Enzyme Immunofiltration Technique for Rapid Diagnosis of Herpes Simplex Virus Eye Infections in a Rabbit Model

PATRICK H. CLEVELAND,^{1,2*} DOUGLAS D. RICHMAN,^{2,3,4} DAVID C. REDFIELD,⁴ DALE R. DISHAROON,¹ PERRY S. BINDER,¹ AND MICHAEL N. OXMAN^{2,3,4}

Department of Surgery (Ophthalmology),¹ Department of Pathology,³ and Department of Medicine,⁴ University of California, San Diego, and Research Service Veterans Administration Medical Center,² San Diego, California 92161

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A rapid enzyme immunofiltration assay for herpes simplex virus (HSV) has been developed which is sensitive enough to detect viral antigens in eye swabs from rabbits with primary herpes keratitis. This assay employs a specially designed filter manifold to immobilize whole cells and cell debris dissociated from the swabs. Viral antigens trapped on the filters are then detected in an indirect immunoassay utilizing staphylococcal protein A conjugated with horseradish peroxidase. The assay required only 2.5 h to perform and could be read visually. Reconstruction experiments indicated that antigen from as few as 49 HSV-infected cells could be detected. Calcium alginate swabs were shown to recover more viral antigen than dacron swabs. The enzyme immunofiltration assay detected HSV antigens on 95% of the eye swabs from which infectious virus was recovered. In addition, HSV antigen was also detected in several swabs from infected eyes which did not yield infectious virus, presumably because the virus was neutralized by native antibody present in the lacrimal fluid. This enzyme immunofiltration assay technique lends itself to the elution of native antibody bound to the viral antigens, and this may be especially applicable in the diagnosis of recurrent HSV keratitis, where antiviral antibody in the lacrimal fluid may interfere with virus isolation and fluorescent-antibody or other virus detection assays.

The signs and symptoms of many important ocular infections overlap significantly, and thus it is often difficult to establish the correct etiological diagnosis on purely clinical grounds. For example, acute follicular conjunctivitis, although commonly caused by herpes simplex virus (HSV), is also frequently caused by varicella-zoster virus, adenovirus, chlamydia, and, recently in the United States, by enterovirus 70, the agent of acute hemorrhagic conjunctivitis (1, 6, 7, 12, 18). Recurrent disease produced by HSV also poses a problem in diagnosis, especially when the typical epithelial dendrite is not present (1). Because of the potentially serious consequences of ocular infections with HSV and the requirement for organism- and stage-specific therapy, the physician must be able to make a rapid and accurate diagnosis.

Diagnostic tests that involve direct or indirect fluorescent-antibody staining of corneal and conjunctival scrapings have been valuable in detecting HSV (11, 14, 17, 19). Although these assays are rapid and may be quite useful, they have certain limitations, such as the requirement for a sufficient quantity of material, intact cells,

and a skilled and experienced technician; furthermore, interpretation, in the case of clinical specimens, is often difficult because of nonspecific fluorescence (8, 10).

At present, the most reliable method of diagnosis is the isolation of virus in cell culture. The typical cytopathic effects of HSV are usually seen within 2 to 5 days. The major problems with virus isolation as a diagnostic technique are that the requisite laboratory facilities are not widely available, and the results often are not available to the physician in time to influence therapeutic decisions.

To improve the speed, sensitivity, availability, and accuracy of laboratory diagnosis, we have developed an enzyme immunofiltration assay (EIA) for viral antigens. This assay employs a specially designed filter manifold which permits the rapid performance of immunoassays by efficiently immobilizing whole cells and fragments of disrupted cells (which are an important source of viral antigen in clinical specimens) on filters (3, 4, 16). By keeping these particulate antigens immobilized during numerous incubations and washes, this technique overcomes an

FIG. 1. Flow diagram of specimen processing and SPA-HRP immunofiltration assay for virus antigen in eye swabs.

obstacle that has complicated and slowed the performance of most other viral diagnostic tests. This nonspecific immobilization technique also avoids the need for a separate "capture" antibody for each virus to be detected and lends itself to the preelution of native antibody bound to viral antigens in clinical specimens, a procedure which may improve diagnostic sensitivity in patients first evaluated several days after the onset of symptoms.

