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Evaluation of the Virocult Transport Tube for Isolation of Herpes Simplex Virus from Clinical Specimens

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Herpes simplex virus survived in Virocult transport tubes and had a half-life of 3.5 days at 2°C and 2.75 days at 22°C. Of 2,000 consecutive clinical specimens transported on Virocult tubes and cultured for herpes simplex virus, 448 (22.4%) were positive. Comparison of the holding times between positive and negative cultures, up to 12 days, revealed no significant loss of positive cultures with time.

Information on active herpes simplex virus (HSV) infection obtained from frequent third-trimester viral cultures on pregnant women can be used to manage pregnancy and reduce the need for cesarean section while avoiding neonatal HSV morbidity (2, 5). Diagnosis of HSV infection in males and in several tissues and organs in both males and females of all ages is also of importance. Tissue culture isolation of virus has been the most sensitive and reliable method for detecting HSV (1, 3). The usual practice for handling the specimen is to collect the specimen on a swab by vigorously rubbing the affected area and placing the swab into a viral transport medium. The medium typically contains a buffered salt solution with some protein (serum) or gelatin or both, along with antibacterial and antifungal antibiotics. Tryptic soy broth and brain heart infusion broth have also been employed as transport media for HSV (6). The specimen is transported to the laboratory, where cell cultures are inoculated as soon as possible after collection of the specimen.

Genital HSV infections have become widespread. However, many physicians and the staffs of small hospitals who have need of HSV diagnostic work do not have ready access to a laboratory capable of doing HSV culturing. There are now commercial sources of convenient collection swabs and transport tubes that can be used for both collection of the specimen and transport to a central laboratory. This study reports on an evaluation of the Virocult transport tube.

Two thousand consecutive clinical specimens that were submitted for evaluation were cultured for HSV. The specimens were collected on Virocult transport swabs (Medical Wire and Equipment Co., Cleveland, Ohio) and shipped at ambient temperatures. The Virocult transport tube consists of a swab that is inserted into a plastic tube which contains a foam pad saturated with a balanced salt solution that contains glucose (1.0 g/liter), lactalbumin hydrolysate (3.0 g/liter), chloramphenicol (2.5 μg/ml), and cycloheximide (10 μg/ml). Cycloheximide is added as an antifungal agent and, at the concentration used, was not significantly inhibitory to the cell cultures or virus replication. Cell culture inoculation was done on the same day that the specimen arrived at the laboratory. The specimens were submitted by private physicians, hospital laboratories, and obstetrical-gynecological clinics from throughout a three-state area in the western United States. Most of the specimens were collected from urogenital sites and most were from pregnant women.

Virus isolation was carried out in Vero cells in flat-sided tissue culture tubes containing a glass cover slip (11 by 22 mm). The cell culture medium was Dulbecco modified minimal essential medium (Flow Laboratories, Inglewood, Calif.) with 10% fetal bovine serum (Sterile Systems, Logan, Utah), 0.11% sodium bicarbonate, 2 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, and 50 μg of gentamicin per ml. The cultures were changed to a maintenance medium immediately before specimen inoculation. This medium was Dulbecco modified minimal essential medium with sodium bicarbonate and HEPES buffers as described above, 2% fetal bovine serum, and 50 μg of gentamicin, 50 U of penicillin, 50 μg of streptomycin, 2.5 μg of amphotericin B, and 30 μg of nystatin per ml. The specimens were inoculated directly into the culture tubes by swirling the Virocult swab 50 times in the cell culture medium of the tube. Cell cultures were incubated for 24 h, examined for cytopathic effect, and then renewed with fresh medium if no cytopathic effect was evident. The cultures were examined daily for cytopathic effect and held a total of 7 days before being declared negative. When cytopathic effect developed, the cells on the cover slip were fixed in cold acetone, and the identity of the virus isolate was confirmed by direct immunofluorescence staining with a bivalent antiserum containing antibodies to both HSV type 1 (HSV-1) and HSV-2 (M. A. Bioproducts, Walkersville, Md.).

