Concentration of Enteroviruses from Large Volumes of Water

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An improved method for concentrating viruses from large volumes of clean waters is described. It was found that, by acidification, viruses in large volumes of water could be efficiently adsorbed to epoxy-fiber-glass and nitrocellulose filters in the absence of exogenously added salts. Based upon this finding, a modified version of our previously described virus concentration system was developed for virus monitoring of clean waters. In this procedure the water being tested is acidified by injection of N HCl prior to passage through a virus adsorber consisting of a fiber-glass cartridge depth filter and an epoxy-fiber-glass membrane filter in series. The adsorbed viruses are then eluted with a 1-liter volume of pH 11.5 eluent and reconcentrated by adsorption to and elution from a small epoxy-fiber-glass filter series. With this method small quantities of poliovirus in 100-gallon (378.5-liter) volumes of tapwater were concentrated nearly 40,000-fold with an average virus recovery efficiency of 77%.

To evaluate the potential public health hazard of enteric viruses in water, methods must be developed for detecting small quantities of virus in large volumes of water. We initially reported that viruses in aqueous fluids could be concentrated on membrane and other reactive surfaces in the presence of divalent cations (optimally 0.05 M Mg or Ca ions) (10). Other workers subsequently confirmed these findings (1, 3, 4). We also have shown that, in isotonic solutions containing organic compounds, viruses adsorbed to membranes more efficiently at lower pH levels than at neutral levels (10). These results were attributed to the fact that, in the presence of excess hydrogen ions, interfering organic compounds were not able to compete for membrane surface sites required by the virus. Recently, we showed that the use of salts for concentrating viruses from water in the field could be made logistically practical by the use of AlCl₃, a trivalent cation salt which required 100- to 200-fold less salt concentration than divalent cation salts for virus attachment to membranes or textile filters (5). Studies with insoluble polyelectrolytes revealed that salts interfered with virus adsorption at increased hydrogen ion levels (6, 11). A study was therefore conducted to determine if, by mere acidification, viruses in water could be concentrated in the absence of added salts on surfaces heretofore reported to require metallic ions for virus attachment.

MATERIALS AND METHODS

Baboon kidney cells. Kidneys obtained from immature baboons were trypsinized and grown as described (2).

Virus and virus assays. Plaque-purified lines of poliovirus type 1, strains Mahoney and LSc, echovirus type 7 (Wallace), and coxsackievirus types A9 (Grigg) and B3 (Nancy) were used. Stock viruses were grown in baboon kidney cells by using an input of 1 to 10 plaque-forming units (PFU) per cell and stored at −70 C. Viruses were diluted in tris(hydroxymethyl)-aminomethane-buffered saline containing 2% fetal calf serum and were assayed by the PFU method as used in this laboratory (2).

Virus adsorbents. Nitrocellulose filters (type HA, Millipore Corp., Bedford, Mass.), epoxy-fiber-glass filters (series AA, Cox Instrument Corp., Detroit, Mich.), and 10-inch (25.4-cm) honeycomb, fiber-glass depth filters, 5-μm nominal porosity (model K27, Commercial Filters Division, Carborundum Co., Lebanon, Ind.) were used. The general methods for concentrating viruses on filter surfaces (5, 10) and the characteristics of the honeycomb depth filters (7) have been described in detail elsewhere. The K27 filters were housed in see-through cartridge holders fitted with a relief valve and rated at 100 psi in normal temperature ranges (Fig. 1).

