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Processing and Transport of Environmental Virus Samples

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Poliovirus-seeded tap water, conditioned with $MgCl_2$ and passed through virus-adsorbing filters, gave better poliovirus recovery than water identically treated but conditioned with $AlCl_3$. Elution of several filter types with beef extract yielded higher recoveries than did elution with glycine. Seeded samples filtered through various filters and stored showed considerable virus loss in 2 days when stored at 4°C, whereas those stored at −70°C gave stable virus recovery up to 4 days. Additionally, the use of antifoam during the elution process reduced foaming and increased virus recovery by 28%.

A virus-monitoring program can often be restricted by lack of trained personnel on the one hand and the distance of sampling sites from adequate laboratory facilities on the other. When the need arises to sample volumes of water in excess of 1,900 liters (7, 15, 17), it becomes impractical to transport such samples to the laboratory; thus, field sampling becomes a necessity. Filters must then be processed in the field or transferred to a laboratory for processing. The transport of virus-laden filters to a laboratory (8) has the advantage of reducing equipment needs in the field, decreasing possible contamination, and thus freeing field personnel to process more samples.

The survival of virus adsorbed to Filterite, Balston, K-27, and Zeta Plus 50SP cartridge filters stored at 4 and −70°C was assessed together with the effects of various salt-conditioned waters. Additionally, the elution procedure was studied, including the use of antifoam to reduce virus loss from foaming. We report here a procedure whereby viruses adsorbed onto cartridge filters can be transported from the field to a laboratory without loss of titer.

MATERIALS AND METHODS

Cell culture and virus assay. The continuous African green (*Cercopithecus aethiops*) monkey kidney cell line, designated BGM, was used throughout the study at passage levels 113 through 200. The methods of propagation were previously described (3, 5, 6).

A plaque-purified strain of poliovirus 1 (Mahoney LP) was used for all experimental work. Virus assays were performed in screw-capped bottles by the plaque technique. A 1.0-ml sample was inoculated onto BGM monolayers and overlaid as described elsewhere (5, 6).

Filters tested. Filters used in this study included the Filterite DUO-FN 10-E-0.45A with 2879.9 cm^2 of surface area (Filterite Inc., Timonium, Md.); Balston 200-35-C with 120.77 cm^2 of surface area (Balston Inc., Lexington, Mass.); K-27R10S with 6735.25 cm^2 of surface area (Carborundum Co., Lebanon, Ind.); and the Zeta Plus 50SP with 929 cm^2 of surface area (AMF Corp. Cuno Division, Meriden, Conn.). All filters were 25.4 cm in length and were sterilized by autoclaving according to the manufacturer's instruction. It should be noted, however, that comparison of filters by surface area does not always reflect the true capability of a filter to retain viruses. The Filterite, for example, is basically a thin-surface, pleated filter, whereas the K-27 filter is considered a depth filter.

Sample preparation and test procedure. Cincinnati tap water was used throughout this study. Batch samples were prepared for each series of tests as follows. Sample water was placed into 20-liter Nalgene carboys with spigots. Either $AlCl_3$ or $MgCl_2$ was added to a final concentration of 0.0005 or 0.05 M, respectively. Residual chlorine was neutralized by adding $Na_2S_2O_3$ to a final concentration of 0.00003 M, and the pH of each sample was adjusted to 3.5 with 12 N HCl. With constant stirring of samples, virus was added and allowed to mix thoroughly. Four-liter aliquots were then withdrawn from the carboys and filtered under pressure through the test filters. Once samples were filtered, cartridges were either eluted immediately or stored for later elution. The latter cartridges were aseptically removed from filter holders, placed into sterile plastic bags, sealed, and stored at either 4 or −70°C.

Cartridge filters were eluted under positive pressure. Eluents consisted of 1,600 ml of either 0.05 M glycine or 3% beef extract. The resulting glycine eluate was reconcentrated as previously described (7, 15, 17), and reconcentration of the beef extract was by organic flocculation (9). Successive elutions were done by collecting the original 1,600-ml eluent and recycling it back through the cartridge two, three, four, or five times. Cartridges stored at 4°C were allowed to warm to room temperature and then were eluted in a similar manner. Those cartridges frozen at −70°C were first allowed to sit at room temperature for 30 min (to allow bags to warm to avoid cracking during subsequent steps), followed by 30 min in a 36°C water bath. Cartridges were then removed from the bag and placed into a cartridge housing, which was then filled with 3% beef extract. The housing was placed into a 36°C incubator for 60 min. After this treatment, filters were eluted as previously described with three successive elutions.