Typing of clinical isolates was performed by indirect immunofluorescence with commercially available monoclonal antibody reagents (Immulok, Inc., Carpinteria, Calif.).

HSV was titrated by standard plaque assay procedures on Vero cells. The cells were planted in 60-mm tissue culture dishes and incubated at 37°C until confluency was obtained. The medium was Dulbecco modified minimal essential medium with 10% fetal bovine serum. Virus (0.1 ml) was placed upon the cells after removal of the medium and allowed to adsorb for 1 h at 37°C, and then cells were overlaid with 1% methylcellulose in growth medium. The cultures were incubated for 4 days at 37°C, fixed in 4% Formalin, and then stained with 1% crystal violet to visualize the plaques. HSV-2 strain 333 contained in cell culture medium was applied in two different concentrations (PFU) to dry Virocult swabs (0.1 ml per swab) and then inserted into the transport tubes. Two different stock preparations of the virus were used, one containing 1.2×10^5 PFU/ml and the other 1.3×10^4 PFU/ml. Separate tubes were prepared for each sampling period. Each series of transport tubes was divided into two sets; one set was stored at 2°C and the other at ambient temperature (22°C). After an equilibration period of 4 h, the virus remaining on the swab was recovered in cell culture

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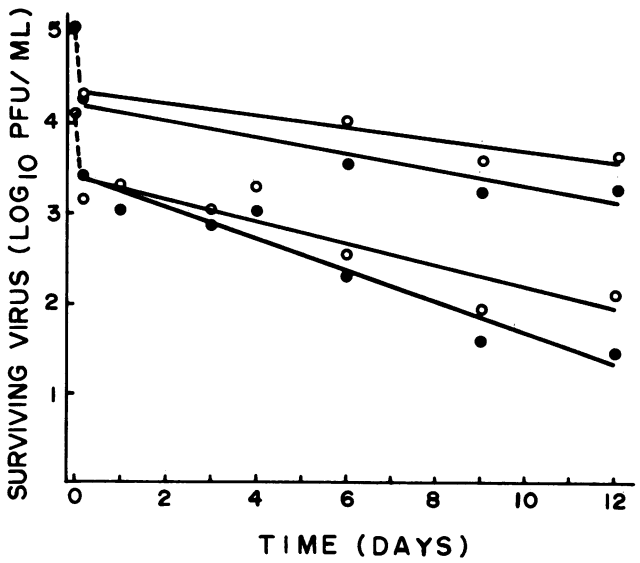

FIG. 1. Persistence of HSV on Virocult swabs at 2°C (○) and ambient temperature (22°C) (●). Known quantities of two different lots of HSV-2 strain 333 were applied to Virocult swabs. Viable virus was recovered and quantitated in plaque assays after the swabs were held up to 12 days at either 2 or 22°C. The initial drop in virus titer was due to equilibration of the virus in the transport medium. The average half-life of the virus at 2°C was 3.5 days and at room temperature was 2.75 days.

medium and then titrated by plaque assay. During the equilibration period, the virus contained on the swab diffused into the ca. 1 ml of liquid and the foam pad in the transport tube (Fig. 1). During the equilibration period, there was a ca. 1:10 dilution of the virus. At subsequent sampling periods, the swab-associated virus was recovered and titrated without regard to the virus contained in the remaining liquid phase. Statistical analysis of the data was performed by linear regression analysis by the method of least squares. The resultant straight line decay curves are shown in Fig. 1. As expected, the virus in specimens held at 2°C persisted at higher titers than that in the specimens held at 22°C; however, the difference in loss of virus viability at the two temperatures was surprisingly small. The titer of the virus held at 22°C was only ca. 0.5 log lower than that of virus held at 2°C for 12 days. The average half-life of the virus was 3.5 days at 2°C and 2.75 days at 22°C.

