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Concentration of Enteroviruses from Large Volumes of Tap Water, Treated Sewage, and Seawater

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Methods are described for the efficient concentration of an enterovirus from large volumes of tap water, sewage, and seawater. Virus in acidified water (pH 3.5) in the presence of aluminum chloride was adsorbed to a 10-inch (ca. 25.4 cm) fiberglass depth cartridge and a 10-inch pleated epoxy-fiberglass filter in a series at flow rates of up to 37.8 liters (10 gallons) per min. Adsorbed viruses were eluted from the filters with glycine buffer (pH 10.5 to 11.5), and the eluate was reconcentrated by using a combination of aluminum flocculation followed by hydroextraction. With this procedure, poliovirus in large volumes of tap water, seawater, and sewage could be concentrated with an average efficiency of 52, 53, and 50%, respectively. It was demonstrated that this method is capable of detecting surface solid-associated viruses originating from sewage treatment plants. No difference in virus recovery between laboratory batch studies and a set-up with acid-salt injection was found. This unified scheme for the concentration of viruses has many advantages over previously described systems. These include: high operating flow rates, low weight and small size, effectiveness with a variety of waters with widely varying qualities, and filters with a high resistance to clogging.

The membrane filter adsorption-elution method (29) continues to be the most promising method for the concentration of enteric viruses from large volumes of water and wastewater (24). The first portable field system with membrane filters for virus concentration (28) has had several modifications described by other investigators (12, 14, 16, 17); they include the use of different filters as primary virus adsorbents and a proportioner pump for adding salts and acid to enhance virus adsorption. These systems have efficiently concentrated a variety of viruses from up to 500 gallons (1,900 liters) of finished tap water (14), but difficulties have been encountered with waters containing large amounts of suspended inorganic matter and soluble organic matter (7, 14, 15, 25). Suspended matter tends to clog the adsorbent filters, thus reducing the amount of water that can be processed. The use of clarifying filters in front of the adsorbing filters reduces the magnitude of this problem, but can result in the decreased efficiency of virus recovery because of the loss of solid-associated virus (15, 20). In addition, as solids accumulate on the clarifying filters, the overall efficiency of virus recovery is further reduced (15, 25). Soluble organic matter in certain waters, most notably sewage and seawater, also interferes with the adsorption of viruses by competing with the virus for adsorption sites on the filter surface (24). Organic matter can also be a problem when reconcentration of the initial eluate is attempted, because of its tendency to form precipitates that result in clogging of the next set of filters used to reconcentrate the initial eluate (4, 6, 7, 22, 25).

Another limitation has been the long processing time necessary to sample large volumes of water because of the relatively slow flow rates with the microporous filters previously used (14, 26, 27). Even with finished tap water, average operating flow rates range from only 1 to 3 gallons (2 to 4 liters) per min because of the limited filter surface area (14, 26, 27).

We reported recently on the advantages of pleated membrane filters for the concentration of viruses from tap water (4) and seawater (22). Use of these filters eliminates many of the problems encountered with previous models of the virus concentrator. The current study is concerned with the development of a unified scheme, with pleated membrane filters, for concentration of viruses from large volumes of test waters of different constitutions, namely, tap, waste, and marine waters.

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MATERIALS AND METHODS

Virus and viral assays. Plaque-purified poliovirus type 1 (strain LSc) was used in all experiments. The BGM cell line (2), kindly supplied by Gerald Berg (Environmental Protection Agency, Cincinnati, Ohio), was used for viral assays. Samples were assayed after being made isotonic and after fetal calf serum was added to make a final concentration of 2% or after dilution in tris(hydroxymethyl)aminomethane-buffered saline containing 2% fetal calf serum. Virus stocks were partially purified and concentrated by membrane chromatography (11) before use.

Virus adsorbents. Glass fiber, melamine-impregnated paper, epoxy filters pleated into 10-inch (ca. 25.4-cm) cartridges (Duo-Fine series, Filterite Corp., Timonium, Md.) with nominal porosities of 0.25-, 0.45-, and 3-μm were used. Ten-inch (ca. 25.4-cm) honeycomb, fiberglass depth filters (model K27, available from the Water Management Group, The Carborundum Co., Niagara Falls, N.Y.) were also used. The characteristics of the Filterite pleated filter and the Carborundum honeycomb depth filter have been described previously (4, 27).