Reagents. Beef extract (lot 91190; GIBCO Diagnostics, Madison, Wis.) was prepared at 3% concentration, adjusted to pH 9.0, and autoclaved. Glycine (reagent grade lot 781300; Fisher Scientific Co., Pittsburgh, Pa.) was prepared at a concentration of 0.05 M, adjusted to pH 10.5, and autoclaved. To virus-seeded samples of 3% beef extract, increasing concentrations of antifoam (Dow Corning Medical Antifoam C, emulsion lot H118038) were added and allowed to mix at room temperature for a period of 1 h. This is approximately the time period involved in the total elution and reconcentration procedure with filter elution.

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TABLE 1. Comparison of virus recovery from cartridge filters eluted with high-pH glycine and 3% beef extract

Filter type	Test no.	Total virus input (PFU/sample)	% Virus recovery: $AlCl_3$-conditioned water[a] Glycine elution		% Virus recovery: $MgCl_2$-conditioned water[b] Glycine elution	
			Glycine elution	3% beef elution	Glycine elution	3% beef elution
Filterite	1	144	48	44	28	69
	2	381	29	51	23	77
	3	437	31	63	26	91
Avg		321	36	53	26	79
K-27	1	332	33	61	31	67
	2	365	30	63	32	105
	3	112	52	57	2	91
Avg		270	38	60	22	88
Balston	1	370	26	45	0	56
	2	344	22	59	2	52
	3	256	33	57	28	59
Avg		323	27	54	10	56

[a] Tap water conditioned with 0.0005 M $AlCl_3$–0.00003 M $Na_2S_2O_3$ and acidified to pH 3.5.

[b] Tap water conditioned with 0.05 M $MgCl_2$–0.00003 M $Na_2S_2O_3$ and acidified to pH 3.5.

RESULTS

To determine the best conditions for virus adsorption and, subsequently, the best eluent for recovery of the adsorbed viruses, comparisons were conducted with the Filterite filter, which was the preferred filter (4, 10, 12), and the Balston and K-27 filters, which are less commonly used for virus concentration (Table 1). Cartridge filters, challenged with Cincinnati tap water seeded with virus and conditioned with $MgCl_2$, gave higher viral recoveries when eluted with beef extract than when eluted with glycine. Similar studies involving tap water conditioned with $AlCl_3$ generally gave the same results: higher virus recovery with beef extract elution than with glycine elution. The noticeable difference arose when glycine or beef extract elutions were compared between $AlCl_3$- and $MgCl_2$-conditioned waters. In almost all cases, glycine elution of filters challenged with virus-seeded $AlCl_3$-conditioned water gave higher virus recoveries than comparable filters treated with $MgCl_2$; conversely, almost all filters eluted with beef extract gave higher virus recoveries with $MgCl_2$-conditioned virus water than with $AlCl_3$ waters. However, overall comparison did show that Cincinnati tap water conditioned with $MgCl_2$ combined with beef extract filter elution always gave the highest virus recovery. Consequently, all remaining studies were done by using $MgCl_2$-conditioned water and beef extract elutions.

TABLE 2. Comparison of virus recovery from filter cartridges held at 4 and 23°C

Test no.	Total virus input (PFU/sample)	% Virus recovery from filters[a] 23°C	4°C
1	211	80	67
2	190	94	75
3	181	50	42
4	125	111	65
Avg	177	84	62

[a] All filters were Filterite 0.45 μm.

TABLE 3. Effectiveness of single and successive elution procedures on recovering viruses adsorbed to Filterite cartridge filters

Test no.	Total virus input (PFU/sample)	Single elution: Method one[a]	Single elution: Method two[b]	Successive elutions[c]: Two	Three	Four	Five
1	264	50	52	40	62	45	45
2	169	59	61	52	72	69	66
3	205	60	63	53	85	70	66
Avg	213	56	59	48	73	61	59

[a] Pressure applied to force the eluent directly through the filter.

[b] Eluent placed in filter holder and held for 1 min in contact with the filter, and then pressure applied to force the eluent through the filter.

[c] Initial elution same as in method two, followed by two, three, four, or five successive elutions with the same eluent.

In Table 2, we compare elution of virus-laden filters at room temperature (23°C) and at 4°C before elution. Filters at 23°C gave an average of 22% better viral recovery than filters stored at 4°C. Thus, all frozen or cooled filters were processed at room temperature in this study.

