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Modified Membrane-Filter Procedure for Concentration of Enteroviruses from Tap Water†

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Enteroviruses added to 114 liters of dechlorinated tap water were recovered in a 16-ml sample by a two-stage concentration procedure in which different types of membrane filters were used in each concentration stage. Viruses in tap water at pH 3.5 were first adsorbed to 10-in. (ca. 25.4-cm) epoxy-fiber glass filters (Filterite). Viruses adsorbed to these filters were eluted with a solution of 0.2 M sodium trichloroaceate buffered at pH 9 with 0.2 M lysine. Viruses in this solution were adsorbed to 47-mm asbestos filters (Seitz) without pH adjustment or other modification of the solution. Viruses were recovered from the Seitz filters with 16 ml of either Casitone or fetal calf serum at pH 9. With these procedures ca. 45% of several types of enteroviruses added to 114 liters of tap water could be recovered in the final 16-ml sample.

A number of methods have been developed to detect viruses in water (1); however, methods in which adsorption to and subsequent elution from membrane filters are used are still considered to be among the most promising for detecting viruses in large volumes of water (4, 13). When relatively small volumes of tap water are processed, one-step adsorption-elution procedures with membrane filters or other solids to concentrate the viruses have been shown to be quite efficient (4, 13). As the volume of water processed is increased, a two-stage concentration procedure becomes necessary to reduce the final sample that must be assayed.

Wallis et al. (12) and Sobsey et al. (10) described two-stage concentration procedures in which the same type (but different sizes) of filters were used in both stages. Difficulties were encountered in applying these procedures to the detection of viruses in estuarine water (8) or in larger volumes of tap water (2). Both organic compounds and viruses were adsorbed to and eluted from the filters in the first stage. These organic compounds formed flocs at the low pH values that were required for the adsorption of viruses to negatively charged filters. These flocs clogged the filters and made a second stage of concentration with negatively charged filters impractical. This problem was overcome by the use of an inorganic flocculation step for reconcentration (2), yet viral recovery was low. Katzenelson et al. (5) increased the amount of viral recovery by using a second-stage organic flocculation procedure with beef extract. Although these procedures have been useful, some problems still exist. One is that in some cases these flocculation procedures produce large final volumes to be assayed. In addition, recent reports from different laboratories have indicated that different preparations and supplies of beef extract vary in their ability to flocculate at a low pH.

In this communication, we introduce three modifications of existing procdures for concentrating viruses in water. These modifications make the pH adjustment step easier and permit detection of viruses in water by a two-stage concentration procedure in which different types of membrane filters are used in each stage. These modifications are as follows: (i) the use of acetic acid in place of hydrochloric acid to adjust the pH of the tap water to pH 3.5; (ii) the use of sodium trichloroacetic acid as the primary eluent; and (iii) a second-stage concentration step with positively charged Seitz filters.

Poliovirus 1 (LSc), echovirus 1 (Farouk), coxsackievirus B3 (Nancy), coxsackievirus B4 (natural isolate), and coxsackievirus B5 (ATCC VR-689) were the enteroviruses used in this study. Tap water (114 liters) was dechlorinated by the addition of sodium thiosulfate and adjusted to a pH between 3.3 and 3.5 by the addition of acetic acid. Ten milliliters of the tap water was removed and seeded with a known amount of one of the enteroviruses. From 10^4 to 10^7 PFU of virus was added in each trial. When low numbers of viruses were added, the eluate from the Seitz filter was assayed directly after an adjustment to pH 7 to 7.5. When higher numbers of viruses were added, viruses were assayed after dilution in phosphate-buffered saline with 2% fetal calf serum (FCS). A 5-ml portion of the viral suspension was added to the 114 liters, and the remaining 5 ml was assayed. Viruses recovered in the final sample were compared with viruses remaining in the 5-ml sample to determine the efficiency of virus recovery. Viruses were assayed with Buffalo Green monkey (BGM) kidney cells and a plaque-forming procedure previously described (7). The seeded tap water was passed through a 10-in. (ca. 25.4-cm), 0.25-μm-pore filter (Filterite Corp., Timonium, Md). The adsorbed viruses were eluted with 1 liter of 0.2 M trichloroacetic acid–0.2 M lysine (Sigma Chemical Co., St. Louis, Mo.) that had been adjusted to pH 9 to 9.3 with sodium hydroxide. This eluate was passed through a 47-mm Seitz S filter (Republic Corp., Milldale, Conn.) under positive pressure without a pH adjustment. Viruses adsorbed to the Seitz filter were recovered by one of two methods. In the first procedure, the filter was removed from the holder and placed in a holder used for vacuum filtration (Gelman Sciences, Inc., Ann Arbor Mich.). Two 8-ml portions of 3% Casitone (Difco Laboratories, Detroit, Mich.) were pulled through the filter with a vacuum. After the first 2 to 3 ml of each portion had passed through the filter, the vacuum was removed and the solution was allowed to soak the filter for 5 min. After the soaking period, the remaining part of each portion was pulled through the filter. The two portions were pooled, neutralized, and assayed. In the second procedure, two 8-ml portions of FCS (pH 9) were pulled through the filter with a