Batch method of virus concentration. In this procedure, 19 to 1,000 liters (5 to 265 gallons) of the water being tested were placed in 40- to 1,900-liter (10- to 500-gallon) plastic tanks and adjusted to pH 3.5 by addition of 12 N HCl. Concentrated acid was used for experimental convenience since the pH was adjusted before the addition of the virus. Lower concentrations of acid must be used in field work to prevent viral inactivation (20). Aluminum chloride (1 M) was then added to the final concentrations indicated in Table 1. A 100- to 1,000-ml sample of the water to be processed was removed from the tank, and seed virus was added. One-half of the sample was then returned to the tank, and virus in the other half was titered at the beginning and conclusion of the concentration step to determine viral die-off in the tank during the virus concentration procedure. Usually, no viral die-off occurred during the concentration procedure or was too small to be considered in determining concentration efficiency. The water in the tank was pumped through the virus concentrator diagrammed in Fig. 2, but without the acid-salt injector in operation. High concentrations of poliovirus were added to the treated sewage samples when determining efficiency to prevent interference from viruses naturally occurring in the sewage.

In-line injection method of virus concentration. In this procedure, 1 liter of the water to be sampled was first titered with 0.2 N HCl to determine the amount of acid necessary to adjust the pH to 3.5. The number of milliliters of 0.2 N HCl was then multiplied by the number of liters to be sampled, and this volume was added to a stainless steel pressure vessel along with 0.5 ml of 1 M $AlCl_3$ (1.5 ml when seawater was sampled) for each liter of water to be sampled. In practice, a 10% excess of acid and salt was added to prevent the acid-salt reservoir from being completely depleted at the termination of sampling. After complete mixing of the acid and salt, the pressure vessel was connected to a virus concentrator. (For details of the injector, filter housings, etc., see Wallis et al. [28].) The entire concentrator (excluding the pump) was mounted on a flat board (45 by 70 cm), allowing it to be easily transported as a compact unit. Virus was then added to the tank as described in the batch method, and the water in the tank was passed through the virus concentrator. Table 1 gives the details of concentration, elution, and reconcentration procedures for the three types of water studied.

Virus elution from filters. When tap water was processed, the virus that adsorbed to the filters was eluted by passing 1,600 ml of 0.05 M glycine (adjusted to pH 10.5 by addition of 10 N NaOH) through the filters five times. The glycine was permitted to remain in contact with the filters for about 1 min during each passage. Eluates were neutralized immediately after collection with 0.05 M glycine adjusted to pH 2 with 12 N HCl.

When seawater or sewage was processed, the viruses that adsorbed to the filters were eluted by passing 1,600 to 2,000 ml of glycine buffer (adjusted to pH 11.5) once through the filters. The glycine was permitted to remain in contact with the filters for about 1 to 2 min before being forced out of the filter housing with positive pressure. Again, the eluates were immediately neutralized after collection.

Because of the sensitivity of viruses to inactivation at high pH, the pH electrode should be carefully standardized before use (7).

Water samples. Estuarine water samples were obtained from coastal canal communities in the Galveston Bay area, about 50 miles (ca. 80.46 km) from Houston, Tex., on the Gulf of Mexico. Turbidity was measured with a turbidimeter (model 2100A, Hach Chemical Co., Ames, Iowa), and salinity was measured with an AO T/C refractometer (AO Instruments Corp., Buffalo, N.Y.). Turbidity of samples ranged from 6 to 19 Jackson turbidity units (JTU), whereas salinity ranged from 15 to 20 g/kg.

Chlorinated secondary effluent was obtained from a local activated sludge sewage treatment plant servicing a residential area of Houston. Turbidity of this sewage ranged from 4 to 8 JTU. In the experiment shown in Fig. 3, sewage was obtained from a trickling filter plant and had a JTU of 13.

Finished drinking water was obtained directly from a Houston tap (dissolved solids, 460 ppm; solids in suspension, 337 mg/liter). Before use, a solution of sodium thiosulfate was added to tap water and sewage in sufficient quantity to eliminate any free chlorine.

Reconcentration of eluates. Viruses were reconcentrated from the initial eluates by using a combination of aluminum flocculation followed by hydroextraction as described by Farrah et al. (5).

