

## Suitability of New Chlamydia Transport Medium for Transport of Herpes Simplex Virus

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Received 6 May 1986/Accepted 16 July 1986

A new chlamydia transport medium (ChlamydiaPort; Scott Laboratories, Inc., Fiskeville, R.I.) was evaluated for its suitability as a transport medium for herpes simplex virus (HSV). Two laboratory HSV strains (McIntyre and 333) and two clinical isolates (AO218 and AO301) were suspended in ChlamydiaPort, ViraPort (Scott Laboratories), and cell culture medium and maintained at 2 and 22°C. Samples were tested at various time intervals to determine surviving virus. The range of half-lives of the HSV strains held at 2°C in ChlamydiaPort medium was from 3.5 to 10 days, while virus stability was greater in ViraPort and less in cell culture medium. These HSV strains held at 22°C in ChlamydiaPort had half-lives from 1.5 to 6 days, which were significantly greater than the half-lives of the viruses held in either tissue culture medium or ViraPort. Clinical specimens were tested for virus by using the Selecticult-HSV (Scott Laboratories) system to determine the performance of the transport medium under field conditions. Clinical specimens maintained up to 5 days at ambient temperatures in ChlamydiaPort medium appeared suitable for diagnostic testing without detectable loss of positive specimens. In addition, there was a significant decrease in the average time required for diagnosis when compared with a standard transport system, Virocult (Microdiagnostics, Cleveland, Ohio). These results show that HSV infections can be successfully diagnosed in distant virology laboratories by shipping specimens in ChlamydiaPort transport medium at ambient temperatures.

It is important to obtain accurate diagnostic information concerning active herpes simplex virus (HSV) infections, especially on samples collected from pregnant women in the third trimester. With accurate testing, the trauma of cesarean section in both mother and child may be avoided (6). One hindrance to accurate diagnosis of HSV infections can be the loss of virus viability in the specimens during transit to the diagnostic laboratory. Therefore, optimal specimen transport is of major concern to many laboratories, especially reference laboratories where specimens often arrive 2 to 7 days after collection and sometimes at ambient temperature. For these laboratories the task of trying to standardize the way specimens are collected, handled, and transported to them from various clinics and hospitals in the region is almost impossible. As a result, a variety of transport media have been developed and tested (3, 4, 8, 9, 11-16) to minimize virus inactivation during transport. However, many of these media still require refrigeration to prevent significant loss of virus (2, 5). Thus, the problem of samples arriving at ambient temperatures remains an impediment to accurate diagnosis. In recent years, two new transport systems have been evaluated (9, 14) that appear to be effective in stabilizing viruses at ambient temperatures. The Transporter (Bartels Immunodiagnostics, Bellevue, Wash.) system is based on "bedside" inoculation directly into a cell culture which enhances viral titers of labile enveloped viruses 500-fold over those in conventional transport media (7). The Virocult system (Microdiagnostics, Cleveland, Ohio) is based upon shipment of the swab used for specimen collection in transport medium to the processing laboratory. In the latter system, viruses may be easily detected in specimens despite holding times of 2 to 3 days and even up to 11 days with some specimens (9). Recently, a chlamydial

transport medium, sucrose phosphate glutamate, was also shown to be effective in stabilizing HSV isolates (15). The reasoning behind its use was to simplify the types of transport media needed to transport specimens, such that the same medium could be used for chlamydial and most viral specimens.

In this paper we evaluate another chlamydial transport medium, ChlamydiaPort (Scott Laboratories, Inc., Fiskeville, R.I.), for the ability to stabilize HSV in specimens held at ambient temperatures during transit to the laboratory.

### MATERIALS AND METHODS

**Cell culture and media.** All virus isolations as well as propagations were performed in Vero cells (9). The cells were routinely grown in Dulbecco modified Eagle medium (Sigma Chemical Co., St. Louis, Mo.) supplemented with 0.11% sodium bicarbonate, 10% fetal bovine serum (Hyclone Laboratories, Logan, Utah), and 50 µg of gentamicin per ml.

**Viruses and clinical specimens.** HSV type 2 strain 333 and HSV type 1 strain McIntyre were propagated in Vero cells. Clinical isolates HSV type 1 strain AO218 and HSV type 2 strain AO301 (1) were also propagated and passaged in Vero cells. Clinical specimens were obtained primarily from urogenital sites and were inoculated directly into Vero cell cultures as soon as possible after receipt. The clinical specimens employed in this study were submitted during the fall, winter, spring, and early summer months by clinics, hospitals, and private physicians from the southwestern United States.