The filter manifold and its operation were originally described for an 125 I-labeled staphylococcal protein A (SPA) radioimmunoassay for HSV antigens and antibodies to them (3). In that study, the immunofiltration assay system was shown to be at least 100 times more sensitive than conventional complement fixation, micro-neutralization, or direct fluorescent-antibody tests. First with 125 I-labeled SPA and more recently with SPA conjugated with horseradish peroxidase (SPA-HRP), we have refined the assay to the point where it can detect as few as 49 HSV-infected tissue culture cells (P. H. Cleveland, D. D. Richman, D. R. Disharoon, and M. N. Oxman, manuscript in preparation).

We describe herein the use of this method to detect HSV antigens on calcium alginate and dacron swabs taken from rabbits with HSV keratoconjunctivitis.

MATERIALS AND METHODS

Antiserum. Rabbit antiserum to HSV was obtained

from an adult male rabbit 3 months after corneal inoculation with 200 PFU of HSV type 1 (HSV-1) (Sanchez strain) which had been propagated in rabbit kidney cells (RK-13). This serum, which has an EIA anti-HSV titer of 12,000, was used at a dilution of 1:500.

Cell culture. HSV-infected human foreskin fibroblast cells (350Q) were prepared as previously described (3); more than 90% of these cells contained HSV antigens on their surfaces, as determined by immunofluorescence staining. The HSV-infected 350Q cells were rendered noninfectious by long-wave UV light and psoralen as previously described (15).

Rabbits. Young adult male albino rabbits, weighing from 2.5 to 3 kg, were infected by topical application of 20 μ l of medium containing 200 PFU of HSV-1 Sanchez into the eye and massaging gently for 1 min. The eyes were then swabbed daily with either calcium alginate type I swabs or dacron type I swabs (Inolex, Glenwood, Ill.) starting at day 3 postinfection. Before swabbing, 10 μ l of fluorescein (Fluores; Barnes-Hind, Sunnyvale, Calif.) was applied to each eye to disclose the presence of corneal or conjunctival lesions. The fluorescein was shown not to interfere with either virus isolation or the EIA. If a lesion was present, the swabs were used to debride it, with an attempt to obtain as much cellular material as possible. If no lesions were seen, both the cornea and conjunctiva were swabbed. The swabs were placed into 2 ml of transport medium (Hanks balanced salt solution with 0.3% gelatin and 100 U of penicillin G, 100 μ g of streptomycin, and 5 μ g of amphotericin B per ml). The specimens were then transported on ice, and serial 10-fold dilutions were made in transport medium. Fifty microliters of the dilutions were inoculated onto 350Q

FIG. 2. SPA-HRP immunofiltration assay: a standard curve with HSV-1-infected 350Q cells.

cells which were grown in the wells of a microtiter plate. The wells were examined daily for cytopathic effects. The remainder of the viral transport medium plus the swab was then tested for viral antigen in the EIA.

Immunofiltration assay of eye swabs. A microfiltration device, consisting of a vacuum manifold and microtiter plates modified to function as both incubation chambers and filter manifolds (V & P Enterprises, San Diego, Calif.), was used as previously described (3, 4, 16). The bottom of each of the wells contained a glass fiber filter disk and a drain hole. Because the hole is small, surface tension prevents liquid within the wells from draining through. When vacuum is applied, the liquid is quickly drawn through the filter disks and out of the wells. This efficiently removes soluble

material and leaves particulate matter larger than 1.5 μ m trapped on the filters. Free virions and particles smaller than 1.5 μ m will not be retained by the filter. All 96 filters can be rapidly washed by adding 0.3 ml of buffer to each well with a Cornwall repeating syringe with an eight-prong dispensing manifold. The immunofiltration assay for HSV antigens was performed as illustrated in Fig. 1. The calcium alginate swabs were dissolved by adding 75 μ l of a 1.0 M sodium citrate solution to the viral transport medium, incubating for 30 min at 22°C, and vigorously mixing on a Vortex apparatus for 1 min. This procedure completely dissolved the calcium alginate and released the antigen-bearing cell debris into the transport medium. The dacron swabs were treated in an identical fashion. The filter disks were conditioned for 10 min with 0.1 ml of