The clinical specimens were shipped to a central laboratory for virological testing. The specimens were cultured on Vero cells, and HSV isolates were confirmed by immunofluorescence staining. The days after inoculation that the cultures showed cytopathic effect in 2,000 consecutive tests is shown in Table 1. Of the 2,000 specimens tested, 448 (22.4%) were positive. More than 85% of the positive cultures were identified by day 3 in culture (Table 1). The other positive cultures gradually appeared on days 4 through 7.

The length of time the viral specimens were held on the Virocult transport swabs before being cultured is shown on Table 2. During the transport time, the specimens were shipped at ambient temperatures but were held at refrigerator temperatures before being shipped and again in the laboratory between their receipt and inoculation into cell cultures. The specimens were not frozen at any time. Comparison of the frequencies of positive and negative

TABLE 1. Day of onset of HSV cytopathic effect in 2,000 cultures of clinical specimens

Days post-inoculation	No. (%) of HSV-positive cultures	Cumulative % HSV-positive cultures
1	108 (24.1)	24.1
2	211 (47.1)	71.2
3	65 (14.5)	85.7
4	34 (7.6)	93.3
5	20 (4.5)	97.8
6	9 (2.0)	99.8
7	1 (0.2)	100.0
Total		
Positive	448 (22.4)	
Negative	1,552 (77.6)	

cultures at the various holding times revealed that the virus in the specimens remained viable during the holding times. If the infectious virus in the specimens was decaying at a significant rate so that complete loss of viability occurred before culturing, there would be significantly fewer positive cultures at longer holding times as compared with the distribution of negative cultures. The distribution of positive and negative cultures with respect to holding time was statistically analyzed by the chi-square test of independence. This analysis showed that the distribution of positive and negative cultures was statistically identical; therefore, significant loss of virus viability was not a problem in identifying positive cultures, even when held for up to 12 days.

Fifty-five of the isolates were serotyped to determine whether HSV-1 as well as HSV-2 remained viable in the Virocult transport tubes. The results indicated that persistence of virus viability was unrelated to the viral serotype. Twenty percent of the isolates that were serotyped were HSV-1 and 80% were HSV-2.

Tryptic soy broth and brain heart infusion broth successfully maintained HSV for 1 to 3 days at 4°C in a study reported by Yeager et al. (6), whereas freezing the specimens resulted in a loss of titer of 10^2 or more 50% tissue culture infective doses. The results of the present study suggest that HSV can be successfully isolated after holding times of up to 12 days when the virus specimen is held in a

TABLE 2. Recovery of HSV isolates from clinical specimens held on Virocult transport swabs for various times

Days specimen on Virocult	No. (% of total)	
	Positive	Negative
Same day[a]	30 (6.7)	129 (8.3)
1	73 (16.3)	236 (15.2)
2	167 (37.3)	512 (33.0)
3	66 (14.7)	259 (16.7)
4	68 (15.2)	237 (15.3)
5	29 (6.5)	110 (7.1)
6	9 (2.0)	41 (2.6)
7	4 (0.9)	16 (1.0)
8	1 (0.2)	6 (0.4)
9	0 (0)	5 (0.3)
10	0 (0)	1 (0.1)
11	0 (0)	0 (0)
12	1 (0.2)	0 (0)

[a] Cell culture inoculation was done on the same day that the specimen was collected.

Virocult transport tube. Such conditions would be preferable to even one freeze-thaw cycle. Normally, the virological results of the culture of clinical specimens would be desired much sooner than would be available with such long holding times; thus, these results show that normal shipping times of 2 to 3 days from outlying areas are well within the useful survival times of the virus.

The effects of cell debris and other materials in the clinical specimen on the survival of the virus were not directly studied. However, our data on isolation of virus from clinical specimens (Table 2) suggest that nothing in the specimen adversely affected virus viability during transport in the Virocult tube.

The most definitive and sensitive diagnostic laboratory test for HSV infections is viral isolation in cell culture (1, 3, 4). The Virocult transport tube is compact, enclosed, and resistant to breakage or damage during shipping. Our results indicate that the viral specimens can be shipped in Virocult tubes at ambient temperatures and that HSV infections can be routinely diagnosed by culture in central virological laboratories.

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