Virus eluent and elution procedure for K27 filters. Glycine (0.05 M) adjusted to pH 11.5 with NaOH was used to elute viruses off virus adsorbents (5). The pH 11.5 eluate obtained was made neutral with 0.05 M glycine adjusted to pH 1.0 with HCl. Viruses were eluted off the 10-inch, K27 depth filters by passing a 1-liter volume of eluent into the filter holder. The relief valve on the holder was opened so
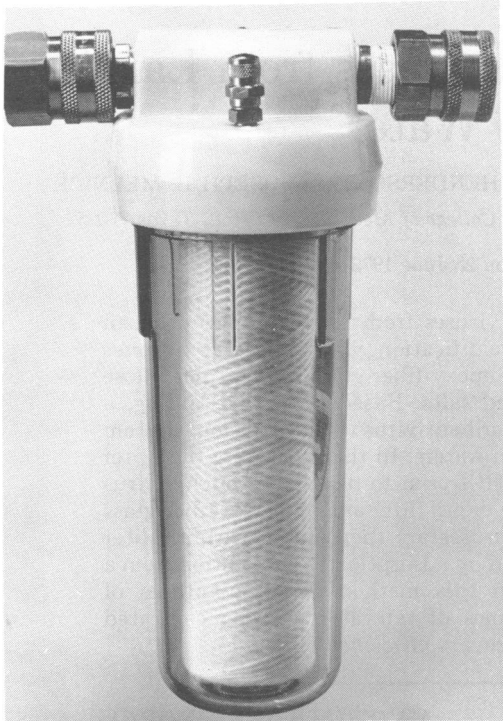
FIG. 1. *K27 fiber-glass depth clarifier in see-through holder.*

that the eluent entered and completely filled the holder, and then the valve was closed. If this valve is not opened, the fluids rise rapidly in the outlet channel and very slowly on the inlet side of the filter. The holder was inverted, positive pressure was applied via a nitrogen tank, and the eluate was then collected in a reservoir and neutralized with pH 1.0 glycine-hydrochloric acid.

Tapwater. Houston tapwater contains an average of about 450 mg of total dissolved solids per liter. When used, it was first dechlorinated with 2 mg of sodium thiosulfate per liter.

RESULTS

Effect of pH on virus adsorption to nitro-cellulose and epoxy-fiber-glass filters in salt-free buffer and in tapwater. A series of experiments was conducted to determine the effect of increased hydrogen ion concentration on virus adsorption from salt-free buffer and tapwater to both Millipore and Cox filters, 0.45-μm porosity. Volumes (100 ml) of tapwater or 0.05 M glycine in distilled water containing poliovirus type 1, strain Mahoney, were adjusted to pH 7, 6, 5, 4, and 3 with 0.1 N HCl. Additional 100-ml volumes of pH 7 glycine and pH 7.8 tapwater were treated with $MgCl_2$ to a final 0.05 M concentration. Volumes (50 ml) of each sample were filtered at 5 psi through

25-mm diameter Millipore or Cox filters, and the filtrates were assayed for unadsorbed virus. The results of this experiment are shown in Table 1. In 0.05 M glycine the Millipore filters removed more than 99% of the virus at pH 3.0 and less than 20% of the virus at higher pH levels, whereas the Cox filters removed more than 99% of the virus at pH 3 and 4, 90% at pH 5, and less than 30% at pH 6 or 7. In tapwater the Millipore filters removed more than 99% of the virus at pH 3.0, 95% at pH 4.0, and less than 55% at higher pH levels, whereas the Cox filters removed more than 99% of the virus at pH 3 and 4, and 98, 94, 94, and 42% at pH 5, 6, 7, and 7.8, respectively. Both filters removed virus efficiently from pH 7 glycine and pH 7.8 tapwater containing 0.05 M $MgCl_2$. The improved removal of virus from tapwater by both Cox and Millipore filters as compared to 0.05 M glycine may have been due to the enhancing effects of the salts which are naturally present in the tapwater.

Other representative enteroviruses, including poliovirus type 1, strain LSc, echovirus type 7, and coxsackieviruses A9 and B3, were tested to determine if they were efficiently adsorbed to Cox filters from tapwater at acid pH levels. Volumes (100 ml) of pH 7.6, 6.0, 5.0, and 4.0 tapwater, experimentally contaminated with

TABLE 1. *Comparative adsorption of poliovirus to membrane surfaces in salt-free buffer and tapwater at different pH levels*

pH	Average no. of PFU/0.1 ml		
		Membrane filtrates	
	Unfiltered control	Fiber glass-epoxy	Nitro-cellulose
0.05 M glycine			
pH 7.0 + 0.05 M MgCl$_2$	48	0	0
7.0	42	44	44
6.0	46	33	50
5.0	50	5	47
4.0	51	0	40
3.0	51	0	0
Tapwater			
pH 7.8[a] + 0.05 M MgCl$_2$	50	0	0
7.8[a]	52	30	41
7.0	49	3	22
6.0	52	3	24
5.0	49	1	23
4.0	56	0	3
3.0	51	0	0

[a] Natural pH of the tapwater.

the aforementioned viruses, were passed through 25-mm diameter, 0.45-μm porosity Cox filters at 5 psi, and the filtrates were assayed for remaining virus. Coxsackieviruses A9 and B3 were efficiently adsorbed to Cox filters at all pH levels tested, whereas poliovirus type 1, strain LSc, and echovirus type 7 were efficiently adsorbed only at pH 4.0 (Table 2).