The elution process is generally a single step in which the eluent is forced under pressure through the filter. We compared this procedure with a modified version in which the cartridge holder was filled with eluent and allowed to remain in contact with the filter surface for 1 min before the elution process was completed. Also studied were successive elutions in which the same eluent was collected and passed through the filter two, three, four, or five additional times. The best recoveries were obtained from 1 min of contact of the eluent with the filter, followed by three successive elutions (Table 3). Therefore, all subsequent elutions were carried out by following this procedure.

The transport of virus-laden filters from the field to a laboratory would require that they be kept either at ca. 4°C by packing in ice or at ca. −70°C with dry ice. We examined a method for thawing filters transported at −70°C. Frozen filters were allowed to thaw at room temperature for 30 min and then were placed (while still in the plastic bag) into a 36°C water bath for the times indicated in Table 4. Those

TABLE 4. Comparison of virus elution procedures for frozen Filterite cartridge filters

Test no.	Total virus input (PFU/sample)	% Virus recovery: Unfrozen filter[b]	Total time (min) frozen filter held at 36°C[a]: 5[c]	15[c]	30[c]	60[d]	75[d]	90[d]
1	177	62	14	19	32	25	NT[e]	NT
2	177	62	25	43	51	47	NT	NT
3	169	41	NT	NT	32	NT	NT	50
4	224	53	NT	NT	32	NT	41	51
5	207	41	NT	NT	49	NT	53	53

[a] All test filters frozen for 1 day at −70°C were thawed for 30 min at room temperature before further treatment.

[b] Unfrozen filters were eluted on the day of the test.

[c] Filters were warmed in a 36°C water bath for the time indicated and eluted. Filters in test no. 3, when held at 36°C for 45 and 60 min, both yielded 28% virus recovery.

[d] Filters were treated as described in footnote c for 30 min, followed by filling filter holder (with filter in place) with eluent and placing in a 36°C incubator for an additional 30, 45, or 60 min.

[e] NT, Not tested.

TABLE 5. Recovery efficiency of virus adsorbed to cartridge filters after storage for 1 and 2 days at 4°C

Filter type	Total virus input (PFU/sample)	% Virus recovery on day:		
		0[a]	1	2
Filterite	162	61	23	17
K-27	133	73	59	50
Balston	197	48	42	31

[a] Control filters were eluted immediately after filtering virus-seeded samples.

held in the bath for 30 min and then eluted with three successive elutions gave the highest viral recoveries. Table 4 also shows the results of further treating the filters by placing them into cartridge holders, filling the holders with beef extract, and placing them in a 36°C incubator for 30, 45, or 60 min, followed by equilibration to room temperature (approximately 30 min), before successive elution. Holding the filters for an additional 60 min before elution increased virus recoveries an average of 6% over unfrozen filters, 13% over filters eluted for 30 min, and 4% over those filters eluted for 75 min.

Sets of filters were challenged with virus-seeded water samples and stored at either 4 or −70°C for various time periods (Tables 5 and 6). All cartridges stored at 4°C showed a consistent decline in virus titer. By contrast, Filterite and K-27 filter cartridges stored at −70°C generally showed some increase, or at least a stable recovery, for up to 4 days. At day 5, there was significant virus loss, as was the case at 14 days. On the other hand, Balston filter cartridges consistently showed a loss of virus. Increased virus recovery from Filterite and K-27 filters can be attributed to the freeze-thaw effect, which may have either released some trapped virus particles that would not otherwise have been eluted or broken up aggregates.

In a separate series of tests, the survival of viruses was compared on Filterite cartridges and Zeta Plus 50SP cartridge filters (Table 7). When tested with $MgCl_2$-conditioned

TABLE 6. Recovery efficiency of virus adsorbed to cartridge filters after storage at −70°C

Total virus input (PFU/sample)	Filter Type	Control filter recovery[a] (%)	Storage time (days)	Virus recovery (%)	Difference (%)
291	Filterite	55	1	68	+13
	K-27	50	1	48	−2
	Balston	68	1	41	−27
136	Filterite	43	2	60	+23
	K-27	56	2	84	+28
	Balston	71	2	52	−19
274	Filterite	62	3	73	+11
	K-27	48	3	114	+66
	Balston	38	3	32	−6
214	Filterite	27	4	27	0
	K-27	55	4	56	+1
	Balston	40	4	35	−5
413	Filterite	66	5	27	−39
	K-27	50	5	23	−27
	Balston	22	5	10	−12
261	Filterite	89	14	33	−56
	K-27	67	14	46	−21
	Balston	45	14	36	−9

[a] Unfrozen control filters were eluted immediately after filtering virus-seeded samples.