TABLE 1. Comparison of specific and nonspecific binding in the EIA

Days after HSV inoculation ^a	Absorbance values ^b of SPA-HRP bound with:		Specific absorbance	Binding ratio	2 $\times$ SD
	HSV-1 antiserum	Preimmune serum			
-10	0.053 $\pm$ 0.004	0.054 $\pm$ 0.003	-0.001	1.0	0.008
3	0.090 $\pm$ 0.004	0.050 $\pm$ 0.004	0.040	1.8	0.008
4	0.386 $\pm$ 0.005 ^c	0.053 $\pm$ 0.006	0.343	7.3	0.012
5	0.315 $\pm$ 0.004 ^c	0.085 $\pm$ 0.013	0.231	3.7	0.026
6	0.270 $\pm$ 0.025 ^c	0.068 $\pm$ 0.005	0.202	4.0	0.050
7	0.400 $\pm$ 0.010 ^c	0.056 $\pm$ 0.000	0.344	7.1	0.020
8	0.392 $\pm$ 0.009 ^c	0.070 $\pm$ 0.015	0.322	5.6	0.030
9	0.450 $\pm$ 0.032 ^c	0.058 $\pm$ 0.004	0.393	7.8	0.064
10	0.243 $\pm$ 0.020 ^c	0.060 $\pm$ 0.006	0.183	4.1	0.040
11	0.073 $\pm$ 0.004	0.049 $\pm$ 0.004	0.023	1.5	0.008
12	0.066 $\pm$ 0.008	0.049 $\pm$ 0.010	0.016	1.4	0.020

^a Calcium alginate swabs taken from the right eye of rabbit 32 before and after inoculation with HSV-1.

^b The values represent the mean absorbance at 488 nm $\pm$ standard deviation from duplicates.

^c Met criteria for positive antigen test.

TABLE 2. Correlation of HSV antigen detection by EIA and HSV isolation with time course of infection

Detection method	No. positive/no. tested at postinoculation day:									
	3	4	5	6	7	8	9	10	11	12
Calcium alginate swabs										
HSV antigen by EIA	3/11	6/11	6/11	6/11	4/11	6/11	6/11	4/11	5/11	2/11
HSV isolation	6/11	6/10	6/11	5/8	6/9	6/10	6/11	4/11	5/11	4/11
Dacron swabs										
HSV antigen by EIA	1/11	4/11	5/11	4/11	5/11	5/11	4/11	5/11	3/11	3/11
HSV isolation	6/11	5/11	5/11	5/9	5/10	5/10	6/11	5/10	6/11	4/11

FBS buffer (phosphate-buffered saline [pH 7.4] containing 10% fetal bovine serum, 1% bovine serum albumin, and 0.3% swine skin gelatin [Sigma Chemical Co., St. Louis, Mo.] and 0.01% thimerosal). The vacuum was applied to remove the fluid, and 200 μ l of the suspension of cellular debris from the swabs was added to each of four wells. In addition, control antigens (25,000 HSV-infected 350Q cells or uninfected 350Q cells) were added to replicate wells. Viral transport medium containing unused swabs and swabs taken from uninvolved eyes were also run as negative controls. Vacuum was applied, leaving whole cells and cell debris trapped on the filter. The specimens were washed once with 0.3 ml of gelatin buffer (phosphate-buffered saline [pH 7.4] containing 0.3% gelatin and 0.01% thimerosal). Next, 100 μ l of 0.1 M glycine-HCl buffer (pH 2.2) was added to each well, and the wells were incubated for 2.5 min at 22°C to dissociate native antibody from the cell debris. Vacuum was applied, and the filters were washed two times with gelatin buffer. Fifty microliters of a 1:500 dilution of rabbit preimmune serum or rabbit antiserum to HSV diluted in FBS buffer was added to duplicate wells. After incubation at 37°C for 60 min, vacuum was applied, and the filters were washed three times with 0.3 ml of gelatin buffer. Next, 2.4 pmol of SPA-HRP (Zymed Inc., Burlingame, Calif.) in 50 μ l of gelatin buffer containing 1% bovine serum albumin was added to each well. After incubation at 37° for 30 min, the vacuum was reapplied, and the filters were washed three times with 0.3 ml of gelatin buffer to remove

unbound SPA-HRP. The holes in the bottom of the plate were sealed with Parafilm (American Can Co., Greenwich, Conn.) by using a plate-sealing device (V & P Enterprises), and then 300 μ l of substrate solution (0.05 M citrate-0.1 M phosphate buffer [pH 5] containing 0.04% *O*-phenylenediamine and 0.006% hydrogen peroxide) was added to each well. The enzyme reaction was allowed to develop in the dark for 30 min at room temperature. The plates were read visually, and 100 μ l from each well was transferred with a 12-channel Titer-Tek pipettor (Flow Laboratories, Inglewood, Calif.) into the corresponding well of a one-half area EIA plate (Costar, Cambridge, Mass.) containing 50 μ l of 2.0 M H₂SO₄ per well. The absorbance at 488 nm was then measured with a MicroELISA reader (model 530) mated to a printing calculator (Dynatech, Alexandria, Va.).

