A Transport Medium for Diagnostic Virology* (33820)

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It has long been the hope of the diagnostic viral laboratory that a transport medium could be devised that permits the shipment of specimens at ambient temperatures (1). The opportunity to investigate this problem came in conjunction with an adenovirus surveillance program[1] in which specimens were being transmitted from all parts of the United States to a central laboratory. Pilot field studies were performed at Fort Ord, California on hospitalized recruits with upper respiratory disease. Laboratory studies included survival studies of representative members of most major viral groups in various transport media at ambient temperatures. The findings led to the formulation of a simple charcoal transport medium (CVTM) that enables the survival of most viral agents for at least 3 weeks.

Materials and Methods. Charcoal viral transport medium (CVTM). Contains (g): sodium chloride, 4.00; potassium chloride, 0.20; dipotassium phosphate, 1.70; charcoal (bacteriological)[2], 10.00; and agar, 4.00. Dissolve salts in 500 ml distilled water. Add charcoal. Dissolve agar in 500 ml distilled water by boiling. Combine the two solutions and autoclave at 121° (15 psi) for 30 min. Transfer to sterile aspirator bottle containing a magnetic bar. Place on magnestir and allow to cool to about 40°. Dispense about 8 ml in plastic 16 × 125-mm screw cap tubes.[3] Mix by inversion to distribute the charcoal just before the medium gels. The final medium is pH 7.6.

Dextran viral transport medium. Substitute 0.5 g/liter of DEAE-dextran[4] for the charcoal in CVTM.

Amies transport medium. Difco formula used.[5] Amies (2) later modified this formula by reducing the sodium chloride to 3 g/liter and increasing the agar content to 4 g/liter. This medium differs from CVTM by also containing 0.1% thioglycollate and 0.01% magnesium and calcium chloride.

Agar control medium. CVTM minus the charcoal.

Throat wash control medium. The salts in CVTM plus 0.45% bovine albumin.[6]

Selection of patients. Hospitalized Army recruits admitted with a temperature of at least 100°F and still febrile on the following morning when specimens were collected.

Collection of specimens. Throat swab specimens were collected from behind the uvula (oropharynx) with a pair of dry cotton-tipped applicators. These were used for the direct inoculation of primary human embryonic kidney (HEK) tissue cells[7] at the bedside (DIR) and for transport media. Throat wash specimens were obtained by having the patient gargle 15 ml of throat wash medium. The two former specimens were maintained at ambient temperatures. The throat wash specimens were maintained in a frozen state with dry ice. Specimens were collected daily and transmitted to the virus laboratory twice weekly.

Viral isolation and identification procedures. HEK monolayers was the cell strain of choice for the isolation of adenovirus agents and for neutralization tests to identify the

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[1] Program initiated by the Preventive Medicine Division, Office of the Surgeon General, US Army.

[2] Colab Laboratories, Chicago Heights, Illinois.

[3] Falcon Plastics, Los Angeles, California.

[4] Pharmacia Fine Chemicals, Piscataway, New Jersey.

[5] Difco Laboratories, Detroit, Michigan. Technical Bulletin 0996, March, 1965.

[6] Pentex, Inc., Kankakee, Illinois.

[7] Microbiological Associates, Bethesda, Maryland; Flow Laboratories, Rockville, Md., and Grand Island Biochemical, Oakland, California.

isolates. Inoculated cells were maintained with medium L-15[8] (3) containing 2% fetal calf serum and antibiotics (4).

Tubes inoculated at the bedside required no processing and were immediately placed in stationary racks in the 35° incubator. Transport media specimens were processed by removing one swab with sterile hemostats (alcohol flaming) and stirring in sufficient maintenance medium to allow 1.5 ml of inoculum/tissue cell tube; the remaining swab was retained in the transport medium for possible repeat studies. Throat wash specimens were added in the ratio of 0.5 ml of specimen to 1.5 ml of maintenance medium, the pH was adjusted to 7.6 with 0.2 *N* KOH, and 2 ml were inoculated per tissue cell tube. Once inoculated, the HEK monolayers were not fed throughout the 21-day observation period. No blind passages were made.