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TABLE 1. Concentration of viruses from tap water[a]

Eluent	Virus	No. of trials	% of added viruses recovered
Casitone (3%, pH 9)	Poliovirus 1	3	67 ± 8
	Coxsackievirus B3	3	46 ± 18
	Coxsackievirus B4	2	43 ± 20
	Echovirus 1	2	63 ± 12
	Total	10	54 ± 18
FCS (pH 9)	Poliovirus 1	4	35 ± 7
	Coxsackievirus B3	2	40 ± 7
	Coxsackievirus B5	2	44 ± 5
	Echovirus 1	2	58 ± 9
	Total	10	43 ± 11

[a] Tap water was dechlorinated, adjusted to pH 3.3 to 3.5 by the addition of acetic acid, and seeded with the indicated virus. The sample was passed through a 10-in. (ca. 25.4-cm), 0.25-μm-pore Filterite filter at 3 to 5 gal. (ca. 11.36 to 18.92 liters) per min. Next, 1,000 ml of 0.2 M sodium trichloroacetic acid–0.2 M lysine (pH 9) was passed through the filter. This eluate was then passed through a 47-mm Seitz S filter. Viruses adsorbed to the Seitz filter were recovered by treating the filter with two 8-ml volumes of 3% Casitone or FCS (pH 9) as described in the text.

50-ml syringe. The two portions were pooled, passed through a 0.45-μm-pore HA filter (Millipore Corp., Bedford, Mass.) or two layers of 0.25-μm-pore Filterite filters, neutralized, and assayed.

Adsorption of viruses to negatively charged filters such as Filterite filters is facilitated at a low pH (10). Hydrochloric acid has been used to adjust water samples to the low pH values required for viral adsorption in many studies. We have found that acidification of tap water with either hydrochloric acid or acetic acid permits the adsorption of greater than 99% of the enteroviruses studied to Filterite filters in our laboratory studies. However, the acidification of water under field conditions with Dema injectors (6) is easier when acetic acid is used in place of HCl. Changes in the flow rate or pressure may cause fluctuations in the amount of acid injected when Dema or pressure injectors are used to adjust water pH. With HCl, these changes may cause wide fluctuations in the pH unless the injection rate is carefully controlled. We have found that these fluctuations are minimized when 1 or 2 M acetic acid is used for acidification. Acetic acid (pK_a, 4.75) will buffer more closely to the desired pH range than will HCl, which dissociates completely.

The association of viruses with solids such as membrane filters has been shown to be influenced by both hydrophobic and electrostatic interactions (3, 4, 7, 13). Previous studies have shown that detergents, which disrupt hydrophobic interactions, can be used to elute viruses adsorbed to membrane filters (3, 7). In addition, recent studies have shown that certain salts may disrupt hydrophobic interactions (3). These salts, called chaotropic salts, are relatively large, singly charged ions such as trichloroacetate and thiocyanate. It has been suggested that these compounds disrupt hydrophobic interactions by decreasing the structure of water and making aqueous solutions more lipophilic. Chaotropic agents have been used to elute viruses adsorbed to various solids by disrupting hydrophobic interactions between the viruses and the solids (3, 11). A solution of the chaotropic salt sodium trichloroacetic acid eluted 77 ± 20% of the enteroviruses used in this study that were adsorbed to Filterite filters. Reconcentration of the viruses in the primary eluate with a second, smaller electronegative filter requires acidification of the high-pH eluent used to recover viruses adsorbed to the primary virus-adsorbing filter (10, 12). It was previously shown (2) that such a drop in pH may be accompanied by the precipitation of organic compounds that may have been concentrated along with the viruses. These organic compounds may interfere with the reconcentration process by reducing the adsorption of viruses to the filters or by clogging the filters (2). Therefore, readsorption of viruses to second filter at the same pH that was used for the elution of viruses from the first filter was studied.

