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Transport of Viral Specimens

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INTRODUCTION

Viral agents vary widely in composition, structure, morphology, size, and stability. For those viruses that infect humans, the loosely packaged enveloped viruses are generally more labile than the nonenveloped dense viruses. However, some enveloped viruses, such as herpes simplex viruses (HSV), are surprisingly stable in the nonfrozen state when placed in a suitable holding environment. It is commonly recommended that clinical specimens for virus isolation either be inoculated into cell culture at bedside or be immediately placed in a refrigerator or on wet ice and then inoculated into cell culture with as little delay as possible. However, in reality many common viral agents withstand both storage at room temperature and transport for extended periods of time (days) when placed in a suitable transport medium.

Perhaps better and more complete diagnostic analysis of some infectious diseases would be accomplished if it were more widely recognized that viral specimens can be collected and transported to the laboratory for successful testing. The infectivity of viruses does, in fact, decrease over time and generally the decay rate is a function of temperature, so that stability is enhanced by cooling (30). Therefore, viral specimens generally should not be stored at room temperature or incubator temperature when a refrigerator is available. The probability of a successful isolation is increased when the time interval between collection and inoculation of the culture is not prolonged and when the specimen contains as many virus particles as possible. To maximize the amount of virus in the specimen, it should be collected early in the patient's illness. If a suitable transport medium is used, successful and reliable isolations can be accomplished when viral specimens are sent to either a local laboratory or a reference laboratory.

A transport medium is not always essential for virus isolation in the laboratory. In some situations, the virus can be transported in the specimen itself. Examples of this include urine, cerebral spinal fluid, blood, washings of the respiratory tract, and stool (16). A variety of viruses can be isolated from urine during phases of an infection when they are being shed from the urinary system. Similarly, viruses are found in cerebral spinal fluid, blood, and stool during certain phases of some infections. These kinds of specimens can be transported without placing them in an exogenous transport medium.

The purpose of this article is to review the transport media that have been reported in the literature and have been used successfully to carry infectious viruses from the patient to the laboratory for culture.

TRANSPORT SYSTEMS

The constituents of suitable viral transport media are designed to provide an isotonic solution containing protective protein, antibiotics to control microbial contamination, and one or more buffers to control the pH. Isotonicity, however, is not an absolute requirement; some highly successful transport media contain hypertonic solutions of sucrose. Liquid transport media are used primarily for transporting swabs or materials released into the medium from a collection swab. Liquid media may be added to other specimens when inactivation of the viral agent is likely and when the resultant dilution is acceptable.

The specimen should be collected as soon as possible after the onset of symptoms, preferably within 3 to 7 days (42). The specimen contains the maximum amount of virus at the time of collection. To maintain viral infectivity, the specimen should be placed in a good transport medium.

Swabs

For transport of viral specimens, the swab may play one of two possible roles. First, it might be both the collection device and the vehicle of transport to the laboratory or to the cell culture or to both. Second, the swab may function solely as a collection device upon which the virus remains only as long as it takes to deposit the swab in a tube of transport medium. In this case, another parameter that must be considered is whether the swab is to remain in or be removed from the transport medium after dispersal of the specimen. The materials in the swab or the swab shaft may be toxic to the virus in the specimen.

The toxicity of calcium alginate for HSV was reported by Crane and associates in 1980 (18). Swab materials shredded from rayon, cotton, Dacron, polyester, and calcium alginate swabs were mixed with virus (HSV type 2 [HSV-2]) in suspension. The suspensions were agitated with a magnetic stirrer. At intervals up to 72 h, samples were removed for virus titration. Over this period of time the virus titers from the samples exposed to rayon, cotton, Dacron, and polyester dropped a little more than 10-fold, an amount equal to the virus control, which was exposed to no swab material. In contrast, the virus exposed to calcium alginate dropped more than 1,000-fold in 48 h. In follow-up experiments, these authors showed that calcium alginate binds directly to the HSV virion, rendering it noninfectious. Thus, for the collection of herpesviruses, calcium alginate swabs should be avoided.

Another study examining the effect of swab materials on the recovery of HSV from clinical specimens (9) compared cotton swabs with calcium alginate swabs. There was no difference in the recovery of virus from cotton or calcium alginate swabs when the samples were inoculated into cell culture immediately following collection. However, when the swabs were allowed to remain in contact with the specimen, the recovery of virus from cotton swabs was significantly greater than that from the calcium alginate swabs. Further, recovery of virus was higher when the specimens were stored at refrigerator temperature rather than frozen and then thawed. It might be advisable, as a routine measure, to avoid the use of calcium alginate swabs entirely for the collection of virus specimens. Certainly, calcium alginate swabs should not be in contact with the specimen during transport.

Swab-Tube Combinations

The Culturette (formerly Marion Laboratories, Kansas City, Mo.; currently BBL Microbiology Systems, Cockeysville, Md.) consists of a plastic tube containing a sterile rayon-tipped applicator and an ampoule of modified Stuart transport medium (43, 60). The Culturette was formulated to serve as a single swab for the isolation of bacteria as well as viruses. Extensive experience with the Culturette (25, 27, 34, 60, 61) has shown isolation rates of up to 50% in tests for the usual viruses obtained in clinical laboratories. Specimens are transported in a nonfrozen state to avoid exposing viruses to harmful freeze-thaw cycles. Before inoculation, materials on the swab can be extracted in another medium such as serum-free cell culture medium (27) or Hanks balanced salt solution (BSS) with 0.5% fetal bovine serum with antibiotics (25). This is accomplished by vigorously swirling the swab in 2 ml of medium.

A study comparing Stuart, Hanks BSS, and Leibovitz-Emory media (LEM) in Culturettes was reported by Huntoon et al. and Smith (34, 61). The double-blind prospective study was carried out to compare the rates of virus recovery from the upper respiratory tracts of children, using the three different transport media. The media were packaged in the Culturette ampoules and labeled in code by the manufacturer. Each specimen was collected on the three different swabs, and one each was inserted into the three different Culturette systems, which were transported to the laboratory at room temperature in 30 min to 21 h for inoculation into cell cultures. Specimens were collected during all four seasons of the year. Eighty isolates were obtained from 200 specimens for a positive rate of 40%. Viruses recovered were parainfluenza virus, enterovirus, adenovirus, HSV, influenza virus types A and B, respiratory syncytial virus (RSV), varicella-zoster virus (VZV), and rhinovirus. Of the isolates, 90% were recovered in Hanks BSS, 80% were recovered in Stuart, and 79% were recovered in Leibovitz-Emory media, but the differences were not statistically significant.