The results of these experiments indicate that enteroviruses are efficiently adsorbed to both Cox and Millipore filters in the absence of added polyvalent cations at acid pH levels. Because Cox filters adsorbed poliovirus type 1, strain Mahoney, more efficiently than Millipore filters at acidic pH levels, and because they are more durable and less susceptible to clogging than Millipore filters, Cox filters were used in all further experiments. Based on the observation that echovirus type 7 and poliovirus type 1, strain LSc, efficiently adsorbed to Cox filters only at pH 4.0, all further experiments were conducted at pH 4.0 or lower.

Adsorption of poliovirus in large volumes of tapwater to fiber-glass filters at acidic pH levels. In preliminary experiments it was observed that the filtration of large volumes of Houston tapwater, which contains high levels of ferric compounds, would readily clog membrane filters of the Cox or Millipore type. Therefore, a 10-inch fiber-glass K27 depth filter was used to clarify Houston tapwater with subsequent concentration of viruses on a Cox filter mounted downstream from the K27. We have previously described the sparing effects of K27 filters on downstream membrane-type filters by their avidity to adsorb ferric complexes (7). Houston tapwater (100 gallons) was placed in a polyethylene drum and dechlorinated. Type 1 poliovirus, strain LSc, was added so that the virus concentration was 1,680 PFU/0.1 ml. The tap-

water was adjusted to pH 3.5 by slowly adding 1 N HCl while mixing the water with bubbling compressed air. The water was pumped through a K27 filter and then through a 142-mm diameter, 0.65-μm porosity Cox filter in series at a flow rate of 1 gallon (3.785 liters) per minute, and filtrate samples were monitored for virus after the first gallon and at 20-gallon (75.7-liter) intervals thereafter. Samples of the K27 filtrate were obtained prior to entering the Cox filter by means of a bleed valve. A Cox filter of 0.65-μm porosity was used in preference to one of 0.45-μm porosity because the former was capable of processing tapwater more rapidly than the latter while still adsorbing viruses efficiently (unpublished results). The experimental results are shown in Fig. 2. Although the K27 filter adsorbed 50% or more of the virus, the remaining portion passed this filter and was completely adsorbed to the Cox filter, as no virus was detectable in the final effluent. We have previously reported that K27 filters will avidly adsorb viruses in the presence of polyvalent cations such as Mg or Al ions (A. Homma, M. D. Sobsey, C. Wallis, and J. L. Melnick, Water Res., in press; reference 8). The inefficiency of the K27 to adsorb 100% of the virus under test in the presence of excess hydrogen ions as in the experiment above may be due to the absence of added polyvalent cations. The somewhat increased adsorption of virus to the K27 filter as filtration proceeded in this experiment may be due to the accumulation of ferric complexes on the fiber-glass surface, which acted as an additional virus adsorbent. From the results of this experiment it was concluded that a K27 fiber-glass filter and a 142-mm diameter, 0.65-μm porosity Cox filter in series would serve as an effective virus adsorbent for large volumes of tapwater.

Elution and reconcentration of virus from K27 and Cox filters in series. We previously reported that viruses adsorbed to K27 filters can be eluted with a 1-liter volume of pH 11.5 eluent and that the viruses in this eluate can be reconcentrated by adsorption to smaller, membrane-type filters at pH 5 to 6 and in the presence of Mg ions, followed by elution with less than 10 ml of pH 11.5 eluent (8). In preliminary experiments it was observed that the eluate obtained from the K27-Cox filter series described above also contained suspended material of tapwater origin which deposited on the 47-mm diameter Cox filters used for reconcentration. A series of experiments was conducted to identify these suspended solids and determine if they would interfere with virus reconcentration.