Expression of results and statistical analysis. The absorbance values were corrected for nonspecific binding of the serum and for residual native antibody by subtracting the absorbance value of the swab material incubated with preimmune serum from the absorbance value of the same swab material incubated with immune serum (i.e., specific absorbance). A positive test for antigen was defined as one that fulfilled all three of the following criteria: (i) it yielded a specific absorbance of greater than 0.05 absorbance units; (ii) the specific absorbance was at least two times greater than the standard deviation of either the preimmune or the immune absorbance value; and (iii) the binding ratio was greater than 1.4 (binding ratio = the absorbance of the swab material incubated with immune serum divided by the absorbance value of the same swab material incubated with the normal serum). The rigorous requirement for all three criteria to be met for a positive result reduced the possibility of reporting false-positive results. For each experiment, HSV-1-infected 350Q cells were used in reconstruction experiments to generate a standard curve of HSV-specific absorbance versus number of HSV-infected cells per well (i.e., per 200 μ l of sample); this permitted the expression of the antigen content of the swabs in terms of HSV-infected cell equivalents. A typical standard curve is presented in Fig. 2.

RESULTS

Clinical observations. Eleven rabbits were inoculated in both eyes with HSV-1. The eyes were observed daily and scored for the presence of conjunctivitis, exudate, and epithelial lesions as detected by fluorescein staining. At 3 days postinfection, 11 of 22 eyes appeared to have

TABLE 3. Correlation of HSV antigen detection by EIA with virus isolation^a

Virus isolation	EIA	
	Antigen positive	Antigen negative
Calcium alginate swabs		
Virus isolation positive	38 (95)	2 (5)
Virus isolation negative	0 (0)	30 (100)
Not cultured	2	5
Dacron swabs		
Virus isolation positive	29 (78)	8 (22)
Virus isolation negative	3 (9)	32 (91)
Not cultured	0	5

^a Swabs were collected 4 to 10 days after HSV inoculation. Data are reported as numbers of swabs. Numbers in parentheses indicate percent correlation between EIA and virus isolation data.

FIG. 3. Comparison of EIA and virus culture results from calcium alginate swabs, and epithelial lesion score on the right eye of rabbit 32 during the course of HSV keratitis.

clinical conjunctivitis; however, 3 of those 11 eyes failed to yield infectious virus throughout the course of the study. At 4 days postinoculation, 9 of 22 eyes had exudate; however, one of the 9 failed to yield infectious virus. Epithelial lesions on the conjunctiva or cornea were not seen until 5 days postinoculation, at which time such lesions were observed in 4 of 22 eyes. By 7 days postinfection, 10 of 22 eyes had epithelial lesions, and by 8 days postinfection, lesions were present in 12 of 22 eyes. New lesions did not appear after day 8.

Antigen and virus detection. Typical EIA results demonstrating the recovery of HSV antigen from sequential eye swabs and the statistical analysis of the data are presented in Table 1, which also shows the low level of nonspecific binding associated with the assay.

Between days 3 and 12 after HSV inoculation, HSV antigens were detected by EIA, and infectious HSV was recovered from 13 of 22 eyes. The correlation of virus isolation, HSV antigen detection by EIA, and time after HSV inoculation is shown in Table 2.

Between 4 and 10 days postinfection, antigen detection by EIA correlated closely with virus isolation. This time interval extended from just before the appearance of epithelial lesions through the time of maximum clinical involvement. Of significance was the detection of HSV antigen by EIA in four eyes 2 days before the appearance of epithelial lesions.

The overall results between days 4 and 10 postinfection demonstrated a 95% correlation between antigen detection by EIA and virus isolation when calcium alginate swabs were employed and a 78% correlation when dacron swabs were used (Table 3). An observation of interest was that three dacron swabs which were positive for HSV antigens by EIA but negative by virus isolation were all taken from the left eye of the same rabbit on days 7, 8, and 10. Virus isolation results on the same eye were positive on day 9 (virus titer = $10^{1.5}$ 50% tissue culture infective dose [TCID₅₀] per 50 µl), day 11 (virus titer = $10^{3.0}$ TCID₅₀ per 50 µl), and day 12 (virus titer = $10^{3.5}$ TCID₅₀ per 50 µl). This eye was also unique in that it never demonstrated any clinical signs of infection.