RESULTS

Comparison of virus recovery from tap water, treated sewage, and seawater. It has been recommended previously that less than one infectious unit of virus per 10 gallons (ca. 37.85 liters) be present in recreational water, and less than one virus in 100 to 1,000 gallons (ca. 378 to 3,785 liters) of drinking water (19). Although such standards are arbitrary, it is clear that sensitive methods are needed to evaluate the occurrence of these agents in drinking and natural waters so that appropriate decisions on the

need, if any, for viral standards are justifiable. In addition, methods are needed for evaluating the effectiveness of present and proposed sewage treatment processes in removing pathogenic viruses. Since concentration methods are less than 100% efficient, systems capable of processing relatively large amounts are required. For these reasons, sample sizes of 472 liters (125 gallons) of tap water, 378 liters (100 gallons) of seawater, and 19 to 190 liters (5 to 50 gallons) of secondarily treated sewage were used to evaluate the efficiency of virus recovery from these waters. The water samples were placed in either 378-liter (100-gallon) or 1,900-liter (500-gallon) polyethylene tanks. After adjusting the pH and adding salts and virus, the water samples were pumped through the concentrator described in Fig. 1.

We have described previously the initial concentration step using pleated membrane filters for tap water (4) and seawater (22). These steps are summarized in Fig. 1 and 2 and in Table 1. The initial concentration step for sewage in this study was identical to that used for seawater. Reconcentration was conducted by a combination of aluminum flocculation followed by hydroextraction (5). The overall virus recovery was 52% from 472 to 1,000 liters (125 to 265 gallons) of tap water, 53% from 378 liters (100 gallons) of seawater, and 50% from 19 to 190 liters (5 to 50 gallons) of secondarily treated sewage (Table 2).

In-line injection versus batch method. The previous experiments were performed without the in-line injection of acid and salts; but under field conditions and when very large volumes are processed, in-line injection is necessary. The work reported here and in our previous studies on virus concentration methodology (4, 22, 26) has been conducted largely with the batch method because of its simplicity. To validate this procedure for comparison to the in-line injection method, a series of side-by-side experiments was performed in which two tanks were filled with 472 liters (125 gallons) each of Houston tap water. After addition of virus, the water in one tank was processed by the in-line injection method (Fig. 2) and the other by the batch method. The results shown in Table 3 indicate that both methods gave comparable results in efficiency of virus concentration.

Resistance of microporous pleated and tube filters to clogging. Previously, we have compared the membrane material of the pleated filters to other commercially available filter material and found the pleated filter to be far superior in its resistance to clogging (4). A microporous tube filter (Balston Inc., Lexington, Mass.) has also been suggested as an alternative to flat-disk membrane filters (17). These filters were compared to pleated membrane filters to determine their ability to resist clogging with treated sewage. The pleated filters are far more resistant to clogging and offer a decided advantage over the tube filter (Fig. 3). In addition, the tube filters cannot be used when the differential pressure exceeds 25 lb/in^2, whereas the pleated filters can be used against a differential pressure of 100 lb/in^2. This allows for much greater flow rates with the pleated filters, without the filter rupturing. Rupturing was a major problem when the Balston filters were used with sewage and seawater, i.e., the pressure built up very rapidly as the membrane clogged and resulted in rupturing the filter (Fig. 3).