TABLE 2. *Adsorption of other enteroviruses to Cox membranes in acidified tapwater*

Viruses[a]	Unfiltered control, pH 7.6[b]	Average no. PFU/0.1 ml in Cox filtrates at pH			
		4	5	6	7.6[b]
Type 1 poliovirus, LSc	67	0	11	24	31
Echovirus type 7	52	1	11	22	42
Coxsackievirus A9	110	0	0	0	4
Coxsackievirus B3	102	0	0	0	1

[a] Samples of viruses at the acidified pH levels were assayed prior to filtration and found to be essentially the same as the pH 7.6 unfiltered control tabulated in the table. Therefore, these control data were omitted for the sake of clarity.

[b] Natural pH of tapwater.

Average no. of
PFU/0.1 ml

100 gallons (378.5 liters)
of dechlorinated tap-
water containing polio-
virus

├─Input virus───────────   1,680

Adjusted to pH 3.5

│─────────────────────   1,700

Filtered through K27
clarifier-0.65-μm Cox
filter in series at 1   Filtrates
gallon per minute   K27   0.65-μm Cox

├─1 gallon (3.785 liters)───── 810   0

├─20 gallons (75.7 liters)───── 790   0

├─40 gallons (151.4 liters)──── 610   0

├─60 gallons (227.1 liters)──── 540   0

├─80 gallons (302.8 liters)──── 490   0

└─100 gallons (378.5 liters)─── 450   0

FIG. 2. *Concentration of virus from large volumes of tapwater.*

An experiment was performed to determine the effect of K27-Cox eluates on virus interaction with the Cox filters used for virus reconcentration. A 3-liter eluate from the processing of 1,000 gallons (3,785 liters) of virus-free tapwater through a K27-Cox filter series was adjusted to pH 3.5 and divided into three 1-liter volumes. To test for direct interference with virus adsorption to Cox filters, type 1 poliovirus, strain LSc, was added to one volume of the eluate to give a virus concentration of about 5×10^4 PFU/ml. The eluate was filtered through a 47-mm diameter, 5- and 0.45-μm porosity Cox filter series, and the filtrate was assayed for virus. It was found that about 65% of the virus was present in the filtrate, indicating that there was substantial interference with virus adsorption.

To determine if these interfering components were membrane-coating components (10), a second 1-liter volume of eluate, containing no virus, was filtered through another 47-mm diameter Cox filter series and the filtrate was discarded. The filters were then challenged with 100 ml of pH 3.5 tapwater containing 5×10^4 PFU/ml of poliovirus, and the tapwater filtrate was assayed for virus. Only 3% of the total amount of virus was found in the filtrate, indicating that the interfering components were not membrane-coating components.

To determine if these virus-interfering components would elute virus from the Cox filters used for virus reconcentration, 100 ml of pH 3.5 tapwater containing 5×10^4 PFU/ml of poliovirus was filtered through another set of freshly prepared, 47-mm diameter Cox filters. The

tapwater filtrate was assayed for virus and none was found, indicating that all virus had adsorbed to the filters. The filters were then challenged with the third 1-liter volume of K27-Cox eluate, and the filtrate was assayed for virus. Only 3% of the adsorbed virus was found in the filtrate.

From the results of this series of experiments, it was concluded that components of tapwater origin in K27-Cox eluates would, upon reconcentration, interfere with virus adsorption but would not coat filters or elute viruses already adsorbed to filters.

To further elucidate the nature of the interfering material in K27-Cox filter eluates obtained from processing large volumes of tapwater, an experiment was conducted in which eluates were treated with a variety of ion exchange resins and absorbents. Volumes (1-liter) of pooled K27-Cox eluates from 250-gallon (946.25-liter) volumes of virus-free tapwater were adjusted to pH 7.5 and treated with 200-g quantities of ion exchange resins in columns, including anion, cation, mixed bed and organic adsorbent types. Poliovirus was added to the treated eluates, they were adjusted to pH 3.5, and filtered through 47-mm diameter, 5- and 0.45-μm porosity Cox filters in series. The filtrates were assayed for virus. The results of this experiment are shown in Table 3. Significant amounts of virus were found in the filtrates of untreated and cation resin-treated eluates. Cox filtrates of eluates treated with anion, mixed bed or organic adsorbent resins contained little or no virus, indicating that the virus-interfering components are anionic, organic compounds. It should be noted that the removal of virus-interfering components from wastewater (9) and from municipal solid waste extracts and municipal solid waste landfill leachate (M. L. L. Peterson, Ph.D. thesis, Univ.