Prototype viral survival studies in various transport media. Adenovirus types 4 and 7, Coxsackie virus types B-5 and A-21, herpesvirus, vaccinia, parainfluenza type 2, and influenza "A" type PR-8 were subjected to survival studies at ambient temperatures (22–25°). Titrations of the former seven agents were made in tissue cultures to obtain the 10, 100, and 1000 fifty percent infective dose after the formula of Reed and Muench (5). The influenze virus was titrated in 9-day-old embryonated chicken eggs. Sterile swabs were placed in each desired dilution for 1 min and inoculated into CVTM, dextran-CVTM, Amies, dextran-Amies, agar control medium, and throat wash control medium. The agar medium was the control medium for the tissue culture studies; the throat wash medium for the egg studies.

Inoculated cell cultures were observed for viral survival as determined by specific cytopathogenicity up to 7 days; the fertile eggs were inoculated via the allantoic route and viral survival was determined by the hemagglutination test after 44-hr incubation. Survival was determined after 1, 7, 14, and 21 days of storage.

Results. Field study comparing direct inoculation of tissue cells at bedside (DIR), Amies' (Am) transport medium, and throat wash specimens (TW). The initial thought in this project was to field test a commercially available transport medium. Amies' (2) modification of Stuart's (6) medium and Cary–Blair's (7) medium was selected.

A pilot study of 71 specimens indicated that the AM medium not only yielded a similar isolation rate (DIR and TW 87%; AM 93%) as the other two methods, but that the charcoal in this medium also reduced the toxicity of the specimens for cell cultures. Whereas 9% of the TW specimens and 50% of the DIR specimens showed nonspecific degeneration of the monolayers within 72 hr, this only occurred with 3% of the AM specimens. This degeneration was usually caused by the growth of Candida species (Table I). Viral cytopathogenic effect was usually evident several days earlier with AM, thus enabling more rapid identification of isolates. The direct inoculation of cell cultures was discontinued and further comparison was made between AM and TW. Adenovirus disease was lessening and upper respiratory disease caused by Coxsackie virus A-21 was starting to appear. Isolation attempts on 325 specimens indicated that AM was as efficient as TW not only for adenovirus but also for the Coxsackie virus. However, the value of the transport medium was not as conclusive for this virus as only 80% of the isolates were obtained from AM specimens (Table II).

Survival studies on prototype viruses in

TABLE I. Comparative Isolation Rate of Adenovirus from 71 Specimens Taken Simultaneously by Throat Wash (TW), by Throat Swabs Inoculated Directly into Tissue Culture[a] (DIR), and by Throat Swabs Inoculated in Amies Transport Medium (AM).

	Adenovirus			
	4	7	Toxic (%)	Positive (%)
TW	11	51	9[b]	87.3
DIR	11	51	50	87.3
AM	12	54	3	93.0

[a] Primary human embryonic kidney monolayers.
[b] Monolayers requiring subculture within 3-days postinoculation due to nonspecific degeneration.

[8] Difco Laboratories, Detroit, Michigan.

TABLE II. Primary Isolations[a] Obtained from Throat Wash (TW) and Amies Transport Media (AM) Specimens Simultaneously Obtained from 325 Hospitalized Patients.

Specimens	Adenovirus 4	Adenovirus 7	Coxsackie A-21	Poliovirus	Herpes simplex	Positive (%)
TW	39	154	12	2	4	65
AM	41	165	20	4	2	71
Pos TW, neg AM	3	2	5	0	2	
Neg TW, pos AM	5	13	13	2	0	

[a] Primary human embryo kidney cultures.

various transport media. Although Amies' transport medium has definite value for the study of adenovirus disease, comparative isolation rates of Coxsackie A-21 as noted above caused concern as to its value with other viral agents. Pilot studies were carried out at the Walter Reed Army Institute of Research (Colonel E. L. Buescher, personal communication) to determine the value of Amies' transport medium for influenza viruses. They found the A_2/Rockville/65 strain of influenza to be inactivated much more rapidly in Amies' transport medium than in throat wash medium at ambient temperatures.