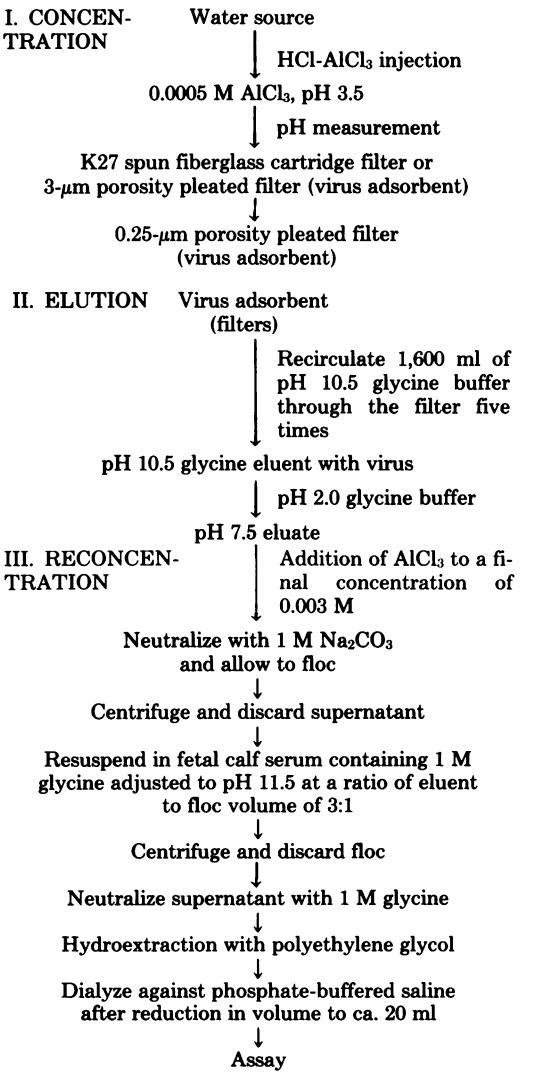

FIG. 1. *Scheme for virus concentration from tap water.*

Recovery of solid-associated virus. Recent findings indicate that a significant number of viruses found in sewage (31) and other waters (9, 23) may be associated with suspended solids. Thus, an experiment was performed to determine whether viruses associated with sewage solids could be detected by the described filter system. Virus was first adsorbed to activated sludge solids obtained from a local wastewater treatment plant by mixing the solids at pH 3.5 and 0.0005 M $AlCl_3$ with virus for 30 min. The solids were collected by centrifugation, suspended in 20 liters of dechlorinated tap water at pH 3.5 and 0.0005 M $AlCl_3$ and passed through a K27 and a 0.25-μm, 25-cm Filterite filter. Virus associated with the solids entrapped on the filters was eluted by passing 1,600 ml of pH 11.5 glycine through the filters. Essentially all of the virus was eluted from the solids during treatment of the filters with the glycine eluent (Table 4).

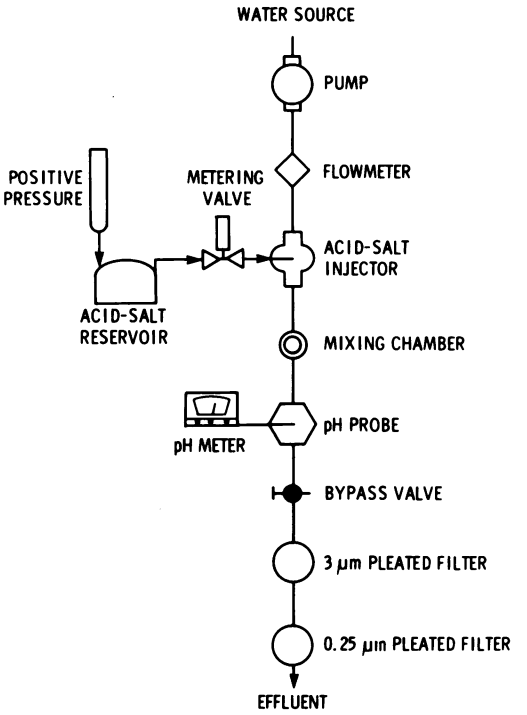

FIG. 2. *Diagrammatic representation of the virus concentrator.*

DISCUSSION

The first field system for concentrating enteroviruses from large volumes of water was described by Wallis et al. (27) and was based on methods they had developed for concentrating enteroviruses on membrane filters (29). The procedure for virus concentration involved passing the water being examined through a series of five textile filters followed by treatment with an anion-exchange resin to remove organic matter before adsorption of the virus onto a 293-mm diameter cellulose nitrate disk filter (27). This device was limited to finished tap water and was much too bulky for routine field use. Further improvements led to a system with only a fiberglass depth filter followed by a series of flat disk, epoxy-fiberglass membrane filters (26). For use with sewage and turbid seawater, an additional set of three clarifying filters of various porosities was added to the system (3, 20). This was the

TABLE 1. *Concentration, elution, and reconcentration procedures for tap water, seawater, and treated sewage*

Type of water	Concn			Elution		Reconcn	
	$AlCl_3$ concn (M)	pH	Adsorbent filters	pH	Times eluent passed through filters	1- to 100-gallon sample	100- to 500-gallon sample
Tap water	0.0005	3.5	Fiberglass depth filter (K27) and/or 0.25-μm porosity pleated filter	10.5 or 11.5	5 or 1	Membrane filters (28)[a]	$AlCl_3$ flocculation followed by hydroextraction (5)
Seawater	0.0015	3.5	Fiberglass depth filter (K27) or 3.0-μm porosity pleated filter and 0.45-μm porosity pleated filter	11.5	1	$AlCl_3$ flocculation followed by hydroextraction (5)	$AlCl_3$ flocculation followed by hydroextraction (5)
Secondarily treated sewage	0.0005	3.5	Fiberglass depth filter (K27) or 3.0-μm porosity pleated filter and 0.45-μm porosity pleated filter	11.5	1	$AlCl_3$ flocculation followed by hydroextraction (5)	$AlCl_3$ flocculation followed by hydroextraction (5)

[a] Numbers in parentheses represent reference numbers.

