-1	89.9	68.7
575-A-2	82.6	74.9
580-A-2	89.5	54.8
587-A	85.3	68.9
602-A-2	100.0	QNS
622-A	100.0	80.9

[a] All specimens described in this table were tissue culture negative but HSV positive in the capture B/SA ELISA.
[b] A_{405}, Absorbance at 405 nm.
[c] Titer of >1:512.
[d] Titer of 1:256.
[e] Quantity not sufficient for assay.

In all the experiments discussed in this report, 2 h of incubation was used with specimens.

The optimum time of incubation with the biotin-linked antibody and streptavidin-alkaline phosphatase conjugate was determined. The optimum time for the biotin-antibody step was 60 min, and that for the streptavidin-alkaline phosphatase conjugate step was 30 min. Again, longer incubation times did not add to the sensitivity of detection, but they increased absorbance readings for both antigen and control materials. The use of a fluorogenic substrate did not improve sensitivity of detection of HSV antigen in our assay, as has been reported elsewhere (21).

Table 2 indicates the sensitivity of detection of gradient-purified HSV-1 and -2 stock preparations by the capture B/SA ELISA. The method allowed detection of the virus at infectivity concentrations of 5×10^3 $TCID_{50}$ per ml for HSV-1 (i.e., 500 $TCID_{50}$ per 100 μl of sample) and 5×10^4 $TCID_{50}$ per ml for HSV-2 (i.e., 5,000 $TCID_{50}$ per 100 μl of sample).

Simultaneous use of HSV-1 and -2 reagents in the initial experiments showed greater sensitivity for detection of HSV in clinical specimens. To confirm that the capture B/SA ELISA was indeed detecting HSV antigens alone and not other proteins, several specimens were tested in the blocking experiments. The typing of HSV in these specimens was performed by restriction enzyme analysis (L. S. Nerurkar and N. Miller, personal communication) and by fluorescence staining (12). The results of a representative experiment are given in Table 3. Most of the specimens (9 of 12, as shown in Table 3) showed no inhibition by negative human serum and low-level inhibition (0 to 23%) with preimmune rabbit serum. The inhibition by positive sera ranged from 78 to 99% for hyperimmune rabbit serum, 46 to 77% for low-positive human serum, and 62 to 83% for high-positive human serum. This confirmed that the capture B/SA ELISA detects HSV antigens.

For a comparison of the detection of HSV or HSV antigen in clinical specimens by tissue culture and by the capture B/SA ELISA, 325 clinical specimens were studied by both techniques in 11 different experiments. With tissue culture technique as a standard the ELISA showed complete correlation of results, with 107 positive specimens (sensitivity, 95.6%) and 195 negative specimens (specificity, 91.4%). Five specimens were tissue culture positive and antigen negative. These five specimens required five to seven days to show the presence of virus, and only one of three to four replicate cultures showed cytopathic effects. This suggests that the virus concentration was very low in these specimens. The presence of HSV and viral antigens in the isolates prepared from these five specimens was confirmed by tissue culture on rabbit kidney cells and by the capture B/SA ELISA, respectively. There were 18 specimens which were tissue culture negative and antigen positive. Of these 18 specimens, 16 were further studied by inhibition assay with rabbit and human sera and were found to contain HSV antigens (Table 4).

Figure 2 compares infectivity of virus determined by endpoint titrations as $TCID_{50}$ per milliliter and ELISA antigen value (absorbance at 405 nm). In general, specimens with higher infectivity titers showed higher absorbance values; however, there were a few specimens with low infectivity which showed evidence of considerable antigenicity. Thus, the infectivity and antigen level of the clinical specimens could not be well correlated.