A four-site evaluation of a laboratory test kit for rapid diagnosis of HSV infections was reported by Smith (62). All four laboratories processed specimens sent to their specific laboratories with the request for HSV culture. One laboratory used the Culturette for transport, one used Hanks BSS containing 0.5% gelatin, one used Eagle minimal essential medium (MEM) with 2% bovine serum, and the fourth used veal infusion broth with 0.5% gelatin. The transport times were approximately 24 h or less. The recoveries of HSV from the specimens were essentially comparable. Direct comparison could not be made because the same specimen was not tested in all transport media. Yet, the Culturette had the highest recovery rate, suggesting that the inclusion of protein in the other three media was not a factor that dramatically increased the isolation rate in specimens cultured for HSV.

The Virocult, a transport system devised for the collection of virological specimens (35, 43, 53) (Medical Wire and Equipment Co., Cleveland, Ohio), consists of a sterile pack containing a collection swab and a plastic tube. The tube contains a small sponge saturated with a phosphate-buffered balanced salt solution at pH 7.2, glucose (1.0 g/liter), lactalbumin hydrolysate (3.0 g/liter), chloramphenicol (2.5 μg/ml), and cycloheximide (10 μg/ml). The cycloheximide is added as an antifungal agent and appears not to inhibit significantly cell cultures or virus replication at this concentration when carried over in the inoculum. At ambient temperature, the Virocult has a 2-year shelf life.

Perez et al. (53) compared viral isolation rates from specimens transported in the Virocult system with those from specimens transported in tryptose phosphate broth and in no transport medium. A total of 1,192 specimens were collected with the Virocult system, 281 were collected in broth, and 288 were collected in no transport medium. The viral isolation rates were 20.8, 18.9, and 5.9%, respectively. Herpesviruses were most commonly isolated, followed by enteroviruses and adenoviruses. Microbial contamination rates, determined by the number of contaminated cell cultures, were also noted. Virocult had the lowest contamination rate (2.8%), followed by broth (4.3%) and no transport medium (8.3%). Although this study did not directly compare the transport systems on a specimen-by-specimen basis, it demonstrated that favorable isolation rates and contamination rates could be achieved with the Virocult system.

An evaluation of the Virocult tube was also reported by Johnson and associates (35). In their study, survival times at 2 and 22°C of a laboratory strain of HSV were examined.

Also, 2,000 consecutive clinical specimens that were collected with the Virocult system were cultured for HSV. The HSV-2 laboratory strain had a half-life of 3.5 days in Virocult at 2°C and 2.75 days at 22°C, indicating a good level of stability in the Virocult tube. Of the 2,000 clinical specimens tested, 448 (22.4%) were positive. The clinical specimens were refrigerated until they were shipped at ambient temperatures and then were refrigerated again in the laboratory while awaiting inoculation into culture. A statistical comparison of the frequencies of positive and negative cultures at various holding times up to 12 days showed that the virus remained sufficiently active during these holding times to reveal the virus-positive specimens. The analysis showed that the distributions of positive and negative cultures were statistically identical over time; that is, a statistically insignificant number of positive specimens became culture negative. Thus, loss of virus activity did not prevent virus recovery in culture from positive specimens within this time frame. Serotyping of the isolates revealed that both HSV-1 and HSV-2 remained active in Virocult tubes. Overall, it was apparent that HSV infections can be routinely diagnosed by culture when the specimens are shipped in Virocult tubes to a central laboratory, even when transport requires several days.

In a subsequent study (46), Virocult tubes were used successfully to transport specimens for the diagnosis of HSV infections to a state department of health laboratory. Of 16,779 specimens received for HSV isolation, 26.6% were positive. Thus, several reports have documented the effectiveness of Culturette and Virocult for the transport of viral specimens.

Liquid Media

Liquid media represent valuable systems to transport viruses in active form to the laboratory for isolation, especially when collection of the specimen on a swab is appropriate. Dermal, nasopharyngeal, cervical, and urethral specimens fall into this category. Relatively high titers of virus particles are collected in these specimens. The material from the swab is dispersed into the liquid medium. Some laboratories recommend removal and disposal of the swab, while others suggest transporting the swab along with the medium. Typically, liquid media are composed of buffers to control pH, protein to stabilize the virus, and often other substances to control osmolality or onto which the viruses can adsorb. Proteins, such as bovine serum albumin (21), gelatin, skimmed milk, normal serum (65), or complex broth bases, are used as protective substances. For the composition of various transport media, see Table 1.

Cell culture medium. Specimens containing HSV held in either a cell culture medium containing 10% fetal bovine serum or a buffered sucrose-based medium have been tested for survival of the virus (7). The cell culture medium was much less effective in maintaining virus activity. The half-life of virus infectivity was in the range of 0.6 to 4 days depending on the virus strain and holding temperature, compared with a half-life of 1.5 to 10 days in sucrose media. Cell culture medium is acceptable in situations in which the specimen will be held for a relatively short time (1 or 2 days) before testing. However, when the specimen must be transported over long distances, or at ambient temperature, and held for several days before testing, transport systems better than cell culture media are available. Furthermore, in relation to cell culture media, one of the early transport media, Stuart medium, was compared with Eagle MEM containing 10% fetal bovine serum and was found to be even less effective than cell culture medium for the transport of HSV (56).

Cell culture medium is effective when transport is in-house and virus is contained in the medium for only a limited period (55) or when the specimen is transported in-house directly on the swab and then the material is released from the swab into cell culture medium in the laboratory (54). In these cases, because virus is contained in the medium for only a short time before cell culture inoculation, survival of the virus can be expected.

BSS and charcoal. Stabilization of acid-base conditions at or near physiological pH is considered desirable for the preservation of virus activity in clinical specimens. Traditionally, phosphate-buffered saline or Hanks BSS (31) has been used as a buffer in a wide variety of transport media (Table 1). These solutions are considered to approximate the osmotic pressure required by many viruses for optimal stability. Some workers have included organic buffers such as *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (15) in their media to provide additional buffering capacity. This may be particularly useful for collection of specimens with high acidity, such as vaginal swabs. Other additives such as the proteins bovine serum albumin, bovine serum, gelatin, skimmed milk, and lactalbumin are thought to promote viral stability. Although agar was included in some of the early transport media (4, 15, 41, 50, 64), agarose was substituted when the acid mucopolysaccharides in agar were discovered to be inhibitory to HSV (50). The stabilizing effect of charcoal was originally demonstrated with bacteria, and the inclusion of charcoal in early viral transport media was a holdover from these bacteriological studies. It was hoped that a single medium could be used for the transport of many, if not all, bacterial and viral agents (4, 15, 41, 50). The Leibovitz agar-charcoal medium, referred to as charcoal viral transport medium (CVTM), was devised in an effort to formulate a single all-purpose medium (41). It was useful for several viruses, including adenovirus, but herpesviruses and influenza virus were not well preserved.