TABLE 7. Virus recovery from Filterite and Zeta Plus 50SP filters after storage for 1, 2, and 3 days

Test sample	Total virus input (PFU/sample)	Filter type	Control filter recovery[a] (%)	Storage temp (°C)	% Virus recovery on day:		
					1	2	3
Water + $MgCl_2$ (pH 3.5)	288	Filterite	65	−70	68	65	64
Water without $MgCl_2$ (pH 3.5)	288	Filterite	59	−70	53	19	18
Water without $MgCl_2$ (pH 7.2)	112	Zeta Plus	31	4	16	29	20
				−70	12	12	21
	128	Zeta Plus	14	4	7	7	12
				−70	2	6	9

[a] Unfrozen control filters were eluted immediately after filtering virus-seeded samples.

and unconditioned water, the survival rate on Filterite filters was far greater in $MgCl_2$-conditioned water (3.5 times more at days 2 and 3) than in untreated water. Survival of viruses on the Zeta Plus 50SP filters, which were used in the pH range 7.0 to 7.5 with unconditioned water, was not only low in the stored samples but also low in the controls. Although recoveries were low, Zeta Plus filters did not show the extreme variation between filters stored at 4 and at −70°C that was seen with the other filters.

In addition to the storage studies, we tested the Filterite, K-27, and Balston filters (Table 8) in a replicate sampling series to determine the average virus recovery from these filters. Although Filterite is the filter recommended for this type of sampling, the K-27 gave comparable results; the Balston filters averaged 34% lower than the other two.

Elution of cartridge filters, especially successive elutions with 3% beef extract, generated considerable foam. As noted by other investigators (2, 11), the formation of foam can serve to concentrate viruses. In our case, this foam formation would cause a loss of viruses as this portion of the eluent would not be processed upon reconcentration. In an effort to reduce or eliminate this problem, we tested various concentrations of antifoam (Tables 9 and 10) for their effect on virus survival and their ability to improve virus recovery during elution. Even at a concentration of 1.0 ml per 100 ml of eluent, there was no substantial virus loss (Table 9). Subsequent tests conducted with elution of Filterite filters showed that only 0.1 ml of antifoam per 100 ml of eluent was necessary to control foaming; therefore, this was the concentration used for all testing. The addition of antifoam at this concentration increased viral recovery by an average of 29% (Table 10).

Physicochemical analyses were routinely performed on sample waters used for these tests (Table 11). Of 36 samples analyzed, none of the 22 test parameters cited could be correlated with any of the variations observed in virus

TABLE 8. Virus recovery from cartridge filters in a replicate sampling series

Filter type	Total virus input (PFU/sample)	% Virus recovery						
		Filter no.						Avg
		1	2	3	4	5	6	
Filterite	195	76	79	96	77	82	75	81
K-27	261	105	92	87	81	70	74	85
Balston	485	67	36	52	40	NT[a]	NT	49

[a] NT, Not tested.

TABLE 9. Effect of various antifoam concentrations on virus survival

Test no.	Total virus input (PFU/sample)	PFU recovered at the following concn of antifoam (ml/100 ml):									
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
1	214	247	234	211	203	204	207	210	220	208	181
2	107	109	116	112	112	93	119	130	111	91	NT[a]
3	45	53	43	45	42	62	51	47	50	41	44
Avg	122	136	131	123	119	120	126	129	127	113	113

[a] NT, Not tested.

recovery efficiencies of the test methods. The most interesting observation concerns the turbidity of tap water with the addition of either $MgCl_2$ or $AlCl_3$. The addition of 0.05 M $MgCl_2$ to tap water only slightly increased turbidity, whereas the addition of 0.0005 M $AlCl_3$ caused a 13-fold increase in turbidity above the average for water only. Also of note was the high pH of the water before any conditioning.

DISCUSSION

The ability to filter water samples in the field and subsequently transport the virus-laden filters to a laboratory for processing has several advantages. It reduces the equipment needed in the field, reduces the possibility of contamination, and permits greater sampling within a given time. During the course of this study, we encountered several problems that required some additional testing in conjunction with the transport part of the study. The preferred method for virus concentration required the addition of $AlCl_3$ salt to waters, followed by pH adjustment to 3.5 (7, 17). Using this procedure with Cincinnati tap water caused the water to become cloudy, but the addition of $MgCl_2$ did not. Chemical analysis of the local water (Table 11) showed that there was a substantial amount of sulfate present in the water. As noted by Sawyer and McCarty (13), its presence together with aluminum chloride under acid conditions will lead to formation of $Al_2(SO_4)_3$. Further chemical analysis verified that the precipitate was $Al_2(SO_4)_3$.

