

Transport Media for Herpes Simplex Virus Types 1 and 2

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An evaluation was made of the recovery rate of herpes simplex virus (HSV) type 1 or 2 from 197 clinical specimens obtained in two or three charcoal transport media: Leibovitz viral transport medium, a modified Leibovitz-Emory medium (LEM), in which agarose was used instead of agar, and Amies bacterial transport medium. The specimens were stored and shipped for 1 to 19 days in these media at ambient temperature or in Hanks buffered-salt solution in dry ice. The results indicate that the LEM was most effective, particularly in the recovery of HSV type 2 from clinical specimens held at ambient temperature. In vitro and in vivo studies in genitally infected mice corroborated the observations obtained with human clinical specimens. The availability of transport media which can be used for shipment at ambient temperature offers clinicians easier accessibility to laboratory confirmation and antigenic typing of HSV from suspect herpetic infections.

The expanding awareness of the wide clinical spectrum of disease caused by herpes simplex virus (HSV types 1 and 2) has increased the need for laboratory confirmation and antigenic typing of the virus (4). Since recovery of HSV is best accomplished when clinical specimens are stored and shipped at -70°C (7), shipment under these conditions is often too cumbersome for physicians to attempt, even though diagnostic virology services are available. An approach to this problem was suggested by Leibovitz, who developed a charcoal viral transport medium (CVTM) which permitted the survival of several viral agents for at least 3 weeks under in vitro testing conditions (1).

An evaluation was therefore begun in August 1969 to determine the efficacy of recovering HSV type 1 or 2 from clinical specimens shipped at ambient temperature in two transport media: Leibovitz CVTM and Amies bacterial transport medium. Preliminary observations suggested that the recovery of HSV type 2 from clinical specimens in these transport media was not as effective as from duplicate specimens submitted in Hanks buffered-salt solution (BSS) at -70°C . Since it is known (8) that the acid mucopolysaccharides found in agar inhibit HSV, a modified Leibovitz CVTM (Leibovitz-Emory medium, LEM) was prepared, in which agarose was substituted for agar. A comparison between these two transport media was also made as to the recovery rate of

HSV type 1 or 2 in genitally infected mice and under in vitro conditions.

MATERIALS AND METHODS

Transport media. Leibovitz CVTM contains 4 g of sodium chloride, 0.20 g of potassium chloride, 1.70 g of dipotassium phosphate, 10.00 g of charcoal (bacteriological; Colab, Chicago, Ill.), and 4 g of agar. The salts are dissolved in 500 ml of distilled water after which the charcoal is added. The agar is dissolved separately in 500 ml of distilled water by boiling, and the two solutions are combined and autoclaved at 121°C (15 psi) for 30 min. The mixture is transferred to a bottle containing a magnetic bar, placed on a magnetic stirrer, and allowed to cool to about 40°C before dispensing into screw-capped tubes (6 by 125 mm).

LEM was prepared similarly to the Leibovitz medium, except that agarose (Fisher Co.) was substituted for the agar.

Amies medium (Difco) contains 3 g of sodium chloride, 0.20 g of potassium chloride, 0.10 g of calcium chloride, 0.10 g of magnesium chloride, 0.20 g of normal potassium phosphate, 1.15 g of disodium phosphate, 1.00 g of sodium thioglycolate, 10.00 g of charcoal, and 4.00 g of agar (Difco).

In vitro studies. Cotton swabs were saturated in suspensions of HSV type 1 (VR₃ strain) and HSV type 2 (MS strain) of known high- and low-titered viruses. The swabs were placed in Leibovitz CVTM or LEM and kept at ambient temperature (20 to 28°C). On the same day and 1 to 8 days thereafter, the swabs were removed and placed in 1.8 ml of Hanks BSS. The solutions were then titered in triplicate in primary rabbit kidney tissue culture (PRK).