The correlation of clinical disease, the amount of infectious virus recovered, and the amount of HSV antigen present as detected by EIA over the course of infection was typified by results from the right eye of rabbit 32, as shown in Fig. 3. Although antigen detection was somewhat less sensitive than virus isolation, there was a general correlation between the amount of HSV antigen detected and the titer of infectious HSV recovered from the same swab between days 4 and 10 postinfection. Moreover, HSV antigen could be detected 2 days before the appearance of a recognizable lesion. The ability to recover HSV antigens and infectious HSV with calcium alginate swabs was compared with recovery

FIG. 4. (A) Comparison of mean EIA and culture results from all eyes swabbed with calcium alginate which were virus culture positive during the course of disease. (B) Comparison of mean EIA and culture results from all eyes swabbed with dacron which were virus culture positive during the course of disease.

with dacron swabs over the course of infection (Fig. 4). Slightly higher titers of infectious virus were recovered with dacron than with calcium alginate swabs; however, the mean recovery of HSV antigen was significantly greater with calcium alginate swabs. The superiority of calcium alginate swabs for the recovery of antigen was also demonstrated by comparing antigen detection with a ranked order of virus isolation titers obtained with each type of swab (Table 4). The

amount of HSV antigen recovered with calcium alginate swabs was approximately twice that recovered with dacron swabs at almost all levels of infectious virus. This same twofold difference was also demonstrated by the combined means of all the EIA-positive swabs: 6,208 HSV-infected cell equivalents per calcium alginate swab versus 3,694 HSV-infected cell equivalents per dacron swab. Although the mean antigen recovery from calcium alginate swabs was twice that

TABLE 4. Correlation of infectious HSV titer with HSV antigen recovery

HSV infectious titer (TCID ₅₀ per 50 μ l)	Mean HSV infected-cell equivalents per swab in EIA (no. of swabs)	
	Calcium alginate	Dacron
10 ^{5.5}	12,000 (1)	6,114 (7)
10 ^{5.0}	8,271 (7)	4,789 (9)
10 ^{4.5}	8,421 (14)	3,325 (8)
10 ^{4.0}	5,050 (7)	2,089 (11)
10 ^{3.5}	3,078 (9)	0 (2)
10 ^{3.0}	1,343 (7)	450 (2)
10 ^{2.5}	960 (5)	0 (3)
10 ^{2.0}	550 (2)	0 (1)
10 ^{1.5}	0 (1)	240 (5)
10 ^{1.0}	2,800 (1)	0 (1)

from dacron swabs, the dacron swabs yielded more infectious virus; the geometric mean titer of recovered HSV was 10^{4.46} TCID₅₀ per 50 μ l for all positive dacron swabs versus 10^{4.01} TCID₅₀ per 50 μ l for all positive calcium alginate swabs. It is not clear whether this difference in recovery of infectious virus was due to a larger amount of infectious virus present in the eyes swabbed with dacron swabs or to some inactivation of virus during the processing of the calcium alginate swabs.

Recovery of infectious virus. To determine whether calcium alginate swabs inactivated HSV and thereby interfered with virus isolation, as has been suggested by Crane et al. (5), we inoculated 1.7×10^4 HSV-infected 350Q cells (about 10^{4.25} PFU/ml) onto calcium alginate type I, dacron type I, rayon, and cotton swabs and placed each of them into 1 ml of transport medium for 1.5 h at 0°C. They were then mixed on a Vortex apparatus, and the medium was titrated for infectivity. Our results showed no significant difference in the recovery of infectious HSV from the calcium alginate and dacron swabs; in fact, recovery of virus from both types of swab was the same as that from HSV cells inoculated directly into the medium (Table 5). However, less virus was recovered from the rayon and cotton swabs (which were larger than the calcium alginate and dacron swabs).