TABLE 2. *Recovery of poliovirus from large volumes of tap water, seawater, and treated sewage*

Type of water	Expt no.	Vol of sample (liters)	PFU of virus added to sample	PFU recovered (%)
Tap water	1	472	1.5×10^6	41
	2	472	1.1×10^6	82
	3	472	4.2×10^5	46
	4	1,000	2.9×10^3	50
	5	1,000	2.2×10^3	41
				Mean 52
Seawater	1	378	2.0×10^7	46
	2	378	4.2×10^6	42
	3	378	1.9×10^4	62
	4	378	9.6×10^3	51
	5	378	1.7×10^3	63
				Mean 53
Secondarily treated sewage	1	19	6.8×10^7	32
	2	19	7.2×10^7	61
	3	19	2.5×10^7	37
	4	38	2.5×10^7	47
	5	190	2.0×10^7	70
				Mean 50

TABLE 3. *Comparison of batch method and in-line injection method for virus recovery from large volumes of tap water*

Method	Sample size (liters)	PFU of virus added to sample	PFU recovered (%)
Batch	472	1.5×10^6	41
In-line	472	1.5×10^6	41
Batch	472	1.1×10^6	46
In-line	472	1.1×10^6	40
Batch	472	1.1×10^6	80
In-line	472	1.1×10^6	82

first field system that could be transported easily and used outside of a laboratory. This system was used successfully in later field studies for the isolation of naturally occurring viruses in wastewater and seawater (21). The system proved useful for concentrating enteric viruses from 378 liters (100 gallons) of finished tap water or 189 to 378 liters (50 to 100 gallons) of turbid estuarine water. However, several drawbacks to the system became evident when virus concentration from larger volumes was attempted or when large amounts of organic matter were present in the water being sampled. One problem was the loss of solid-associated virus on the clarifying filters when sampling waters containing large amounts of suspended matter (15, 25). This problem could be reduced by eliminating the clarifying filters, with a concurrent reduction in the amount of water that could be processed, or eluting the clarifying filters in a manner similar to that used for the adsorbent filters. Unfortunately, this resulted in a rather large volume of eluate to be reconcentrated (4, 22). Another drawback was the relatively slow flow rates and thus long processing times for large volumes. Because of the limited surface area of the flat-disk adsorbent filters, maximum flow rates of only 3 gallons (ca. 11 liters) per min could be achieved. Under field conditions with turbid seawater, average flow rates only ranged from 0.5 to 1 gallon (ca. 2 to 4 liters) per min when 50-gallon (189-liter) samples were processed (22).

A final problem was that humic acid and other organic compounds are also concentrated from water onto the filters during operation of the virus concentrator (6). These compounds are eluted from the filters along with the virus and form an insoluble precipitate when the eluate is neutralized. They seriously interfere with any

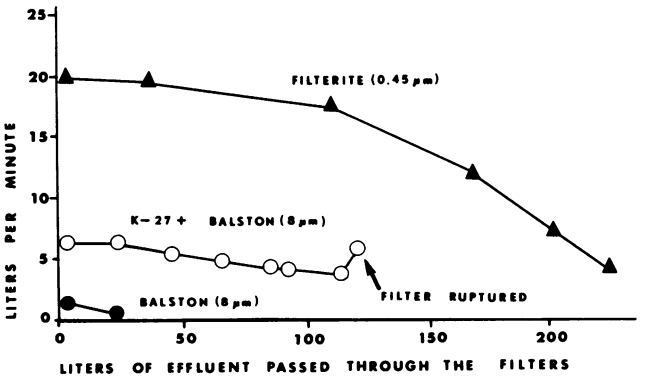

FIG. 3. *Comparison of resistance of Balston and Filterite pleated filters to clogging when processing treated wastewater with a turbidity of 13 JTU.*

TABLE 4. *Recovery of solid-associated viruses from membrane filters*

PFU of virus added to sewage solids[a]	PFU of virus adsorbed to sewage solids[b]	Virus adsorbed to sewage solids (% of added virus)	PFU of virus eluted from sewage solids	Virus eluted from sewage solids (% of adsorbed virus)
1.2×10^7	1.0×10^7	83	9.5×10^6	95
1.1×10^7	9.3×10^6	84	9.0×10^6	97

[a] PFU added to 50 ml of wastewater from the activated sludge aeration tank.