TABLE 3. *Effect of ion exchange resins on the virus-interfering components in tapwater eluates from K27-Cox filters*

Resin	Type	Form	Percentage of virus in Cox filtrate
Control, no resin			25
Duolite[a] ARC-249	cation	H$^+$	42
Ionac[b] C-249	cation	H$^+$	36
Duolite ARM-381	mixed bed	H$^+$ + OH$^-$	0
Duolite ARA-366	anion	OH$^-$	1
Dowex[c] 1-X8	anion	Cl$^-$	1
Duolite S37	organic adsorbent		0

[a] Diamond Shamrock Co., Redwood City, Calif.
[b] Ionac Chemical Co., Birmingham, N.J.
[c] Bio-Rad Laboratories, Richmond, Calif.

of Michigan, 1972) by anion resins has been previously reported.

Because previous studies with sewage have shown that Al ions enhance virus adsorption to filter surfaces in the presence of interfering material (A. Homma et al., in press), an experiment was performed to determine if the virus interference in K27-Cox eluates could be overcome with Al ions. K27-Cox eluate (4 liters) obtained from 1,600 gallons (6,056 liters) of tapwater was dosed with poliovirus to give a concentration of 5×10^4 PFU/ml, adjusted to pH 3.5, and divided into four volumes of 1 liter each. One eluate volume received no $AlCl_3$, and the remaining three volumes received final $AlCl_3$ concentrations of 0.0005, 0.0015, and 0.005 M, respectively. Each eluate was filtered through a 47-mm diameter, 5- and 0.45-μm porosity Cox filter series at 10 psi, and the filtrate was assayed for virus. The filtrates from samples with no $AlCl_3$, 0.0005 M, 0.0015 M, and 0.005 M $AlCl_3$ contained 26, 2, 0, and 0%, respectively, of the total amount of virus present before filtration. These results indicate that the virus-interfering material in K27-Cox eluates from the processing of tapwater can be easily overcome by adding a final 0.0005 or 0.0015 M concentration of $AlCl_3$ to the eluate. Therefore, in all further experiments virus interference was overcome with Al ions rather than removed with resins because the former was economically and logistically more practical.

Concentration of small quantities of poliovirus from large volumes of tapwater. Based on the results of the experiments described above, a modified form of the portable virus concentrator developed in this laboratory (8) was constructed for the processing of clean waters. This modified virus concentrator differed from the original instrument in that it: (i) contained no clarifier filters, (ii) had only a single injection system for acidifying the tapwater with 1 N HCl, and (iii) used a virus adsorber consisting of a K27, 10-inch cartridge depth filter and a 142-mm diameter, 0.65-μm porosity Cox filter in series. The instrument was tested for its ability to concentrate small amounts of exogenously added poliovirus from large volumes of tapwater by the following procedure. In the first step of the procedure (Fig. 3, step A), 100 gallons of dechlorinated Houston tapwater in a polyethylene drum was experimentally contaminated with a small quantity of type 1 poliovirus, strain LSc. As the tapwater was pumped out of the drum at a flow rate of 2 gallons per minute, 1 N HCl was injected into the water via a metering valve as previously