Table 5 compares the tissue culture and capture B/SA ELISA methods for detection of HSV in samples inactivated by different treatments. Infectious virus could not be detected after 24 h of incubation in distilled water or in EMEM at 37°C. In contrast, viral antigens could be detected by the capture B/A ELISA, though in small amounts, beyond 24 h, indicating that HSV antigen could be recognized after the viral infectivity was lost. UV irradiation or treatment of HSV specimens with Triton X-100 destroyed the virus infectivity completely or nearly completely but retained the

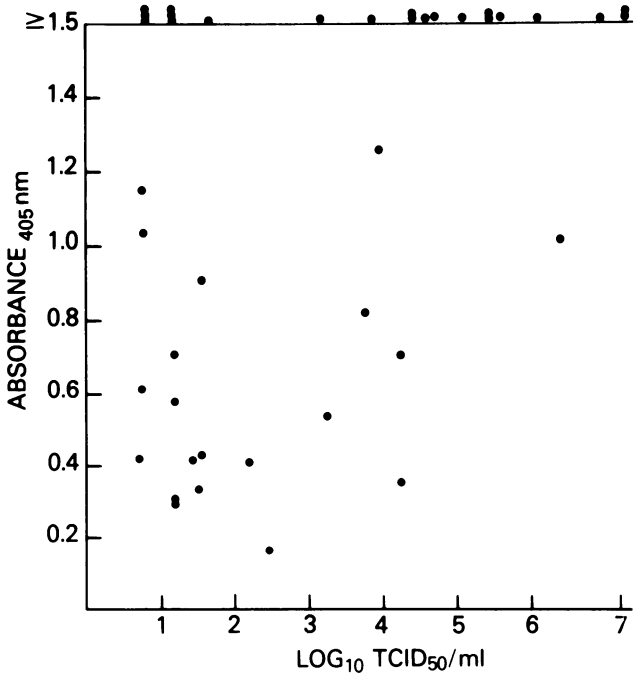

FIG. 2. Comparison of antigen capture B/SA ELISA and tissue culture detection of HSV in genital herpes specimens.

TABLE 5. Effect of different inactivation conditions on HSV detection by tissue culture and the capture B/SA ELISA

Conditions	Time	Infectivity by tissue culture	Antigen detection by B/SA ELISA
Distilled water, 37°C	1 h	+	+
	24 h	+	+
	48 h	−	+
	72 h	−	+
EMEM, 37°C	1 h	+	+
	24 h	+	+
	48 h	−	+
	72 h	−	+
UV irradiation	3 min	Tr	+
Hypochlorite	Instantaneous	−	−
Triton X-100, 1%	3 min	−	+

virion subcomponent anpigenicity. However, on contact with hypochlorite treatment, both the infectivity and the antigenicity were destroyed instantaneously.

Table 6 shows results of experiments in which *S. aureus* and *C. albicans* were added to stock HSV-1 and -2. It was clear that neither *C. albicans* nor *S. aureus* significantly affected the assay. Cytomegalovirus was not detected with these reagents. The culture-negative specimens did not show any false-positive reactions after the addition of *S. aureus*. Also, the contaminated specimens did not show any reaction in the capture B/SA ELISA. This further supported the specificity for HSV antigens of the assay described here.

DISCUSSION

The capture B/SA ELISA method had excellent sensitivity for the detection of HSV in clinical specimens. The method detected HSV antigen in 95.6% of clinical samples which were positive by conventional tissue culture methods. With purified virus, the method was less sensitive than was tissue culture. This is probably related to the smaller amounts of noninfectious viral antigens which are present in the purified samples (Table 2 and Fig. 2). The method also detected HSV in samples which were inactivated at 37°C or by UV irradiation or Triton X-100 treatments, when the infectious virus could no longer be detected.

TABLE 6. Effect of bacterial and fungal contamination on detection of HSV by the capture B/SA ELISA

Virus and added microorganism (concn per 100 μl)	A_{405} (mean ± SD)[a]
HSV-1 (10^4 $TCID_{50}$)	0.593 ± 0.029
Plus *C. albicans* (4×10^4)	0.601 ± 0.022
Plus *S. aureus* (1×10^4)	0.644 ± 0.012
HSV-2 (10^4 $TCID_{50}$)	0.211 ± 0.020
Plus *C. albicans* (4×10^4)	0.195 ± 0.005
Plus *S. aureus* (1×10^4)	0.207 ± 0.003
Culture-negative specimens[b]	0.006 ± 0.004
Plus *S. aureus* (1×10^4)	0.007 ± 0.001
Specimens with contaminations[b]	0.001 ± 0.001
Cytomegalovirus AD169 (10^6 $TCID_{50}$)	0.008 ± 0.000
S. aureus (1×10^4)	0.011 ± 0.009
C. albicans (4×10^4)	0.001 ± 0.001
Medium	0.004 ± 0.008

[a] A_{405}, Absorbance at 405 nm.
[b] $n = 10$.