In the decade prior to 1950, several investigators found sterile (autoclaved) skimmed milk to be an ideal medium in which to store or lyophilize certain rickettsiae. Skimmed milk as a viral holding medium was reported in 1952 by Allen et al. (2). In that study, HSV suspended in skimmed milk lost no detectable activity when stored for as long as 4 months at either −70 or −20°C. However, viral titers decreased by about 2 orders of magnitude when the virus was stored in skimmed milk at 4°C for 4 months.

In a study designed to compare an enzyme-linked immunosorbent assay technique with virus isolation for the diagnosis of HSV, Alexander and colleagues (1) used a transport medium composed of 50% skimmed milk and 50% phosphate-buffered saline for the specimens that were cultured. Virus isolation was successful for specimens held at 4°C for 3 h to 1 day. Longer holding times were not tested.

In two studies reported in 1971, three charcoal-containing transport media (50) and Stuart transport medium (56) were tested. Modified Leibovitz-Emory medium, in which agarose was used instead of agar, was compared with CVTM and Amies bacterial transport medium. Leibovitz-Emory medium was most effective, particularly for recovery of HSV-2 from clinical specimens held at ambient temperature. It is similar to CVTM except that it contains agarose instead of agar. Of the three charcoal media, Amies was the least effective for transporting HSV.

When Stuart transport medium was compared with both

TABLE 1. Composition of viral transport media

Medium	Components	Reference(s)
Swab-tube combination		
Culturette	Swab-tube combination containing modified Stuart medium, antibiotics	MDS[a]
Virocult	Collection swab, plastic transtube, small sponge saturated with phosphate buffer, D-glucose, lactalbumin hydrolysate, chloramphenicol, and cycloheximide	MDS
Cell culture medium		
MEM	MEM with amino acids, fetal bovine serum, buffers, antibiotics	
BSS, charcoal, protein additives		
Buffered BSA[b]	Hanks BSS, 1% BSA, sodium bicarbonate, phenol red, antibiotics	45
Buffered gelatin	Hanks BSS, 0.5% gelatin	42, 45
HH	Hanks BSS, BSA, HEPES and bicarbonate buffers, antibiotics	15
CVTM	Phosphate-buffered saline, potassium chloride, charcoal, ionagar (or agar)	15, 41, 50
Modified Leibovitz-Emory	Similar to CVTM except agarose used in place of agar	50
Modified Stuart (Amies transport medium)	Phosphate-buffered saline, agar, sodium thioglycolate, charcoal, magnesium chloride, calcium chloride	4, 41, 50
Skimmed milk	50% phosphate-buffered saline, 50% skimmed milk	1
Skimmed milk	Phosphate-buffered saline, 10% skimmed milk, sodium bicarbonate, phenol red antibiotics	45
Stuart medium	Agar, thioglycolic acid, sodium glycerophosphate, calcium chloride, methylene blue	64
YLE20CS	Yeast extract, lactalbumin, Earle BSS, 20% calf serum	65
Broth-based media		
Nutrient broth	Beef extract, peptone	8
Tryptic soy broth	Tryptone, soytone, dextrose, sodium chloride, dipotassium phosphate	6
Tryptose phosphate broth	Tryptose phosphate broth, 0.5% BSA or 0.5% gelatin, antibiotics, phenol red (optional)	42, 45
Veal infusion broth	Veal infusion, proteose peptone, sodium chloride	17
Bentonite-containing media		
Bentonite transport	HB597 tissue culture powder, Tris buffer, EDTA, bentonite, antibiotics, with or without rabbit serum-coated bentonite	10
Buffered sucrose-based solutions		
Carr-Scarborough Viral/chlamydial transport	Phosphate-buffered sucrose, L-15 medium, glutamic acid, bovine albumin, gentamicin	MDS
Leibovitz-SPG	Leibovitz #15, sucrose, phosphate buffer, BSA, antibiotics	32
Richards Viral Transport	Phosphate buffer, organic buffers, sucrose, amino acids, bovine serum, phenol red, antibiotics	MDS
2SP	0.2 M sucrose, phosphate buffer, fetal bovine serum, antibiotics	63
SPG	Sucrose, phosphate buffer, glutamate	11
Transporters		
Transporter	MEM, buffers, fetal bovine serum, antibiotics, human diploid fibroblasts	MDS

[a] MDS, Manufacturer's data sheet.
[b] BSA, Bovine serum albumin.

plain swabs and Eagle MEM containing 10% fetal bovine serum (56) for the transport of HSV, the plain swabs were found to be a less effective transport system than either tissue culture media or Stuart medium. Further, it was found that Stuart transport medium was less effective than tissue culture media. If, however, bacteria are to be transported in the same sample, Stuart medium is useful because it contains no antibiotics, unlike the cell culture medium.

In a study reported by Chaniot and co-workers (15), CVTM was compared with Hanks BSS containing albumin and HEPES buffer (HH medium) for the preservation of rhinovirus 2, influenza virus type B, and coxsackievirus A21. Rhinovirus was judged stable in HH medium at 4, −20 and −70°C, while in CVTM it was labile at −20°C. Influenza virus type B was stable only at −70°C in HH but not in CVTM. Coxsackievirus was unstable in either medium at 4°C but was stable in both media at −20 and −70°C. Culture of clinical material collected into both HH and CVTM suggested that HH medium was superior for rhinoviruses. In an interesting part of that study, nasal swabs were collected from 17 men in the Antarctic and transported back to the United Kingdom in HH medium for evaluation. The specimens were stored for 4 months at −28°C, then at −40°C for 4 months, and then at −70°C for 3 months. The value of HH medium was indicated by the results upon examination: 79 of 208 swabs yielded rhinovirus, showing that 15 of the 17 men at camp had been infected; 11 of the swabs contained $10^{3.5}$ 50% tissue culture infective doses of virus per ml.

A new generation of transport media without agar and charcoal has been developed. These media are preferable for specimens collected on swabs and are more compatible with inoculation into cell cultures since they are nontoxic and contain no particulate material to obscure the observation of cytopathic effects. For example, Hanks BSS containing 0.5% gelatin and antibiotics is recommended by some groups

(42, 45), especially for transporting clinical specimens containing HSV (51, 76, 77).