**Determination of surviving virus in ChlamydiaPort and ViraPort transport media.** Stocks of two HSV laboratory strains (McIntyre and 333) and two HSV clinical isolates (AO218 and AO301) were diluted 1:10 in ChlamydiaPort or

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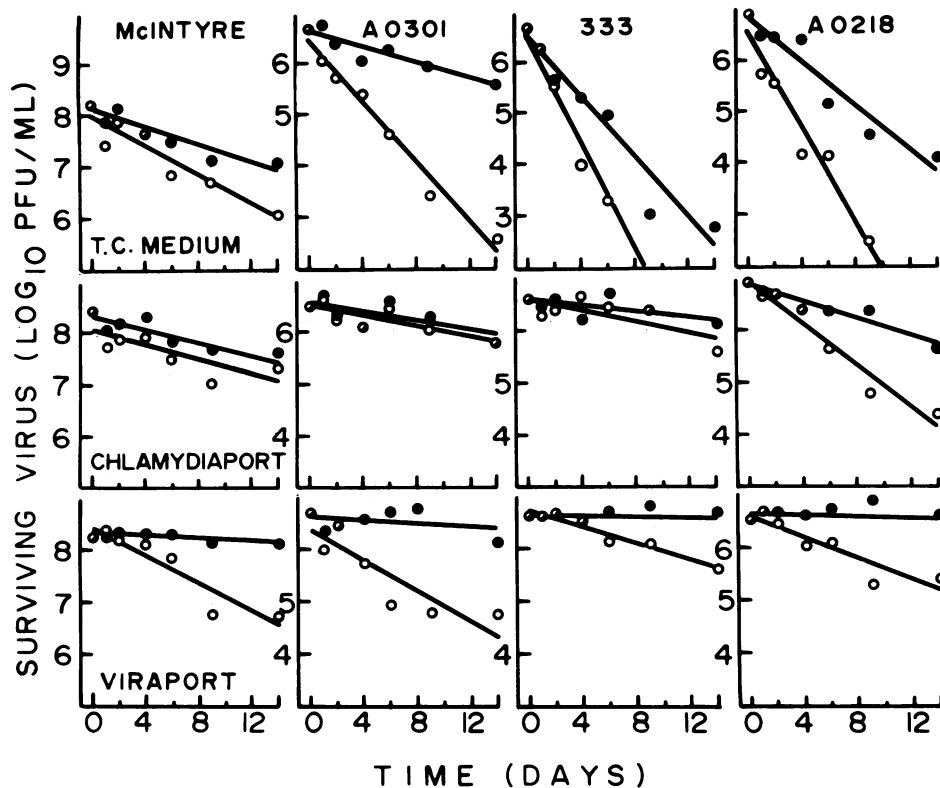


FIG. 1. Survival curves, as determined by standard plaque assay techniques, of four HSV strains held in tissue culture (T.C.) medium or two transport media. Virus strains were held in the appropriate medium at 22°C (○) or at 2°C (●). Virus infectivity was quantitated up to 14 days. McIntyre and AO301 are HSV type 1 strains, while 333 and AO218 are HSV type 2 strains.

ViraPort (Scott Laboratories) transport media and held at 22 or at 2°C. Samples were taken at various time intervals for titration of surviving virus by standard plaque assay procedures. Viral decay curves were determined by using regression analysis. Significant differences between curves were detected by analysis of covariance, as described by Zar (17). The Newman-Keuls multiple range test was used for comparing the half-lives of the virus strains in the three transport media.

**Comparison of the transport characteristics of ChlamydiaPort and the Virocult swab system for transport of HSV-infected specimens.** Clinical specimens were transported in either the Virocult swab system as previously described (9) or in ChlamydiaPort transport medium. The type of transport system used was dictated by the individual clinicians at the various hospitals, clinics, or offices of physicians submitting samples. For transport in ChlamydiaPort, samples were collected on swabs by vigorously rubbing the affected area. The swabs were then swirled several times in tubes containing ChlamydiaPort. The tubes were sealed after removal of the swabs and shipped. Upon arrival at the central processing laboratory (1 h to 7 days), the specimens were inoculated into cell culture. The monolayers were observed daily for cytopathic effects, and HSV infection was verified by using the Selecticult-HSV system (Scott Laboratories) (10). The comparison of the two transport systems was statistically analyzed by using a two-sample Student *t* test.