Capture ELISA methods have been reported for detection of several viruses (2, 4, 6, 8, 10, 19, 22). The method reported previously for the detection of HSV antigens in genital specimens (19) was evaluated in our laboratory but was found to have low sensitivity. This method used antibody-enzyme direct conjugates as the detecting antibody, rather than the biotin-streptavidin system used in our studies. The advantage of the biotin-streptavidin system is that it has a very high binding constant (10^{15}), and this binding ability appears to increase the sensitivity of the test (1). We have previously noted that genital specimens may have very low concentrations of virus (12), and it appears that, unless the sensitivity of the assay is sufficiently high, the capture ELISA will not be able to detect this virus. Preliminary studies with egg avidin-enzyme conjugate showed lower sensitivity, and streptavidin was selected for all of the studies reported in this paper.

Several other methods for rapid detection of HSV have been reported, including direct immunofluorescence (17, 18), direct immunoperoxidase (17), and Papanicolaou or crystal violet staining (3). These tests are direct, do not include tissue culture amplification of virus, and have lower sensitivity than does the tissue culture method. In our laboratory, for example, smears from HSV lesions stained by immunofluorescence with the biotin-avidin system detected the viral antigens with 89% sensitivity and 62% specificity compared with the tissue culture method (13). The disadvantage of non-tissue culture, direct-detection methods, e.g., direct immunofluorescence of clinical smears, is that they require intact tissue or cells which show the presence of viral antigens or morphological change or both. These methods do not detect non-cell-associated virus. The ELISA antigen capture method does not have this disadvantage, since it allows detection of any antigenic material which is present in the sample. The ELISA also detects antigen in the absence of virus infectivity. In contrast, the tissue culture method is strictly based on the infectivity of the virus. The capture ELISA, however, will not detect the antigen when the antigenicity is destroyed by hypochlorite treatment and the antigen can no longer react with the antibody.

The specificity of the capture B/SA ELISA was 91.4%. The discrepant results involved 18 specimens which were negative by tissue culture. Blocking studies conducted with specific rabbit and human sera with the 16 specimens which were available showed interference by the appropriate sera and not by antibody-negative sera. The results suggest that these 16 specimens contained HSV antigen, and the specificity of the test was actually greater than 91.4%. The addition of *S. aureus* or *C. albicans* to stock HSV did not significantly influence the test results, and the contaminated specimens could be assayed satisfactorily. Also, the test was not positive with human cytomegalovirus, further demonstrating the specificity of this method for HSV antigens.

The capture B/SA ELISA could be performed in 4.5 h. This is considerably faster than tissue culture methods (15) and other reliable, comparably sensitive tests (12, 13). Rapid detection of HSV is important in diagnosing infections,

particularly in pregnant women near term, when clinical decisions as to possible delivery by cesarean section must be made. Rapid detection of HSV is also important in the diagnosis of primary infections of newborn infants and of adults, who may benefit by early treatment with antiviral therapy.

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ADDENDUM IN PROOF

After this paper was submitted for review, a report by K. Adler-Storthz, C. Kendall, R. C. Kenndey, R. D. Henkel, and G. R. Dreesman (J. Clin. Microbiol. **18**:1329–1334, 1983) was published. The procedures followed in our study differ from those of the above report in the use of (i) biotinylated rabbit anti-HSV immunoglobulin G instead of a monoclonal anti-HSV immunoglobulin M antibody and (ii) streptavidin instead of egg white avidin in the alkaline phosphatase conjugate. Both of these factors may be responsible for the improved sensitivity and specificity of HSV detection reported in our study.

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