[b] At pH 3.5 and a final $AlCl_3$ concentration of 0.0005 M.

attempted reconcentration of the initial eluate using membrane filters (4) when processing more than 160 gallons of tap water or smaller volumes of seawater and sewage.

Hill et al. (14) described the use of epoxy-fiberglass filter tubes in a parallel series to concentrate virus from 1,900-liter volumes of finished tap water. The tubes were 10 inches (ca. 254 mm) in length with a nominal porosity of 8 μm. Unfortunately, these tube filters suffer from many of the drawbacks inherent with flat-disk membrane filters. The Balston tube filters (Fig. 3) clog more readily than pleated membrane filters and cannot be used even with moderately turbid water. In addition, they cannot be used above an in-line pressure of 25 lb/in^2, and, thus, high flow rates cannot be achieved. This limits their practical use to only finished tap water of good quality. In contrast, pleated filters can operate with an in-line pressure of up to 100 lb/in^2.

Processing time should also be considered when evaluating virus concentration systems. Systems with flat and tube filters were operated at 9.5 liters (2.5 gallons) per min by Hill et al. (14). This would require about 3.5 h to process a 1,900-liter sample of tap water. With pleated fiberglass membrane filters, the operating time would be reduced to 50 min (at the maximum flow rate of 37.8 liters [10 gallons] per min).

Pleated membrane filter systems offer several other advantages over previously described systems, which include: (i) the adsorbent filters can be reused after sterilization by autoclaving or treatment with a strong base (3), with no change in their ability to adsorb virus. (ii) Large volumes of tap water and turbid water can be processed (up to 37.8 liters/min) (4). This system is capable of concentrating virus from 100,000 liters of finished tap water (3) or almost 4,000 liters of turbid seawater (22). (iii) The filter cartridges fit easily into previously described models of the virus concentrator (28). This allows visual inspection of the filters at all times. (iv) The filters used in the construction of the cartridges are the most resistant to clogging of any membrane filter that has been evaluated for virus adsorption and can be used without clarifying filters (4). (v) Filter material can be bent or folded without damage. (vi) The system has been tested and found effective with a variety of waters with widely varying quality (4, 8a). (vii) The filter material is of low weight and size; it does not require bulky or heavy holders, as do flat disk filters. (viii) The system can withstand operating differential pressures as high as 100 lb/in^2.

The use of the batch method for development of concentration methodology was found to yield equivalent efficiencies to those obtained with the in-line method for acid-salt injection (Table 3). These results indicated the appropriateness of the more simple batch method for development of procedures for portable field virus concentrators. The batch method can be used for concentration of naturally occurring viruses (8a) and actually may be more useful when sample sizes are 40 liters (ca. 10 gallons) or less. However, adjustment of pH should be done cautiously, with rapid mixing (i.e., by bubbling compressed air) and dilute acid (1 N or less) to avoid unnecessary viral inactivation (20). Aluminum chloride should be added only after adjustment of the pH below 4.5 to avoid formation of a floc.

Previously published methods for the concentration of viruses from large volumes of estuarine water have been hampered by the often high turbidities characteristic of these waters (13, 20). Hill et al. (12) described a method using Celite as a filtering aid in processing 15- to 100-gallon (ca. 57- to 378-liter) amounts of estuarine water with turbidities of 8.5 to 80 JTU through flat membrane filters. However, virus recoveries were very poor, ranging from 0.4 to 2.2% when low numbers of viruses (49 to 692 plaque-forming units [PFU]) were concentrated. In another modification of the membrane adsorption method, Metcalf et al. (21) first clarified seawater through three clarifying filters with final adsorption occurring on two sets, each containing a fiberglass depth filter and a flat Cox membrane filter. Sample sizes as large as 155 gallons (ca. 587 liters) were processed with this system, but again only low recoveries were achieved. Also, in this system viruses tended to adsorb to suspended matter trapped on the clarifying filters when large volumes were processed (20). The system used by Sobsey et al. (25) to concentrate viruses from tap water was found capable of processing up to 190-liter (50-gallon) volumes of estuarine water. Flow rates averaged only 1.9 liters (0.5 gallon) per min, and efficiency of