**Evaluation of virus stability in clinical specimens maintained in ChlamydiaPort during transit.** Clinical specimens transported in ChlamydiaPort were immediately inoculated into cell culture upon arrival, and the cell culture was

observed daily for cytopathic effect. Transit times varied from 0 to 7 days. HSV infection was verified as previously described (10). Comparison of the distribution of positive and negative specimens was performed by using the Kolmogorov-Smirnov two-sample test.

## RESULTS

To determine the efficacy of ChlamydiaPort in protecting HSV from inactivation, four different strains of HSV were held in the medium for various time periods either at 2 or at 22°C and assayed for surviving virus. ViraPort transport medium and Dulbecco modified Eagle tissue culture medium were also evaluated as comparative transport media. The various strains of virus tested in each medium showed great variability in stability when compared at the high and low temperatures (Fig. 1). There were significant differences in virus stability between 2 and 22°C ( $P < 0.05$ ) with few exceptions. In each type of medium tested, virus was usually much more stable at 2 than at 22°C (Fig. 1). Nevertheless, the McIntyre strain held in tissue culture medium and in ChlamydiaPort, strain 333 held in ChlamydiaPort, and AO301 held in ChlamydiaPort showed no significant differences in virus stability between 2 and 22°C ( $P > 0.05$ , two-sample Student *t* test). In fact, the regression lines for each of these comparisons were coincidental ( $P > 0.05$ , two-sample Student *t* test). When half-lives of the virus strains held in the three media at 2 or at 22°C were compared, it was clear that the virus stability is much better in the two transport media than in tissue culture medium (Table 1). With one exception (AO301), the virus held in ViraPort at 2°C indicated marked stability when compared with the

TABLE 1. Comparison of half-lives of four strains of herpes simplex virus in three different transport media

Transport medium	Half-life of virus strain (days)							
	McIntyre		AO301		333		AO218	
	2°C	22°C	2°C	22°C	2°C	22°C	2°C	22°C
Tissue culture medium <sup>a</sup>	3.6 <sup>b</sup>	2.1 <sup>c</sup>	4.0 <sup>d</sup>	1.0	1.0	0.6	1.4 <sup>e</sup>	0.7
ChlamydiaPort	5.6 <sup>b</sup>	4.3	7.0 <sup>d,f</sup>	6.1	10.0	5.2	3.6 <sup>e</sup>	1.5
ViraPort	25.0	2.3 <sup>c</sup>	19.0 <sup>f</sup>	2.1	44.0	3.7	49.0	3.0

<sup>a</sup> Dulbecco modified Eagle medium containing 0.11% sodium bicarbonate, 10% fetal bovine serum, 50 U of penicillin per ml, and 50 µg of streptomycin per ml.

<sup>b</sup> No significant difference. Regression lines were coincidental; Newman-Keuls multiple range test,  $P > 0.5$ .

<sup>c</sup> No significant difference. Newman-Keuls multiple range test,  $P > 0.5$ .

<sup>d</sup> No significant difference. Regression lines were coincidental. Newman-Keuls multiple range test,  $P > 0.1$ .

<sup>e</sup> No significant difference. Newman-Keuls multiple range test,  $P > 0.05$ .

<sup>f</sup> No significant difference. Newman-Keuls multiple range test,  $P > 0.2$ .

half-lives in tissue culture and ChlamydiaPort media. Statistical analysis of half-lives revealed that tissue culture medium was usually (three of four strains) almost as effective in protecting virus at 2°C as ChlamydiaPort was. The half-lives in ViraPort ranged from 19 to 49 days as compared with 3.5 to 10 days at 2°C in ChlamydiaPort, or 1 to 4 days in tissue culture medium. However, at 22°C, ChlamydiaPort protected AO301, 333, and McIntyre strains better than did ViraPort, and all four strains were significantly more stable in ChlamydiaPort than in tissue culture medium (Newman-Keuls multiple-range test, all  $P$  values  $< 0.05$ ). The half-lives were from 5 to 6 days in ChlamydiaPort and only from 2 to 3.75 days in ViraPort. Only strain AO218 was more stable in ViraPort at 22°C than in ChlamydiaPort.

It was also considered important to determine whether ChlamydiaPort would delay or enhance the detection of HSV-positive cultures when compared with another transport system, the Virocult system. The distributions of positive specimens detected were significantly different ( $P < 0.001$ ), as were the average days to diagnosis ( $P < 0.0004$ ), 1.8 days with ChlamydiaPort compared with 2.2 days with Virocult (Table 2).