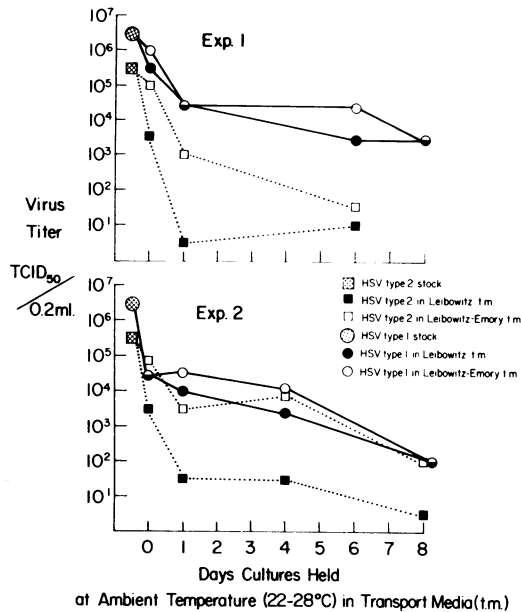


FIG. 1. Recoverability of herpes simplex virus types 1 and 2 in Leibovitz and Leibovitz-Emory transport media held at ambient temperature (22 to 28 C).

In vivo studies. In studies with mice, random-bred Swiss female mice (6 to 12 weeks old) were inoculated intravaginally with cotton pellets soaked in HSV type 1 or 2, as previously reported (5); the pellets were removed the next day. An alternating procedure was adopted so as to preclude the need for taking two swabs from one mouse on the same day, which might bias virus recovery in favor of the first specimen. The vagina was swabbed on day 3, and the swab was placed transiently in Hanks BSS before inoculation directly onto primary rabbit kidney (PRK) tissue culture. On the 4th day, a swab from the same mouse was placed in Leibovitz CVTM or LEM, and on the following day a swab was placed in the alternate medium from the one used on the 4th day. On the 6th day, the swabs were obtained again in Hanks BSS, and, on the 7th day, alternate mice had swabs placed in either Leibovitz or LEM medium. All swabs were held for 5 days in the two CVTM at ambient temperature and processed in PRK tissue culture, as noted above.

In human clinical studies, over the period of August 1969 to June 1971, 197 specimens were tested from a variety of sites, e.g., face, genitals, or eyes, in patients with suspect HSV infections. Almost all of the specimens were obtained from patients seen in a private dermatological clinic in San Antonio, Tex. The samples were collected with cotton swabs from suspect herpetic vesicular or ulcerative lesions in random combinations, utilizing for every patient two to three of the following media: Leibovitz CVTM, LEM, and Amies bacterial transport medium (Difco). A swab was almost always also obtained in Hanks BSS and stored in dry ice or at -70°C before processing. Most

of the clinical specimens were maintained at ambient temperature for 1 or more days until they were sent by air mail to the virology laboratory in Atlanta, Ga. The swabs from the transport media or the specimen in Hanks BSS were inoculated in triplicate in PRK tissue culture tubes. The interval from time of sampling to time of culture ranged from 1 to 19 days. There were 98 specimens received in transport media which were placed in tissue culture within 3 days of collection, 21 specimens cultured between 4 and 6 days, and 10 specimens cultured from 7 to 19 days. The Hanks specimens were inoculated on the day received and had been stored at -70°C for 2 days to 2 months before shipment.

PRK monolayers were observed daily for cytopathic effect, and positive cultures were confirmed and typed by a fluorescent-antibody technique described earlier (2).

RESULTS

In vitro studies. Figure 1 presents two sets of experiments, in which HSV type 1 or type 2 with relatively high titers ($10^{5.5}$ to $10^{6.5}$ $\text{TCID}_{50}/0.2$ ml) was collected with a cotton swab and placed in either Leibovitz CVTM or LEM. Titration of virus recovered from swabs placed in either transport medium, and removed within 2 hr on the same day, revealed an apparent inactivation of both viruses, particularly pronounced with HSV type 2 in the Leibovitz medium. HSV type 1 was recoverable equally well in Leibovitz CVTM or LEM for as long as 8 days. A more pronounced inactivation of HSV type 2 in Leibovitz CVTM as compared to the LEM was again observed.

When either type 1 or type 2 virus at titers below 10^4 TCID_{50} was used to inoculate either the Leibovitz CVTM or LEM, little virus was recoverable on the same day, and no virus could be demonstrated after the 1st day of incubation at ambient temperature.