recovery averaged about 40%. To overcome problems with filter clogging, a reconcentration method using virus adsorption to flocs formed by the addition of ferric chloride was developed. Flocs formed by addition of $FeCl_3$ to the initial eluates were found to be dependent in size on the amount of soluble organic matter present in the eluates. When volumes of seawater larger than 50 gallons (190 liters) were processed, the volume of floc formed became excessively large (22). Flocs formed by the addition of $AlCl_3$ were much smaller than those formed by $FeCl_3$, and when followed by hydroextraction, the final volume was reduced to 10 to 40 ml (5). Using the above procedures in the present study, we were able to recover seeded poliovirus with an average efficiency of 53% in 378-liter (100-gallon) volumes of turbid estuarine water.

It is important to note that optimal adsorption of virus in seawater to the Filterite pleated membranes used in this study requires a minimum $AlCl_3$ concentration of 0.0015 M. This contrasts the 0.0005 M $AlCl_3$ concentration necessary for optimal adsorption in tap water and sewage (4). In addition, the 3-μm porosity pleated filter appears to offer a greater amount of protection to the final adsorbing filter than does a spun fiberglass filter when processing turbid seawater and sewage. This is undoubtedly due to the greater effective surface area of the pleated filter. A pleated 0.25- or 0.45-μm filter can be used by itself for concentration of virus without the need of a larger porosity filter in series. From our experience, 1,000 gallons (3,780 liters) of tap water and 25 to 75 gallons (95 to 284 liters) of sewage or seawater can be processed in this manner without difficulty. Because of the lower cost, the fiberglass depth filter (K27) may be used instead of the 3-μm porosity filter in processing tap water and 50- to 100-gallon amounts of sewage and seawater. The methods described in this report have been used successfully to isolate naturally occurring enteric viruses from seawater along the upper Texas coast (8a).

The use of filters for the concentration of virus from raw sewage was first described by Homma et al. (15). In that study raw sewage was first passed through a series of clarifying filters and adjusted to a pH of 3.5, and $AlCl_3$ was added to enhance virus adsorption to a spun fiberglass depth filter (model K27). No membrane filters were used in this procedure. Elution of seed virus from the fiberglass filter yielded 81% of the total input virus in a 1-liter eluate. Because of the relatively high concentration of virus normally present in raw sewage, reconcentration was not attempted. Approximately 23% of the input virus was removed by the clarifying filters when 132 liters (35 gallons) of raw sewage was sampled. Because of the relatively high concentrations of virus normally found in raw sewage, such large volumes would usually not be needed to detect virus. Other methods that do not use membrane filters may be more useful in detecting both freely suspended and solid-associated viruses in raw sewage, where only small volumes need be sampled to detect virus.

A greater concern for recovering as much solid-associated virus as possible occurs when attempting to detect viruses present in low concentrations. Almost all sewage and water treatment methods reduce the amount of suspended material initially present, thereby reducing the amount of suspended solid-associated virus. Recent studies we have conducted on solid-associated viruses in discharges from activated sludge and trickling filter sewage treatment plants indicate that the percentage of solid-associated bacteriophage ranges from 2 to 21% of the total virus in the discharge and that only a small percentage of total virus may be embedded within solids (i.e., percent increase in virus concentration after sonic treatment) (C. H. Stagg, C. Wallis, C. H. Ward, and C. P. Gerba, Prog. Water Technol., in press). After chlorination, the percent of solid-associated bacteriophage ranged from 6 to >99% of the total virus remaining. The protective effect that solids afford viruses increases the need to have available methods for their detection. As demonstrated in this study, the poliovirus that adsorbed to sewage solids was easily eluted by the high pH buffer used to elute viruses adsorbed to the filters. Thus, surface solid-associated viruses are easily detected by the methods described in this study. Embedded viruses may not be detected by this procedure, but adequate methodology and data on their occurrence and importance in treated sewage discharges and other waters do not exist at present.