The formation of $Al_2(SO_4)_3$ may explain why lower virus recoveries were obtained with waters conditioned with $AlCl_3$ as opposed to $MgCl_2$. A method developed for virus concentration, in which $Al_2(SO_4)_3$ floc was used, showed that to recover the virus, the floc had to be completely dissolved, a process that required from 1 to 2 h (18). Therefore, virus was being trapped not only on the filters, but also in the $Al_2(SO_4)_3$ floc. Since the contact time for eluting virus from the filter is not sufficient to dissolve the $Al_2(SO_4)_3$ floc, sulfate-containing waters should be conditioned with $MgCl_2$ to optimize recovery rates. $MgCl_2$, although needed in larger amounts, was also recommended by Sobsey et al. (14, 16) as the better salt for recovery of certain viruses.

TABLE 10. Effect of antifoam on virus recovery from Filterite cartridge filters

Test no.	Total virus input (PFU/sample)	% Virus recovery	
		Without antifoam	With 0.1% antifoam
1	227	63	67
2	227	52	61
3	311	53	94
4	269	62	120
Avg	259	57	86

TABLE 11. Physicochemical characteristics of Cincinnati tap water

Parameter[a]	Value[b]		
	High	Low	Avg
Ca	47.1	31.1	38.8
Mg	9.8	7.1	8.8
Na	21.7	8.6	14.5
K	4.4	1.7	2.6
Cl	28.0	13.0	20.3
SO_4	122.0	60.0	77.3
NO_2	0.1	0.1	0.1
NO_3	1.6	0.1	0.9
NH_3	1.6	0.1	0.3
PO_4	0.7	0.1	0.2
Fe^{2+}	0.1	0.05	0.8
TOC	4.6	1.0	2.9
COD	14.0	4.0	9.8
TKN	6.0	0.1	0.6
TSS	32.0	0.6	8.8
TS	283.0	191.0	221.0
TVS	224.0	89.0	146.0
VSS	24.0	0.4	5.3
pH	9.0	7.2	8.4
Turbidity in water only	0.6	0.2	0.3
Turbidity in water + $MgCl_2$	1.5	0.2	0.4
Turbidity in water + $AlCl_3$	10.0	0.5	3.8

[a] Abbreviations: TOC, total organic carbon; COD, chemical oxygen demand; TKN, total Kjeldahl nitrogen; TTS, total suspended solids; TS, total solids; TVS, total volatile solids; VSS, volatile suspended solids.

[b] Expressed in parts per million, with the exception of pH, which is expressed as the negative logarithm of the concentration of the hydrogen ion in moles per liter, and turbidity, which is expressed in nephelometric turbidity units.

The high-pH glycine elution that had been the recommended procedure for eluting virus-laden cartridge filters (7, 15, 17) is no longer the method of choice. We subsequently found, as have others (10, 14, 16), that virus recovery was higher when beef extract was used as the eluent. Temperature was also a significant factor in virus recovery. Our studies with Filterite filters showed that viruses should be eluted at room temperature (23°C) as opposed to 4°C. On the other hand, there are only two viable options for shipping virus-laden filters from the field: pack in ice, in which case the temperature is ca. 4°C, or pack in dry ice at ca. −70°C. Joret and Block (8) found no difference between storage of filters at −26°C and at ambient temperatures, whereas Sobsey et al. (16) reported a virus loss of ca. 15% on holding filters at 4°C. References on laboratory storage of virus samples recommend holding temperatures of −70°C (1, 4). Testing virus stability at 4 and −70°C, we subsequently showed −70°C to be the preferred temperature for storage of cartridge filters.

Based on the data presented, the following recommendations are made for the collection, transport, and processing of environmental virus samples. (i) Use K-27 or Filterite cartridge filters for collection and transport of field samples. (ii) Place cartridge filters in sterile plastic bags and transport to the laboratory at −70°C. (iii) Process cartridge filters within 4 days to avoid virus loss. (iv) Thaw frozen filters by holding them at room temperature (23°C) for 30 min and then placing them in a 36°C water bath for 30 min. (v) Elute thawed filters with 1,600 ml of 3% beef extract and then

recycle the eluent twice through the filter. (vi) Control foaming during elution by adding antifoam at a rate of 0.1 ml per 100 ml of eluent.

Finally, we recommend that, where possible, waters be pretested by seeding with viruses. This will provide an analyst with data not only on which salt to use for maximum virus recovery but also on the recovery efficiency of the monitoring system.

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