A medium composed of Earle BSS, 0.5% lactalbumin hydrolysate, 0.1% yeast extract, Eagle MEM, 20% inactivated normal calf serum, and sufficient $NaHCO_3$ to adjust the pH to 7.2 was referred to as YLE20CS (65). The medium also contained unspecified antibiotics. Laboratory strains of HSV-1 and -2 were held in the medium at 20, 4, −20, and −70°C. The estimated half-life of virus infectivity at 20°C was about 1 day, while at 4°C it was about 4 days. Frozen storage of virus in this medium revealed some decay at −20°C, but virus infectivity remained unchanged over a 6-week period at −70°C. In follow-up studies on clinical strains of HSV, these authors showed that YLE20CS can be used successfully as a transport medium for specimens collected for diagnostic purposes.

Broth-based media. Viral transport media composed primarily of bacteriological broth (nutrient, tryptic soy, tryptose phosphate, and veal infusion broths) with some array of additives, such as additional protein, salt, phenol red indicator, or antibiotics, have been used by many laboratories (6, 8, 42, 45, 57, 69). Recommendations for their use have been based somewhat on tradition.

The number of virus isolates obtained from specimens transported in tryptic soy broth as compared with CVTM was determined (57). Throat swab specimens were collected and placed in CVTM. They were maintained at refrigerator temperatures before shipment and then transported at ambient temperatures. The specimens transported in tryptic soy broth were collected as throat washings consisting of a 15-ml gargle. They were stored frozen and then shipped on dry ice. From 3 to 21 days elapsed from the time of collection to the time of processing for isolation. The viral isolates obtained in these specimens consisted of adenoviruses, coxsackievirus A21, influenza viruses, parainfluenza viruses, and polioviruses. Because the specimens held in the two transport media were collected in different ways (swabs and gargled media) and the holding temperatures were different, direct comparisons of the effectiveness of the two transport media could not be made. Nevertheless, the authors reported that approximately twice the number of virus isolates were detected in the broth transport as from the CVTM.

Broth has been used to transport HSV. Positive cultures were obtained when specimens on swabs were placed in tryptic soy broth and then held at 4°C before and during transport (6, 78). Nutrient broth (8), calf infusion broth (12, 39), brain heart infusion broth (78), and tryptose-phosphate broth with bovine serum albumin (79) were also used successfully. Veal infusion broth with 0.1% gelatin and antibiotics was used to transport enteroviruses collected as throat and rectal swabs (17), while RSV was collected in tryptose phosphate broth and transported in the frozen state (74). When tryptic soy and brain heart infusion broths were tested with HSV, viral infectivity was maintained within ±1 serial dilution of the original titer in broths held for 1 to 3 days at 4°C (78). The appearance of cytopathic effect was not delayed after holding periods as long as 5 days before inoculation. Because these broth-based media contained no antibiotics, about 1% of the samples became contaminated by other microbial agents.

In general, broth-based transport media are adequate for most applications. The survival of herpesviruses and other viruses appears to be about the same as in cell culture media. Other transport media, however, provide longer survival times (see Table 2).

Bentonite-containing media. Transport medium containing bentonite was developed because of certain limitations of charcoal-containing media (10). Amies medium, for example, preserves some viruses for up to 3 weeks, but HSV and rubella viruses lose their activity rapidly in this medium. Another limitation is that charcoal media are toxic for some types of cell cultures. The charcoal medium was designed mainly for swab specimens and was unsuitable for transporting such specimens as throat washings. In addition, the charcoal particles seriously interfere with immunofluorescent staining used for demonstrating influenza virus antigen in exfoliated respiratory cells. They also interfere with the detection of virus-specific antibodies in the pharyngeal secretory globulins.

Bentonite, a clay material, is known to act as a cation-exchange resin as well as a good adsorbing agent for electropositive and electronegative proteins. The essential ingredient in bentonite transport medium is bentonite itself, either uncoated or coated with serum proteins (10). Coxsackieviruses A9 and B5, Echovirus 11, adenovirus 5, influenza virus A2, parainfluenza viruses, rubella viruses, and HSV maintained their infectivity without loss of titer in bentonite transport medium for 3 to 21 days, depending on the virus type. For example, approximately 50% of the HSV infectivity remained after 7 days. The protective activity that bentonite has for HSV, influenza viruses, and rubella viruses could be enhanced by coating the bentonite particles with rabbit serum.

Although the mode of action of bentonite on virus particles has not been ascertained, electron micrographs suggest that, besides acting as an adsorbent, it has a monodispersing effect on virus aggregates (10). Even though bentonite-containing media offer some advantages, they have not been widely adopted for use in the clinical laboratory, probably due to difficulty in formulation, lack of commercial availability, and the presence of solid particles which complicates the identification of viral cytopathic effects in cell culture.

Buffered sorbitol and sucrose-based solutions. Sorbitol is a cryoprotectant and thus has been used both for the storage of viruses and as an additive in transport media (33). Among the more frequently isolated viruses are herpesviruses, and, of these, cytomegalovirus (CMV) and VZV are the most labile. In many laboratories these viruses are combined with sorbitol and stored at −70°C. Even so, CMV and VZV recovery after storage is inconsistent, and sorbitol may be toxic for some cell cultures. Sorbitol and 0.2 M sucrose-phosphate (SP) were evaluated for suitability as both storage and transport media (33). CMV and VZV recovery was uniformly better from 0.2 M SP than from 70% sorbitol after storage at 20, 4, and −70°C. Similar stabilizing effects on RSV and HSV-1 were observed when viral stocks were preserved in 0.2 M SP and compared with 70% sorbitol. Although sorbitol has been recommended for the transport and holding of CMV and other enveloped viruses, titers may decrease rapidly. Indeed, CMV and VZV infectivity was lost when these viruses were stored in sorbitol, even for short periods of time (33). Virus recovery was significantly better from 0.2 M SP.

Lennette and Lennette (43) noted that sorbitol has been used primarily for frozen transport of urine samples to be tested for CMV. Even though sorbitol may have some limited use for freezing labile viruses, accumulating evidence shows that CMV is usually recoverable from urine specimens refrigerated for up to 1 week or longer. Thus, there is no need for the routine freezing of urine specimens or the use of added sorbitol.