The use of pH 10.5 and 11.5 glycine buffer has been shown in this and previous studies (4, 22, 27) reported by our laboratory to be effective in the elution of enteroviruses from membrane filters. Rapid passage of pH 11.5 glycine buffer once through the filter series described in this report allows for the effective elution of virus. Buffer adjusted to pH 10.5 is also effective in elution of virus if it is cycled through the filters several times, but a loss of virus may occur during recycling because of prolonged contact of the virus at a high pH. In practice the pH 11.5 buffer usually drops to 10.5 to 11.0 after contact with the filters because of residual low pH water retained in the filters. Multiple passage of the pH 10.5 eluent appears necessary to completely neutralize residual acid in the filter housing. If stronger buffers are used, interference with floc

formation in the reconcentration step may occur. Multiple passage of pH 9.5 eluent also results in the elution of some virus, but is not as efficient (Farrah, unpublished data). Use of clear plastic filter holders (28) allows for observation of the filters during elution, ensuring complete contact of the eluent with the filters. Neutralization of these eluates should be done as quickly as possible to avoid undue viral inactivation. Neutralization should be conducted by using buffered solutions because addition of strong acid solutions may also result in inactivation of virus (20). Typically, elution results in recovery of 70 to 90% of the virus from the filters. High pH eluents have been successfully used in field studies to detect naturally occurring enteroviruses and reoviruses (8a, 10, 21). The use of high pH eluents is not suitable for some enteric viruses, such as adenoviruses (8) and rotaviruses (Farrah, unpublished data), which are rapidly inactivated by high pH. The use of organic eluents may be more suitable (8, 18), although reconcentration is more difficult and volume reduction is not as great. In addition, their effectiveness for detecting naturally occurring viruses in waters of varying quality has yet to be evaluated.

The aim of reconcentration is to reduce the volume to a quantity that can be economically assayed by conventional tissue culture methodology. Reconcentration strategy is determined by the volume of the water being sampled and by the amount of soluble organic matter present. For volumes of 378 liters (100 gallons) or less of finished tap water or other waters containing relatively low concentrations of organic matter, reconcentration can usually be accomplished by adsorption-elution methodology by using small diameter disk membrane filters (26). When large amounts of organic matter are present, a precipitate forms in the initial eluate, making reconcentration by membrane filters difficult (6, 25). To overcome this problem reconcentration with ferric and aluminum flocs was found to be an acceptable alternative, especially when combined with hydroextraction. The present study has shown the usefulness of this method with waters of widely varying quality. In each case, an average efficiency of about 50% was achieved. Previous studies have shown this methodology to achieve the same efficiency even when small amounts of virus are present in the sample and to detect naturally occurring virus (5, 8a, 25). Schemes for virus concentration and reconcentration methodology for the various waters and volumes used in this study are summarized in Table 1.

Balston filters, which are recommended in the proposed tentative method for virus concentration from water in the most recent edition of *Standard Methods for the Examination of Water and Wastewater* (1), were found to be inadequate for concentrating viruses from large volumes of either treated sewage or seawater. These filters were found to clog readily and often ruptured when flow rates exceeding 15 liters (4 gallons) per min were used. The better performance of pleated filters is undoubtedly related to their greater effective surface area. For practical purposes the use of Balston filters appears to be limited to only highly finished tap water, where they have proved to be highly suitable (17). The method described in *Standard Methods for the Examination of Water and Wastewater* also recommended the use of a proportioner pump for injection of acid and salt. This method is not suitable for the high flow rates possible with pleated membrane filters because the orifice in the proportioner previously described (17) limits the maximum flow rate, but it is certainly the best method to use with lower flow rates. Using an acid-salt injector (28), it is possible to efficiently concentrate virus at flow rates of about 40 liters (10 gallons) per min. When chlorinated tap water or treated sewage is being processed, sodium thiosulfate solution can be injected through another injector port before acid adjustment.

The continued usefulness of any procedure or methodology is dependent on its ability to be adapted to the needs of the situation at hand. The filter adsorption-elution methodology has proven to be flexible enough to allow its use with a wide variety of waters and viral types, with appropriate modifications. Thus, each scheme shown in Table 1 is an adaptation to maximize virus recovery from the type of water being studied with the least amount of effort and expense. Further, development and refinement of virus concentration technology are undoubtedly called for to allow detection of new viral groups (e.g., rotaviruses), application of new assay methodology (e.g., radioimmunoassay), and the physical state or association of the virus. We believe this study and previously reported studies indicate that filter adsorption-elution methodology has the flexibility to meet these challenges.

The improved portable virus concentrator and reconcentration procedure described in this study offer many advantages over previous systems (18, 27) and can be used in the field to efficiently monitor large volumes of secondarily treated sewage, tap water, and seawater for enteroviruses.

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