described (8). The pH was monitored with a battery-operated pH meter equipped with a flow-through combination electrode which was mounted downstream from the acid injector. HCl injection was controlled by manual adjustment of the metering valve to give the tapwater a pH of 3.5. The pH 3.5 tapwater passed through the virus adsorber described above. After the entire 100 gallons of tapwater was processed, the viruses on the K27-Cox filters were eluted with 1 liter of pH 11.5 glycine-NaOH eluent (Fig. 3, step B). The eluate was adjusted to pH 3.5 with pH 1.0 glycine-hydrochloric acid, and $AlCl_3$ was added to a final concentration of 0.0005 M to reconcentrate the virus in the 1-liter eluate on a smaller surface. The viruses in the eluate were reconcentrated by filtration through a 47-mm diameter, 5- and 0.45-μm porosity Cox filter series (Fig. 3, step C). The filters were washed with 25 ml of pH 3.5 physiological saline to remove excess Al ions, and the viruses adsorbed to the filters were eluted with 7 ml of pH 11.5 glycine-NaOH. The eluate was adjusted to pH 7.5 with pH 1.0 glycine-hydrochloric acid, rendered isotonic with NaCl, made up to a volume of 10 ml, and assayed for virus. With this procedure the virus particles in 100 gallons of tapwater were concentrated nearly 40,000-fold. The results of five replicate experiments in which low virus con-
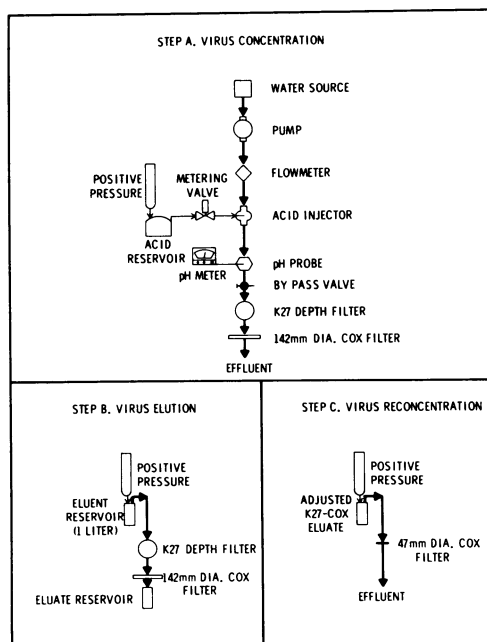

FIG. 3. *Diagrammatic representation of some important steps in the virus concentration and reconcentration procedure for testing water.*

centrations were used indicate that viruses were efficiently concentrated and reconcentrated by this procedure with recoveries ranging from 48 to 95% (Table 4).

DISCUSSION

The results of this study indicate that, by acidification, viruses can be efficiently adsorbed from water to fiber-glass and nitrocellulose filter surfaces in the absence of exogenously added polyvalent cation salts. Epoxy-fiber-glass (Cox) filters were found to be superior to nitrocellulose (Millipore) filters for virus adsorption because they were more efficient under acidic conditions, more durable, and less susceptible to clogging. Under acidic conditions, viruses in large volumes of tapwater were efficiently concentrated on a virus adsorber consisting of a 10-inch K27 fiber-glass depth filter and a 142-mm diameter, 0.65-μm porosity Cox filter in series. Although the eluate from this adsorber series contained components of tapwater origin which were capable of interfering with subsequent virus reconcentration by adsorption to smaller, membrane-type filters, this interference was overcome by adding low concentrations of AlCl$_3$ to the eluate. Based upon the aforementioned findings, a modified, portable virus concentrator was developed which differed from the original unit (8) in that it had: (i) no clarifying filters, (ii) a single injection system for acidifying the water being processed, and (iii) a K27-Cox filter series as a virus adsorber. The modified procedures used for virus concen-

tration has advantages over the previously described procedure (5, 8) because the elimination of clarifying filters makes it less expensive, and the elimination of salt addition makes it operationally easier. When tested with small quantities of virus in 100-gallon volumes of tapwater, this modified unit was capable of concentrating the viruses nearly 40,000-fold with an average recovery efficiency of 77%. We believe this improved portable virus concentrator can be used in the field to efficiently monitor large volumes of clean water for enteric viruses.

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TABLE 4. Concentration of small quantities of poliovirus from 100 gallons of tapwater

Exp no.	Total input virus[a] (PFU)	Calculated tapwater virus concentration (PFU/gal)	Total virus in final eluate (PFU)	Percentage of total input virus in final eluate
1	380	3.8	360	95
2	3,000	30	1,900	63
3	1,600	16	1,400	88
4	1,200	12	1,100	92
5	2,500	25	1,200	48

[a] Based upon assay of the stock poliovirus added to 100 gallons of tapwater.