In 1950, Bovarnick and associates (11) found that the

survival of rickettsiae is favored by the presence of glutamate, a pH near 7.0, a basal salt solution high in potassium ions rather then sodium ions, and the presence of 0.1 to 1.0% bovine or human serum albumin. Thus, the original formulation of sucrose-phosphate-glutamate medium (SPG) contained 0.218 M sucrose, 0.0038 M KH_2PO_4, 0.0072 M K_2HPO_4, 0.0049 M glutamate, and up to 1% serum albumin. With all strains of rickettsia studied, the presence of glutamate was of marked importance in increasing stability at moderate temperatures. Glutamate was one of the few substances then known to act as a substrate for rickettsiae. Although its mechanism of action was unknown, it became traditional to include glutamate in many chlamydial transport media and, hence, viral transport media when the former were adapted for viral applications. Because a medium low in sodium ions was favorable to rickettsial survival, the phosphate salts used for the buffer were potassium salts rather than sodium salts. Thus, it is believed that sodium salts should be avoided in transport media used for both chlamydial and viral agents.

Media of similar composition identified as ChlamydiaPort or Richards Viral Transport (Richards Laboratories, Inc., Pleasant Grove, Utah) were evaluated for suitability as a transport medium for HSV (7). The survival of two laboratory strains and two clinical isolates at 2 and 22°C was tested in both cell culture medium and the sucrose-containing transport medium. Some of the HSV strains were perceptibly more stable than others under similar conditions. However, stability appeared not to correlate with viral serotype. The half-life of the viruses in transport medium ranged from 3.5 to 10 days at 2°C, while in cell culture medium the range was only 1 to 4 days. At 22°C in transport medium, the half-life ranged from 1.5 to 6 days compared with only 0.6 to 2 days in cell culture medium. When clinical specimens were held in the sucrose medium and transported under field conditions, the specimens could be held for up to 5 days at ambient temperatures without decrease in the number of positive specimens. Holding times of longer than 5 days were not tested. Thus, for those specimens that are sent to a reference laboratory, a buffered sucrose transport medium is superior to cell culture medium, even though the cell culture medium is buffered and may contain up to 10% fetal bovine serum.

A medium containing SPG, L-15 medium, and bovine albumin, referred to as Carr-Scarborough or SmithKline Bio-science transport medium, has been used for VZV transport (75). Another medium that was formulated for chlamydial transport, 2SP, has been used for viral transport. This medium, which is similar to SPG but contains no glutamate, has been used successfully to transport herpesviruses (20, 73). The transport of HSV was also accomplished in a serum-free sucrose-containing medium consisting of Leibovitz medium #15, sucrose, phosphate, glutamine, bovine serum albumin, and gentamicin (32).

Tryptose phosphate broth was compared with 2SP as a transport medium for chlamydiae and genital mycoplasmas (63). No significant differences between the isolation rates of mycoplasmas were found; however, greater chlamydial recovery rates and higher inclusion counts were obtained in the specimens transported in 2SP. The authors concluded that 2SP is a better medium for the combined recovery of *Chlamydia trachomatis* and genital mycoplasmas.

Warford et al. (73) reported on a study to determine whether SPG could be used to transport viral as well as chlamydial specimens for culture and, thus, eliminate the need for two different transport media. SPG was tested with stock herpesvirus suspensions and compared with Eagle MEM containing 2% fetal bovine serum. The SPG medium was also tested with herpesvirus patient isolates and used for transporting clinical specimens on wet ice from hospitals and clinics. The stability of the herpesvirus group in SPG was found to be equal to or better than that in MEM after 3 days of incubation at 4 or 23°C. Chlamydiae were also isolated from patient specimens. The authors concluded that SPG is a suitable transport medium for cervical specimens collected for diagnosis of HSV and *C. trachomatis* infections.

Transporters. A viral transport system, the Transporter, was introduced by Bartels Immunodiagnostics, Bellevue, Wash. It consists of a plastic centrifuge tube containing a monolayer of human diploid fibroblast cells in 2 ml of Eagle MEM with 20 mM HEPES buffer, 2% fetal bovine serum, and antibiotics (62, 72).

This system was tested in parallel with SPG for the recovery of viruses (72). Specimens from 47 hospitals and clinics were collected in duplicate and placed in SPG and Transporters. These sites were at distances from the laboratory ranging from 8 to 131 miles (ca. 12.9 to 210.8 km). The Transporters (containing specimens) were held at ambient temperatures, while the specimens in SPG were held at refrigerator temperatures. Time in transport ranged from 3 h to 2 days. Ninety-two (91%) of 101 viral isolates were recovered in cell culture from the Transporter as compared with 82 (81%) from SPG. Twenty-five (24.7%) of the viral isolates were detected by cytopathic effects that developed 1 to 4 days earlier in cultures inoculated with specimens from the Transporter tube as compared with specimens transported in SPG. Of the 101 virus isolates in the study, 9 were recovered exclusively from SPG, while 19 were recovered exclusively from the Transporter tubes. In sum, the authors considered that the Transporter system was superior to SPG because of the earlier development of cytopathic effects and the facilitated transport of clinical material at ambient temperature. Primarily herpesviruses were recovered from the samples tested. Of 101 isolates, 95 were either HSV, CMV, or VZV. These viruses replicate in fibroblasts which are used in the Transporter. It is possible that the Transporter would not be as successful in transporting viruses that do not replicate in fibroblasts. Because the Transporter contains living cells, another disadvantage is its short shelf life. Many laboratories keep transport tubes on hand, either frozen or refrigerated, and use them over time as the occasion demands.

Viruses Adsorbed onto Filters

Virus-monitoring programs designed to assess the virological content of water can be restricted by the distance between sampling sites and adequate laboratory facilities. When large volumes of water need to be sampled and the size of the sample is large, it is impractical to transport such volumes to the laboratory. In such cases, field sampling becomes necessary. Filters may be used to entrap viruses from large volumes of water and then may be transferred to a laboratory for processing. The use of the filter as a mechanical entrapment device and as a viral transport vehicle has the advantage of reducing equipment needs in the field, decreasing possible contamination, and making it convenient for field personnel to process more samples.