Elution of native antibody. Our initial experiments on the detection of HSV antigens in eye swabs indicated that relatively high levels of native antibody were present in the debris from some swabs. If native antibody were bound non-specifically, it would interfere with the assay by raising the background of bound SPA-HRP, and if the native antibody were bound specifically to HSV antigens, it would again raise the background of bound SPA-HRP and also prevent the rabbit anti-HSV serum from binding at those sites. To reduce the level of native antibody

bound to cellular debris in specimens, we eluted with 0.1 M glycine buffer (pH 2.2) (Fig. 1). Reconstruction experiments were performed to test the ability of a 2.5-min glycine buffer elution to remove rabbit antibody from HSV-infected cells. In these experiments, 25,000 HSV-1-infected or uninfected 350Q cells were incubated with antiserum to HSV or FBS buffer, followed by elution with glycine buffer or FBS buffer for 2.5 min. As shown in Table 6, 64% of the bound HSV antibody was removed by acid glycine elution. To determine whether the glycine elution removed or altered the antigenicity of the HSV-infected cells, a parallel experiment was performed in which 25,000 HSV-infected or uninfected 350Q cells were incubated with FBS buffer, eluted with either acid glycine or FBS buffer, and then incubated with HSV antiserum. The results of this experiment indicated that the antigenicity of the HSV-infected cells was not altered by brief acid glycine treatment (Table 6). However, we noted that elution periods of 5 min or more reduced the antigenicity of the cells to about the same extent that it removed antibody (Fig. 5).

We observed that with material from rabbit eye swabs which has a high level of background SPA-HRP binding, presumably due to the presence of native antibody, preelution with acid glycine reduced the background by two- to threefold without reducing HSV-specific absorbance (Table 7).

DISCUSSION

The immunofiltration technique utilized in the assay described here provides a means for the

TABLE 5. Recovery of infectious HSV from different swabs after inoculation with 1.7×10^4 HSV-infected 350Q cells

Swab ^a	Infectivity (TCID ₅₀ per ml)
Calcium alginate.....	10 ^{4.25}
Calcium alginate + citrate ^b	10 ^{4.25}
Dacron.....	10 ^{4.25}
Rayon.....	10 ^{4.0}
Cotton.....	10 ^{3.75}
None ^c	10 ^{4.25}

^a Swabs were placed into 1 ml of transport medium, held on ice for 1.5 h, and then mixed vigorously for 15 s.

^b A 75- μ l amount of 1.0 M sodium citrate solution was added, and the swab was incubated at 0°C for 30 min to dissolve the calcium alginate.

^c HSV-infected 350Q cells (1.7×10^4) were inoculated directly into 1 ml of transport medium, which was held on ice for 1.5 h and then mixed vigorously for 15 s.

TABLE 6. Elution of antibody from HSV-1-infected cells with acid glycine and stability of HSV antigens

Expt	Incubation 1 ^a	Elution solvent ^b	Incubation 2 ^c	HSV-specific absorbance at 488 nm	% of total possible specific absorbance
1. Elution of specific antibody	HSV-1 antiserum	FBS	FBS	0.817	100
	HSV-1 antiserum	Glycine	FBS	0.298	36
2. Stability of HSV antigen	FBS	FBS	HSV-1 antiserum	0.810	100
	FBS	Glycine	HSV-1 antiserum	0.841	104
	HSV-1 antiserum	Glycine	HSV-1 antiserum	0.891	110

^a A total of 25,000 HSV-infected and uninfected 350Q cells were incubated with a 1:500 dilution of HSV-1 antiserum or FBS buffer for 30 min at 37°C.

^b After washing, the antibody was eluted from the cells with 100 μ l of acid glycine buffer or FBS buffer for 2.5 min at 22°C.

^c After washing, the cells were incubated with a 1:500 dilution of HSV-1 antiserum or FBS buffer for 30 min at 37°C; standard SPA-HRP immunofiltration assays were then performed.

nonspecific capture and immobilization of antigen-bearing cells and cell debris present in clinical specimens and permits the efficient elution of native antibody and the subsequent detection and quantitation of viral antigens by indirect SPA-HRP immunoassay.

The studies presented here indicated that the SPA-HRP immunofiltration assay was rapid, sensitive, and accurate in detecting HSV antigens on eye swabs. Furthermore, if one examined calcium alginate swabs collected between days 4 and 10 (an interval corresponding to the time at which patients usually present with clinical symptoms), the correlation between antigen detection and virus isolation was 95%. However, the results of the EIA are available within 3 h, and this assay requires neither the expertise nor the elaborate laboratory facilities required for virus isolation.