Representative prototype viruses were chosen for survival studies in various transport media as noted in "Materials and Methods." The Charcoal viral transport medium (CV TM) proved most successful among media tested in retaining viral survival at approximate peak titer over a 3-week period. Only herpesvirus and influenza "A" PR-8 showed a one-lg drop in titer over this period. The thioglycollate in Amies and dextran-Amies was inhibitory to the herpesvirus, the Coxsackie viruses and the myxoviruses. DEAE-dextran appears to be inhibitory for the myxoviruses (Table III).

TABLE III. Survival of Viral Agents in Various Transport Media at Ambient Temperatures (22–25°) over a 21-Day Storage Period.[a]

Virus	Transport media (days in storage)																			
	CVTM[b]				DVTM				AM				DEAM				Controls[c]			
	1	7	14	21	1	7	14	21	1	7	14	21	1	7	14	21	1	7	14	21
Adenovirus 4	2[d]	2	2	2	2	1	1	1	2	2	1	1	2	2	1	1	2	1	0	0
Adenovirus 7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2
Herpesvirus	2	2	1	1	2	2	1	0	2	2	—[e]	—	2	2	—	—	2	1	—	—
Coxsackie B5	3	3	3	3	3	3	3	3	3	2	1	1	3	3	3	1	3	3	3	3
Coxsackie A21	3	3	3	3	3	3	3	3	3	3	1	1	3	3	1	1	3	3	3	3
Vaccinia	2	2	2	2	2	2	2	1	2	2	2	1	2	2	2	1	2	1	0	—
Parainfluenza 2	2	2	2	2	2	0	—	—	2	2	0	0	2	2	—	—	2	—	—	—
Influenza A PR8	4	4	4	3	4	—	—	—	4	3	—	—	4	2	—	—	4	4	4	4

[a] All but influenza PR8 virus survival determinations performed in tissue culture; influenza PR8 determinations in embryonated chicken eggs.

[b] CVTM = charcoal viral transport medium; DVTM = DEAE-dextran viral transport medium; AM = Amies' transport medium; DEAM = DEAE-dextran Amies' transport medium; see "Materials and Methods."

[c] Controls: All but influenza PR8 in agar control medium; influenza PR8 in throat wash control medium; see "Materials and Methods."

[d] Inverse logarithm to the base 10 of the highest dilution to yield discernible viral growth within 7 days.

[e] No discernible viral activity in 7-day observation period.

Discussion. Charcoal has long been advocated as a detoxifying agent to enable the growth of certain bacterial pathogens under otherwise adverse conditions (6, 8–10). Both field and laboratory studies reported here indicate that such media are also useful for the transporting of specimens at ambient temperatures for viral isolation studies. Modification of presently available charcoal transport media was deemed necessary when survival studies of prototype virus agents indicated the thioglycollate present in such media was inhibitory to certain viral groups, especially the myxoviruses and herpes. The cations, calcium and magnesium, were also eliminated as being unessential to the survival of the myxoviruses (see the formula for the throat wash medium under "Materials and Methods"). Wallis and Melnick (11) demonstrated that cations may enhance deactivation of most virus agents other than the enteroviruses at 50°.

Summary. A simple charcoal viral transport medium (CVTM) has been devised that permits the survival of representative viral agents for at least 3 weeks at ambient temperatures. Amies' bacterial transport medium was useful for the transporting of specimens containing adenovirus agents but the thioglycollate present in this medium is inhibitory to herpesvirus, Cozsackie virus and the myxoviruses. The use of DEAE-dextran as a detoxifying agent is limited because it is inhibitory to the myxoviruses.

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