In a study by Dahling and Wright (19), poliovirus-seeded tap water was used to assess various parameters of the collection and recovery sequence. Conditioning of the water with $MgCl_2$ gave better recovery yields than conditioning

TABLE 2. Stability of viruses in various transport media

Virus	VTM[a]	Time in VTM (days/temp)	Approximate recovery (%)	Estimated half-life (days)[b]	Reference
Adenovirus	Bentonite	14/room	100	NA[c]	10
Coxsackievirus A9, B5	Bentonite	21/room	100	NA	10
CMV	2SP	21/4°C	4	6–10	33
	2SP	7/20°C	9	4	33
	Sorbitol (70%)	3/4°C	4	1.5	33
	Sorbitol (70%)	3/20°C	2	1.5	33
	Cell culture media	3/4°C	1	0.5	73
	Cell culture media	3/23°C	10	1	73
	SPG	3/4°C	10	1	73
	SPG	3/23°C	10	1	73
Echovirus 11	Bentonite	21/room	100	NA	10
HSV	Skimmed milk	30/4°C	25	15	2
	Cell culture media	12/2°C	0.01–1.0	1–4[d]	7
	Cell culture media	12/22°C	0.001–1.0	0.6–2[d]	7
	Cell culture media	3/4°C	10	1	73
	Cell culture media	3/23°C	1	0.5	73
	Richards Transport (ChlamydiaPort)	12/2°C	10–50	4–10[d]	7
	Richards Transport (ChlamydiaPort)	12/22°C	0.1–50	1.5–6[d]	7
	Bentonite	14/room	1	7	10
	YLE20C	42/4°C	1	4	65
	YLE20C	3/20°C	10	1	65
	Virocult	12/2°C	3	3.5	35
	Virocult	12/22°C	1	2.75	35
	Leibovitz and LEM[e]	8/22–28°C	0.01	1	50
	SPG	3/4°C	30	1–1.5	73
	SPG	3/23°C	10	0.5–1	73
Influenza virus type A2	Bentonite	14/room	33	10	10
Parainfluenza virus 3	Bentonite	14/room	50	14	10
RSV	Cell culture media	10/4°C	0.1	1	30
	Cell culture media	4/25°C	0.1	0.5	30
	Sucrose (44.5%)	14/4°C	44	11	40
Rhinovirus 2	HH	30/room	0.6	4	15
Rhinovirus 2	Leibovitz (CVTM)	30/room	0.6	4	15
Rubella virus	Bentonite	14/room	1	7	10
VZV	2SP	7/4°C	0.2	3.5	33
	2SP	3/20°C	4	1.5	33
	Sorbitol (70%)	3/4°C	4	1.5	33
	Sorbitol (70%)	1/20°C	0	NA	33

[a] VTM, Viral transport medium holding the virus.
[b] Half-life, Time in days required for the disappearance of 50% of the virus infectivity.
[c] NA, Not applicable.
[d] Half-life varied with strain being tested.
[e] LEM, Leibovitz-Emory medium.

with $AlCl_3$. Several filter types were tested for virus adsorption and recovery: Filterite (Filterite Inc., Timonium, Md.), Balston (Balston Inc., Lexington, Mass.), K-27 (Carborundum Co., Lebanon, Ind.), and Zeta Plus 50SP (AMF Corp. Cuno Division, Meriden, Conn.) cartridge filters. The effect of transport time and temperature was also assessed, and the elution procedure was studied. From this work several recommendations were made for the collection, transport, and processing of environmental virus samples. First, use K-27 or Filterite cartridge filters for the collection and transport of field samples. Place the filters in sterile plastic bags and transport them to the laboratory at −70°C. Process the cartridge filters within 4 days to avoid virus loss. The filters should be thawed by holding them at room temperature for 30 min and then placing them in a 36°C water bath for 30 min. The attached viruses should be eluted with 1,600 ml of 3% beef extract, and this eluate should be recycled twice through the filter. Foaming should be controlled by adding antifoam (Dow Corning Medical Antifoam C; Dow Corning Corp., Midland, Mich.) at the rate of 0.1 ml per 100 ml of eluant.

Enteric viruses were shown to survive adsorption to glass microfiber filters and subsequent transport through the postal service (36). Poliovirus type 1 as well as indigenous viruses of waste-waters were adsorbed onto the filters. After viruses had been mailed, they were eluted in a 3% beef extract solution (pH 7.5). Virus recovery ranged between 59 and 65%. The results of this study suggested that water quality monitoring efforts could be extended by adsorbing viruses to filters and then shipping the filters by regular mail service to the laboratory for virological evaluation.

Virus Stability in Transport Media: a Comparison

It is important to compare the stability of viruses, within a given group, in various transport media. This information facilitates the selection of a suitable transport medium for clinical use. It is also important to compare the stability of different virus groups to select optimal conditions for the collection and transport of sensitive agents. Table 2 illustrates the stability of viruses in various transport media. These data do not represent direct comparisons under con-

trolled conditions, but are simply a compilation of published information. In some cases, the published data were converted to estimated viral half-lives for the purpose of comparison. The half-life of viral infectivity can be measured in days, and an estimate of virus stability can be quickly obtained. Positive specimens containing multiple $\log_{10}$ concentrations of virus undergo several half-lives of viral decay before the specimens become culture negative. The assumption is made that the decay of viral infectivity follows logarithmic decay kinetics.

Ideally, direct comparisons of transport media that support different viruses should be performed in a clinical setting. However, the information in Table 2 suggests that, for viral culture and isolations, and especially for HSV, specimen transport is not a problem. Even more labile viruses with short half-lives, such as CMV, RSV, and VZV, survive transport for up to 1 to 3 days. However, some specimens with extremely low virus levels could lose infectivity in this time.

Availability of effective transport systems makes viral isolation a realistic approach for diagnosing many clinically significant virus infections including HSV (44), VZV (66), adenoviruses (70), reoviruses (24, 68), togaviruses (13), parainfluenza viruses (28), mumps virus (52), RSV (5), influenza viruses (38), bunyaviruses (14), arenaviruses (48), the enteroviruses (including poliovirus, coxsackieviruses, and echoviruses) (37), and rhinoviruses (3).

C. trachomatis cultures are often performed in virus laboratories. Cell cultures (by the shell vial technique) have been regarded as the standard diagnostic test (58). Traditional transport media for chlamydia have been SPG and 2SP, but other commercial transport media are now available. Because the sites sampled are likely to be contaminated with other microbial agents, the specimen should be collected into a medium that contains appropriate antibiotics. Chlamydiae are resistant to streptomycin, gentamicin, vancomycin, nystatin, and amphotericin B. These antibiotics are often included in chlamydia transport media.