Swabs from virus-infected eyes often contain

clumps of degenerating epithelial cells, mucus, inflammatory cells, and native antibody. This presents two problems: (i) the efficient recovery of viral antigen in the form of a homogeneous preparation that can be divided into replicate samples and (ii) the removal of native antibody bound specifically to viral antigens and non-specifically associated with cell debris. Since native antibody will bind to SPA-HRP, it will elevate the level of nonspecific background binding. We have solved the first problem by using calcium alginate swabs which can be dissolved by sodium citrate and vigorous mixing to produce a homogeneous suspension that is trapped on glass fiber filters. Comparison of calcium alginate and dacron swabs showed a higher recovery of viral antigen with calcium alginate swabs. The reason for this higher recovery was probably our ability to dissolve the calcium alginate swab material by using sodium

FIG. 5. Elution of antibody and antigen activity from HSV-1-infected cells by glycine buffer. The cells were incubated as described in Table 6 with either HSV-1 antiserum or FBS and eluted with glycine buffer (pH 2.2) or FBS for 1.25 to 20 min. The cells were then tested by standard SPA-HRP immunofiltration assays to determine the percent of antibody or antigen activity removed by elution.

TABLE 7. Effect of glycine buffer elution on specific and background binding to eye swab material

Elution solvent ^a	Absorbance values ^b of SPA-HRP bound with:		Specific absorbance	Binding ratio	2 × SD
	HSV-1 antiserum	Preimmune serum			
FBS	0.181 ± 0.024	0.133 ± 0.059	0.048	1.4	0.118
Glycine	0.111 ± 0.016	0.052 ± 0.005	0.059	2.2	0.032

^a Calcium alginate swab from a rabbit eye 12 days after inoculation with HSV-1. The swab material was immobilized on the filter manifold and eluted with either FBS buffer or glycine buffer (pH 2.2) for 2.5 min before EIA.

^b The values represent the mean absorbance at 488 nm ± standard deviation from duplicates.

citrate solutions, thus totally releasing all antigenic debris trapped in the calcium alginate fiber matrix. Because calcium alginate swabs have been reported to inactivate HSV (5), we were concerned about using them in studies which were also designed to recover infectious HSV. However, in the experiments of Crane et al. (5), calcium alginate fiber from the equivalent of 200 swabs (1 g) was incubated with the virus for 12 to 72 h. We repeated these experiments, but used conditions comparable to those actually employed in the collection and processing of clinical specimens. Our results showed no reduction in the recovery of infectious HSV between calcium alginate and dacron swabs; furthermore, these results demonstrated no significant reduction in recovery with any of the swabs tested.

We have approached the problem of native antibody in clinical specimens by eluting with acid glycine buffer before assay. Reconstruction experiments with a 2.5-min acid glycine wash indicated that antibody to HSV could be effectively removed without diminishing the amount of HSV antigen initially present. In HSV infections, as in most other virus infections, the amount of detectable infectious virus and the quantity of viral antigen detected by such immunological techniques as fluorescent-antibody staining usually diminish rapidly after the onset of symptoms. This is presumably due to the appearance of local antibody which interferes with both of these assays (9, 13). An antibody elution step which does not result in the loss or destruction of viral antigens should increase the sensitivity of antigen detection and thus improve the chances of establishing the diagnosis in the latter stages of infection, the time at which patients usually present. We found, with eye swabs from rabbits, that washing with acid glycine buffer often reduced the background in our EIA by two- to threefold; we observed in three rabbits that swabs which were culture negative (from an eye which was culture positive on an earlier or later day) were antigen positive after treatment with acid glycine buffer. Since the lacrimal fluid of this rabbit may have had anti-

body to HSV at this time (the presence of specific antibody on the surface of virus-infected cells has been demonstrated within 72 h of the administration of the virus [2]), it is probable that native antibody neutralized infectious virus, resulting in a falsely negative culture result. By eluting with acid glycine buffer, we were able to remove that native antibody and demonstrate the presence of viral antigen in the specimen. More extensive studies to explore this possibility are under way. Preliminary studies with eye swab specimens from patients with recurrent HSV keratitis have demonstrated the sensitivity and clinical applicability of this diagnostic technique and have also revealed the presence of HSV antigens in culture-negative specimens. Studies are under way to determine whether these represent false-negative culture results or false-positive EIA results.

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