In vivo studies. Table 1 presents results obtained in mice which had been inoculated intravaginally with either HSV type 1 or type 2 strains. A mouse was defined as having an established infection if

TABLE 1. Recovery of HSV type 1 or 2 from mouse vaginal cultures obtained with swabs placed in Leibovitz or Leibovitz-Emory GVTM and maintained at ambient temperature for 5 days

Mice inoculated with	Leibovitz CVTM	Leibovitz-Emory CVTM
HSV type 1	18/26 ^a (70) ^b	24/28 (86)
HSV type 2	13/17 (76)	16/18 (89)
Total	31/43 (72)	40/46 (87)

^a No. of mice with positive viral recovery in CVTM/no. of mice with positive viral recovery in Hanks BSS.

^b Values in parentheses represent percentages.

TABLE 2. Recovery rates of HSV type 1 or 2 from human clinical specimens placed in Leibovitz CVTM, Leibovitz-Emory CVTM, Amies bacterial transport medium, or Hanks BSS

Transport medium	Type 1 HSV		Type 2 HSV		Total	
	No. of cases positive/total	Per cent	No. of cases positive/total	Per cent	No. of cases positive/total	Per cent
Hanks BSS ^a	27/30	90	12/14	86	39/44	89
Amies ^b	19/24	79	5/11	45	24/35	69
Leibovitz ^b	20/22	91	6/10	60	26/32	81
Leibovitz-Emory ^b	17/17	100	13/15	87	30/32	94

^a Held and shipped at -70°C .

^b Held and shipped at ambient temperature.

the culture obtained in Hanks BSS was positive for HSV on the 3rd day. Of 43 mice with HSV recovered in Hanks BSS, 31 swabs collected and held in Leibovitz CVTM were positive (72%). Of 46 mice with HSV recoverable in Hanks BSS, 40 (87%) were positive when the swabs had been held in the LEM. The slightly improved recovery rate with the LEM was noted for both HSV type 1 and HSV type 2.

The human clinical specimens were obtained in two or three different media in alternate fashion, so that swabs would have been placed in one or the other medium without preference. Forty-three specimens in which no virus was recovered in any of the transport media were excluded from this evaluation, because presence of HSV could not be confirmed. Table 2 presents the recovery rate of HSV type 1 or 2 in the various transport media in those instances in which at least one of two or three of the transport media yielded virus. The most striking difference between the media was the apparent low recovery rates of type 2 HSV in Amies medium (45%) and Leibovitz CVTM (60%), as compared with the LEM (87%). The differences in rates of recovery of type 1 HSV strains were not as striking, although here again the LEM appears to be somewhat better than the other transport media.

DISCUSSION

The results obtained in this study indicate that it is possible for the clinician to obtain clinical specimens from suspected cases of HSV type 1 or 2 infections for ambient temperature shipment to appropriate virology diagnostic laboratories. This should permit more ready confirmation of suspect herpetic lesions which might not be detected by cytological techniques (5). Cytological techniques do not permit a differentiation of HSV from varicella-zoster virus or HSV type 1 from HSV type 2. Only a few specialized laboratories can identify and type HSV from clinical specimens directly by immunofluorescence techniques (3).

Our results suggest that, of the three transport media which could be used for shipment (Amies bacterial transport media, Leibovitz CVTM, and LEM), the LEM is the most effective. The *in vitro* studies and the *in vivo* experimental mouse studies offer support for our findings with human clinical material. Agarose was used in the LEM to replace the agar in the Leibovitz medium because agar, which contains acid mucopolysaccharides, is known to inactivate herpes simplex viruses (8). Preliminary experiments that we have recently carried out indicate that HSV type 2 is more susceptible to the effect of acid mucopolysaccharides than HSV type 1.

The *in vitro* studies suggest that specimens which contain low titers of HSV, regardless of antigenic type, might not be able to survive in the transport medium. However, it is possible that even low-titered virus present in a plasma clot or fibrin may survive in the CVTM. Kibrick (*unpublished data*) has shown that herpes simplex virus (presumably type 1), present in skin crusts of patients with eczema herpeticum, is capable of surviving at room temperature for several weeks.

Our earlier results with the use of transport media for adenoviruses (1) and the present findings suggest that diagnostic virology laboratories will be of greater aid to the clinician if the Leibovitz or LEM media, or possibly others, are evaluated for other viruses. Of immediate potential value might be the evaluation of our media for the shipment of other herpesviruses of importance in human and veterinary medicine (6).

ADDENDUM IN PROOF

Rodin et al. have recently reported on the use of Stuart's media for the transport of HSV at ambient temperature (*Brit. J. Vener. Dis.* **47**:198-199, 1971).

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