CONDITIONS FOR SHIPPING OR TRANSPORT OF SPECIMENS TO THE LABORATORY

Temperature and Time

It is commonly recommended that specimens for virus isolation be held at refrigerator temperatures or on wet ice and transported to the laboratory as speedily as possible (22, 23, 43, 49, 59). The specimen should never be left at room temperature or in an incubator. Because virus infectivity decays over time, the chances for successful isolation are at a maximum immediately after specimen collection. However, in many cases it is neither practical nor necessary to inoculate cultures at the patient's bedside. Many viruses are sufficiently stable to allow specimen transport to the laboratory in a transport medium at ambient temperature. The decay rates of some viruses are sufficiently slow to allow several days in transport, if necessary (Table 2), but the detectability of virus in a positive specimen depends on the amount of virus present at the beginning. At a given decay rate, active virus is recoverable over a longer period of time if the specimen contains a higher amount of virus upon collection. Thus, proper collection of the specimen increases the probability of a successful result. Although cool holding temperatures, short transport times, and high virus concentrations are important factors, in practice many viruses can be successfully isolated from a variety of transport media sent to the laboratory at ambient temperatures and requiring up to several days for transport (Table 3). The term ambient temperature does not imply that the sample can be exposed to high temperatures, such as the intense heat found in direct sunlight, on loading docks, in mail trucks, or on refrigerator tops. The specimen should be packaged in an insulated container to protect it from such temperature extremes if it is to be shipped to a laboratory.

The practice of freezing viral and chlamydial specimens for transport has become less common with the realization that most agents remain infectious for at least 1 or 2 days if kept cool (43). Indeed, many viruses lose infectivity rapidly at the temperatures in most freezers (−15 to −20°C) (78). The freeze-thaw cycle itself is harmful to many viral agents. In addition, many investigators have considered the temperature fluctuations in frost-free freezers to be too stressful for adequate viral preservation.

Regulations

The U.S. Public Health Service has published the regulations for shipping specimens (Fed. Regist., vol. 45, no. 141, 21 July 1980), and they are found in the Department of Transportation and Interstate Quarantine regulations (49 CFR, Section 173.386.388, and 42 CFR, Section 72.25, Etiologic Agents). Summaries of the regulations have been published (29, 43, 45). Full copies of the regulations can be obtained from the Biohazards Control Officer, Centers for Disease Control, Atlanta, Ga.

In summary, the regulations require the specimen to be wrapped in sufficient absorbent material to absorb the entire contents of the specimen in case of leakage or breakage. The wrapped specimen must be enclosed in a durable watertight container, which in turn is enclosed in another outer shipping container. The watertight container must be secured with shock-absorbent material or tape so that it does not become loose as the dry ice sublimates or wet ice melts.

The specimen, itself, should be contained in a tightly sealed, preferably screw-cap container (43). For shipment, the caps should be secured with adhesive tape (not transparent or cellophane tape) to prevent loosening due to vibrations during shipping. Stoppered tubes should not be used as they are difficult to seal properly and they may create an infectious aerosol hazard when they are opened (45).

PRESERVATION AND STORAGE OF VIRUSES

Aside from the practical aspects of transporting specimens from collection site to laboratory, holding media are used for the preservation of viruses during storage. Most reports suggest that, for viruses in general, loss of infectivity is greater when storage is at −20°C than when storage is at −70°C or colder. HSV stored in YLE20C lost infectivity at −20°C but not at −70°C in a report by Tada et al. (65). RSV (40) lost infectivity more rapidly when stored at −20°C than when held at −70°C. There may be some exceptions to this rule, however. HSV stored in skimmed milk lost no detectable infectivity during storage at either −70 or −20°C (2).

Dimethyl sulfoxide (DMSO) has long been used in preservation media as a cryoprotectant for the frozen storage of cells and viruses. Other materials including serum, glycerol, sorbitol, sucrose, skim milk, and other proteins have been used as cryprotectants. These stabilizers are believed to act by preventing the formation of destructive ice crystals. Their protective effects on cells have also been attributed to their action in lowering the effective concentrations of electro-

TABLE 3. Successful transport of viruses to the laboratory in clinical specimens contained in various media and under various transport conditions[a]

Virus	Specimen	Transport medium	Transport conditions[b]	Reference(s)
Adenovirus	Oropharyngeal	Culturette	Ambient temp	34
	Oropharyngeal	Amies	Ambient temp	41
	Lab strain	Leibovitz CVTM	Ambient temp	41
	NS[c]	Virocult	NS	53
	Throat	Tryptic soy broth	Frozen	57
	Throat swabs	Eagle MEM	NS	48
Coxsackievirus	Lab strain	Leibovitz CVTM	Ambient temp	41
CMV	Urine	Transporter	Ambient temp	72
Enteroviruses	Oropharyngeal	Culturette	Ambient temp	34
	Throat, rectal	Veal infusion broth	NS	17
	NS	Virocult	NS	53
HSV	Genital	Tryptic soy broth	4°C	6
	Genital	Richards (ChlamydiaPort)	Ambient temp	7
	Genital, nongenital	Virocult	Ambient temp	7
	Genital	Calf infusion broth	Ambient temp	12
	Cervical	2SP	Frozen (liquid nitrogen)	20
	Genital	Culturette	Ambient temp	25, 27, 34
	Genital	Veal infusion broth	NS	39
	Lab strain	Leibovitz CVTM	Ambient temp	41
	Genital, nongenital	Virocult	Ambient temp	46
	Genital, nongenital	Leibovitz-Emory medium	Ambient temp	50
	Genital, nongenital	Buffered gelatin	4°C	51, 76, 77
	Genital, nongenital	Eagle MEM, 1% fetal bovine serum[d]	NS	54
	NS	Virocult	NS	53
	NS	Cell culture medium	NS	55
	Genital	Stuart	Ambient temp	56
	Genital	Hanks BSS + 0.5% gelatin	Ambient temp	62
	Genital	Culturette	Ambient temp	62
	Genital	Eagle MEM + 2% fetal bovine serum	Ambient temp	62
	Genital	Veal infusion + 0.5% gelatin	Ambient temp	62
	Genital	Transporter	Ambient temp	72
	Genital	SPG	4°C	73
	Genital, nongenital	Tryptose phosphate broth + 0.5% BSA[e]	4°C	79
Influenza virus types A, B	Oropharyngeal	Culturette	Ambient temp	34
Influenza virus types A PR8	Lab strain	Leibovitz CVTM	Ambient temp	41
Influenza virus	Nasal washes	Veal or tryptose broth	4°C	38
Parainfluenza virus 2	Lab strain	Leibovitz CVTM	Ambient temp	41
Parainfluenza virus	Oropharyngeal	Culturette	Ambient temp	34
Rhinovirus	Oropharyngeal	Culturette	Ambient temp	34
RSV	Oropharyngeal	Culturette	Ambient temp	34
	Nasopharyngeal aspirates	Tryptose phosphate broth + 0.5% BSA	−70°C or ambient temp	74
Vaccinia virus	Lab strain	Leibovitz CVTM	Ambient temp	41
VZV	Dermal lesions	Culturette	Ambient temp	34
	Cutaneous lesions	Smith Kline/Carr-Scarborough	NS	75