Sobsey and Jones (9) studied the adsorption of polioviruses to positively charged filters at several pH values. They found that both Zeta-plus filters (cellulose-diatomaceous earth-charged modified resin; AMF Cuno Inc., Milldale, Conn.) and Seitz filters (asbestos) efficiently adsorbed polioviruses at a relatively high pH. For the second-stage concentration, we used the Seitz grade S filters. When the sodium trichloroacetic acid-lysine eluate from the Filterite filters was passed through the filters, greater than 95% of the viruses were adsorbed. Viruses adsorbed to the Seitz filters were eluted with pH 9 solutions of either 3% Casitone or FCS. Casitone is less expensive than FCS. However, a soaking period was required when Casitone was used as the eluent. It was found that pulling the eluents through the filters with an apparatus for vacuum filtration or a syringe permitted better elution than pushing the eluent through the filters. It is possible that the eluent solutions do not make contact with the entire filter surface because of air gaps that form during pushing of the eluents through the filters. The mean recovery of enteroviruses with Casitone was 54%, and that with FCS was 43% (Table 1).

In summary, we have devised three modifications of procedures for the concentration of viruses from tap water: (i) the use of acetic acid to adjust the pH of water samples; (ii) the elution of viruses adsorbed to the first filter with a solution that disrupts both electrostatic and hydrophobic interactions between the viruses and the membrane filter; and (iii) a second-stage concentration step in which a second membrane filter that differs from the first membrane filter is used.

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LITERATURE CITED

1. **Farrah, S. R., and G. Bitton.** 1982. Methods (other than microporous filters) for concentration of viruses from water, p. 117–150. *In* C. P. Gerba and S. M. Goyal (ed.), Methods in environmental virology. Marcel Dekker, Inc., New York.
2. **Farrah, S. R., C. P. Gerba, C. Wallis, and J. L. Melnick.** 1976. Concentration of viruses from large volumes of tap water using pleated membrane filters. Appl. Environ. Microbiol. **31**:221–226.
3. **Farrah, S. R., S. O. Shah, and L. O. Ingram.** 1981. Effects of chaotropic and antichaotropic agents on elution of poliovirus adsorbed on membrane filters. Proc. Natl. Acad. Sci. U.S.A. **78**:1229–1232.
4. **Goyal, S. M., and C. P. Gerba.** 1982. Concentration of viruses from water by membrane filters, p. 59–116. *In* C. P. Gerba and S. M. Goyal (ed.), Methods in environmental virology. Marcel Dekker, Inc., New York.
5. **Katzenelson, E., B. Fattal, and T. Hostovesky.** 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. **32**:638–639.
6. **Payment, P., and M. Trudel.** 1980. A simple low cost apparatus for conditioning large volumes of water for virological analysis.

Can. J. Microbiol. **26**:548–550.
7. **Shields, P. A., and S. R. Farrah.** 1983. Influence of salts on electrostatic interactions between poliovirus and membrane filters. Appl. Environ. Microbiol. **45**:526–531.
8. **Sobsey, M. D., C. P. Gerba, C. Wallis, and J. L. Melnick.** 1977. Concentration of enteroviruses from large volumes of turbid estuary water. Can. J. Microbiol. **23**:770–778.
9. **Sobsey, M. D., and B. L. Jones.** 1979. Concentration of poliovirus from tap water using positively charged microporous filters. Appl. Environ. Microbiol. **37**:588–595.
10. **Sobsey, M. D., C. Wallis, M. Henderson, and J. L. Melnick.** 1973. Concentration of enteroviruses from large volumes of water. Appl. Microbiol. **26**:529–534.
11. **Wait, D. A., and M. D. Sobsey.** 1983. Method for recovery of enteric viruses from estuarine sediments with chaotropic agents. Appl. Environ. Microbiol. **46**:379–385.
12. **Wallis, C., M. Henderson, and J. L. Melnick.** 1972. Enterovirus concentration on cellulose membranes. Appl. Microbiol. **23**:476–480.
13. **Wallis, C., J. L. Melnick, and C. P. Gerba.** 1979. Concentration of viruses from water by membrane chromatography. Annu. Rev. Microbiol. **33**:413–437.