Under clinical conditions we examined whether there would be significant loss of virus infectivity that might prevent identification of HSV-containing specimens held in ChlamydiaPort medium during normal transit times. As specimens arrived at the laboratory in ChlamydiaPort medium they were immediately inoculated onto cell cultures and eventually diagnosed as positive or negative. The spec-

imens were held in the transport medium for various times as a normal consequence of the collection and shipping process. The distributions of positive and negative values were very similar during the 7-day period examined (Table 3). Statistical analysis showed that the distributions were identical ( $P > 0.05$ ). Thus, there was no significant decrease in detection of positive cultures, especially at longer holding times in ChlamydiaPort transport medium.

## DISCUSSION

The results of this study show that the stability of two HSV type 1 strains and two HSV type 2 strains is greater in ViraPort at 2°C than in ChlamydiaPort and tissue culture medium at 2°C. However, many laboratories receive samples at ambient temperatures, thus resulting in possible decreases in virus titers (2). Therefore, the observation that the virus was more stable in ChlamydiaPort than in ViraPort at 22°C over a 14-day period of time is significant. For that reason, ChlamydiaPort may be the better transport medium under field conditions. In our experience, maximum shipping times vary up to 7 days with many specimens arriving 3 to 4 days after collection and at ambient temperature. Furthermore, the temperatures to which specimens are exposed during shipping likely exceed 22°C. Thus, a medium that can maintain virus infectivity for up to 5 to 7 days and at ambient temperatures would appear to be the transport medium of choice. Clearly, cell culture medium is an unsuitable transport medium.

Another important parameter of a transport medium is whether the time to diagnosis will be lengthened or shortened when the specimens are held in the transport medium.

TABLE 2. Day of onset of HSV cytopathic effect in cultures of clinical specimens held in either ChlamydiaPort or Virocult transport swabs

Day postinoculation	No. (%) of HSV-positive cultures	
	ChlamydiaPort <sup>a,b</sup>	Virocult <sup>a,c</sup>
1	39 (45)	82 (30)
2	34 (39)	107 (39)
3	9 (10)	45 (17)
4	3 (3)	23 (8)
5	2 (2)	10 (4)
6	0 (0)	5 (2)

<sup>a</sup> Distributions are different. Kolmogorov-Smirnov two-sample test,  $P < 0.001$ .

<sup>b</sup> Total number of positive specimens, 87; total number of specimens, 312 (27.9% positive). Average number of days to diagnosis of positive specimens was 1.8 ( $P < 0.0004$ , two-sample Student  $t$  test).

<sup>c</sup> Total number of positive specimens, 272; total number of specimens, 873 (31.2% positive). Average number of days to diagnosis of positive specimens was 2.2 ( $P < 0.0004$ , two-sample Student  $t$  test).

TABLE 3. Recovery of HSV isolates from clinical specimens held in ChlamydiaPort for various times

No. of days in ChlamydiaPort	No. (% of total)	
	Positive <sup>a</sup>	Negative <sup>a</sup>
Same day <sup>b</sup>	14 (19)	29 (15)
1	28 (38)	64 (33)
2	11 (15)	33 (17)
3	9 (12)	24 (13)
4	7 (10)	23 (12)
5	4 (5)	15 (8)
6	0 (0)	3 (2)
7	0 (0)	1 (0.5)

<sup>a</sup> Distributions were identical. Kolmogorov-Smirnov two-sample test,  $P > 0.05$ .

<sup>b</sup> Cell culture inoculation was done on the same day that the specimen was collected.

Determining the average days to diagnosis is a measure of this parameter. For specimens held in ChlamydiaPort the average time to a positive result was significantly reduced ( $P < 0.0004$ ) when compared with Virocult, a transport system specifically designed for the transport of virus-infected specimens. In addition, comparison of the frequencies of positive and negative cultures at various holding times, dictated by the transit times to the central processing laboratory, revealed that there was no significant loss of infectious virus in the specimens ( $P > 0.05$ ) during the holding times (up to 5 days). If the virus strains had lost infectivity at a significant rate when in ChlamydiaPort, there should have been significantly fewer positive cultures at longer holding times when compared with the distribution of negative cultures. In a previous report (9) strain 333 had half-lives on Virocult swabs of 3.5 and 2.75 days at 2 and 22°C, respectively. This compares with 10 and 5.2 days in ChlamydiaPort. Thus, ChlamydiaPort appears to have the advantage over Virocult regarding both virus stability and shortened time to a positive result.

This study shows that HSV-containing specimens can be successfully held in ChlamydiaPort transport medium for at least 5 days at ambient temperature without significant loss of positive virus samples.

#### ACKNOWLEDGMENTS

We thank Sandy Spradling for technical assistance.

This study was supported in part by the John W. Adkins Memorial Virus Research Fund.

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