[a] Not to imply optimal results nor acceptable preservation of viral viability.
[b] Time in transport varied from a few minutes to several days.
[c] NS, Not specified.
[d] Specimens received as swabs or vesicular fluid.
[e] BSA, Bovine serum albumin.

lytes, preventing the denaturation of cell structures by the toxic effects of concentrated salts and solutes. The substances that stabilize cells in the frozen state are also recognized as preservatives for enveloped viruses (71). DMSO in the preservation medium was shown to stabilize HSV through a series of four freeze-thaw cycles (71). Measles virus, vesicular stomatitis virus, and Sindbis virus were also stabilized. Concentrations of DMSO as low as 5% effectively protected the enveloped viruses against the trauma of freezing, but lower concentrations were less effective.

In a study reported by Gallo and co-workers (26) various freezing media were compared with a standard medium consisting of RPMI 1640 containing 50% fetal bovine serum and 10% DMSO and frozen at −190°C for the preservation of human immunodeficiency virus. Specimens collected for human immunodeficiency virus culture often are frozen until cultures are initiated. The five media compared with the standard medium were (i) tryptose broth with 0.5% gelatin; (ii) RPMI 1640 with 10% fetal bovine serum; (iii) RPMI 1640 with 20% fetal bovine serum, Polybrene, interleukin-2, and anti-alpha interferon; (iv) RPMI 1640, 10% fetal bovine serum, and 10% DMSO; (v) same as medium iii plus 10% DMSO. Peripheral blood lymphocytes in heparinized blood

specimens were obtained from human immunodeficiency virus antibody-positive individuals. These specimens were stored in the test media at −60°C and in the standard medium at −190°C. After 1 week of storage, the samples were cocultivated with phytohemagglutinin-stimulated uninfected peripheral blood lymphocytes, and the presence of human immunodeficiency virus was detected by the reverse transcriptase test. Storage in medium i, ii, or iii, which did not contain DMSO, resulted in unsatisfactory isolation rates, but DMSO-containing media iv and v gave equal or better isolation rates than storage in standard medium at −190°C.

Hypertonic sucrose solutions exert a protective effect on labile viruses. When RSV was placed in different concentrations of sucrose and stored at various temperatures, the survival of virus was enhanced as the concentration of sucrose was increased (40). For retention of virus infectivity, the optimal sucrose concentration and storage temperature were 44.5% and −70°C. Viruses were stored under these conditions for periods in excess of 2 years with no significant loss of infectivity. Storage at −20°C, even in the presence of hypertonic sucrose, was less satisfactory than storage at 4 or −70°C. In a portion of the study with simulated clinical specimens, it was found that cotton swabs containing <10 50% tissue culture infective doses of virus and held in 44.5% sucrose at 4°C retained viable virus for 7 days.

For RSV, and perhaps some other very labile viruses, sucrose provides better stabilization of the virus than does serum or other protein solutions and has the additional advantage of not being antigenic. There are some disadvantages to using hypertonic sucrose solutions for virus stabilization, but in most cases these are easily overcome. At a concentration of 44.5%, sucrose is toxic to cell cultures and produces pain upon injection into animals. A 1:10 dilution reduces the concentration to levels tolerated by cell cultures, and a 1:5 dilution eliminates the difficulty upon injection.

The stability of RSV in SPGA (SPG containing 1% bovine albumin) and in the absence of SPGA was evaluated following freeze-drying of the samples (67). RSV strains freeze-dried in the presence of SPGA showed only small losses of infectivity at 4°C and losses of approximately 2 $\log_{10}$ infectious units when held at 25°C for 45 weeks. Infectious virus persisted for the longest time in those preparations that were freeze-dried in SPGA. The authors suggested that laboratory strains of RSV freeze-dried in the presence of a suitable stabilizer can be transported as unrefrigerated samples without undue loss of infectivity. Although many viruses are very labile and require special storage conditions, some viruses are sufficiently stable so as to require little or no additional protection during storage (47).

RECOMMENDATIONS AND FUTURE DIRECTIONS

There have been no studies reported that comprehensively compare viral transport systems for use with many different kinds of specimens and for their application with many different kinds of viruses. Therefore, recommendations for routine clinical use of viral transport systems can only be made based on available data and experience. Further, the needs of individual laboratories differ; those of reference laboratories are different from those of in-house laboratories. The optimal transport system can be defined as that system which preserves the virus in the specimen (whatever virus is being sought and for whatever length of time is necessary), prevents loss of the specimen or test due to microbial contamination, has a long shelf life, is readily available, and is inexpensive. Currently, many laboratories are finding that the optimum viral transport system for them is a sucrose-based medium such as Richards Viral Transport Medium, one of the broth-based media, or either Culturette or Virocult. Future experiments and experience will be needed to compare the effectiveness of these systems.

Several difficulties have slowed the progress of appropriate studies that compare transport systems. A large number of patients is needed to obtain data with matching samples. Sampling is not always uniform, so some specimens that come from a given patient may contain more or less virus; thus, a valid comparison of the transport systems into which these specimens are placed is difficult. The transport systems should be tested for effectiveness with a broad range of viruses. The expense of comparative studies or lack of personnel time prevents many laboratories from undertaking these studies. However, future studies will need to be done to compare effectively the viral transport systems.

CONCLUSIONS

The successful diagnosis of viral infections by culture is enhanced when the specimen contains as much virus as possible upon collection, is protected from thermal inactivation, and is contained in an effective transport system. An ideal viral transport medium would possess many of the following characteristics. It would preserve the activity of the virus, even at room temperatures; it would be nontoxic to cell cultures and not obscure the appearance of viral cytopathic effects; it would have a long shelf life (either in a frozen or nonfrozen state); and it would be applicable for both culture isolations and direct tests such as enzyme immunoassays or immunofluorescence. Several useful transport systems exist, including sucrose-based liquid media, broth-based media, transtubes, and the Transporter. Using adequate transport systems, successful isolations can be performed by remote reference laboratories as well as in-house virology laboratories. Specimens should be properly packaged to prevent breakage or leakage during transport.

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