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Organic Flocculation: an Efficient Second-Step Concentration Method for the Detection of Viruses in Tap Water

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A method is described for second-step concentration of viruses from water. This method, combined with an adsorption-elution method, yields a mean recovery of about 75%.

A tentative standard method for the detection of viruses in potable water was recently described by Hill et al. (4): the virus is concentrated from the water in two steps by adsorption to and elution from virus adsorbent filters, thus providing a "yes" or "no" answer from a volume of 380 liters of drinking water.

Clogging of the filter during the second or reconcentration step (3) and low virus recovery (about 35%) are two drawbacks inherent in this method. To increase virus recovery, Hill et al. (4) have suggested enlarging the sample volume to 1,900 liters. For a simple yes or no answer this may be the right solution, but a standard method for virus detection should have a much higher recovery efficiency enabling quantitative estimation.

Farrah et al. (3) used inorganic floc in the reconcentration step to eliminate clogging, but virus recovery remained low (40 to 50%). The use of two steps instead of one lowers virus recovery, but with a large volume of water the second step becomes necessary. Furthermore, virus elution in a pH 11.5 buffer may inactivate the virus; a lower pH, however, gives poor results (3).

The utilization of a protein solution for elution of viruses from adsorbent filters, such as 3% beef extract (2, 7), gives a high recovery but does not allow readsorption. Lowering the pH of a protein solution (e.g., beef extract) to 3.5 produces flocculation of proteins. The sediment, obtained by centrifugation of 300 ml of beef extract (3%) at 3,000 × *g* for 10 min, is soluble in 15 ml of 0.15 M Na_2HPO_4 (pH 9.0), i.e., a concentration factor of 20.

To determine whether viruses could be concentrated in the same manner, poliovirus I (6) was seeded into 450 ml of sterile beef extract (3%), and the sample was divided into three equal parts. To one 150-ml sample, 2 N HCl was added dropwise under stirring until a pH of 3.5 was reached. In the same way the pH values of the second and third portions were changed to 4.0 and 4.5, respectively. Stirring was continued for 30 min, followed by centrifugation (3,000 × *g* for 10 min). The supernatant was separated from the sediment, and the latter was redissolved in 7.5 ml of Na_2HPO_4 (0.15 M) by pipetting or stirring with a glass rod for 5 to 10 min. Antibiotics (0.2 ml: neomycin, 25 mg/ml; kanamycin, 200 mg/ml; streptomycin, 200 mg/ml; penicillin, 200,000 U/ml) were then added to the concentrate. The final pH was 7.2. Antibiotics were also added to the supernatant, and the pH was adjusted to 7.2 with NaOH (2 N). The dissolved sediments and the supernatants from the three pH treatments were assayed (8) on BGM cells (1). The results (Table 1) clearly show that nearly total recovery was obtained from the solubilized sediments at pH 3.5 and 4.0 (ranging from 69% to 123%; mean, 100%), whereas at pH 4.5 the majority of the virus remained in the supernatant (ranging from 54% to 89%; mean, 67%).

It now became feasible to test the practicability of the organic flocculation method for the concentration of viruses from water by comparing it with the tentative standard method of Hill et al. (4) using glycine buffer for elution as described by Jakubowski et al. (5) in the following manner. One thousand liters of Jerusalem tap water (Table 2) was collected in a plastic container, seeded with a known concentration of poliovirus I, and mixed by air bubbling; the pH was reduced to 3.5. The sample was then divided into two equal parts (500 liters), and each volume was passed (flow rate, 7 liter/min; pressure, 2 atm) through an AA Cox M780 epoxy-fiber glass-asbestos microfilter (diameter, 293 mm; pore size, 0.45 μm; Cox Instruments, Detroit, Mich.) with a fiber glass prefilter (diameter, 300 mm; SM 13430 membrane filter; Sartorius, West Germany). The adsorbed virus was eluted by 1,000 ml of 0.05 M glycine buffer (pH 11.5) from one filter (9) and from the other by 300 ml of 3% beef extract (pH 9.0). Reconcentration from the first eluent was car-

TABLE 1. *Effect of low pH treatment on the presence of seeded poliovirus in sediment and supernatant of 3% beef extract*

Virus input/150 ml of beef extract	Distribution of virus (% of input)					
	pH 3.5		pH 4.0		pH 4.5	
	Sediment	Supernatant	Sediment	Supernatant	Sediment	Supernatant
4.8×10^3	69	0	77	0.65	25	54
3.4×10^3	112	1.1	106	1.4	23	89
1.8×10^3	123	0	114	5.0		59
Mean	101	0.4	99	2.4	24	67

TABLE 2. *Characteristics of Jerusalem tap water used in virus experiments*

Parameter	Value	
	Mean	Range
Turbidity (formazin units)	0.77	0.3–1.6
pH	7.63	7.5–7.8
Conductivity (μmho)	745	650–850
Chlorides (mg/liter)	109.9	73–141
Calcium and magnesium (meq/liter)	3.05	2.87–3.20
TOC[a] (μg/ml)	0.88	0.2–1.3
TSS[b] (mg/liter)	0.76	0.1–2.4

[a] TOC, Total organic carbon.
[b] TSS, Total suspended solids.

ried out according to Jakubowski et al. (5) and from the second eluent by flocculation at pH 3.5. The results obtained with the glycine buffer method (Table 3) are identical to those described by Hill et al. (4), with recoveries ranging from 30 to 47% (mean, 35%). The organic flocculation method, on the other hand, gave recoveries ranging from 60 to 91% (mean, 74.4%), a number obtained even at the low contamination level of 15 plaque-forming units/500 liters. It should be noted that in all experiments carried out with the organic flocculation method recoveries were at least twice that of the glycine buffer method.

The comparison of the organic flocculation method with the tentative standard method of Hill et al. (4) was done with seeded tap water. Since each water sample used for the comparative experiment was divided into two parts, the results may be assumed to be highly reliable. This reliability is strengthened by the fact that the recoveries obtained by the glycine buffer are very similar to those described by Hill et al. (4). The true test of a given method, however, lies in its efficiency under field conditions. Preliminary results of experiments with water of the River Jordan, presently being carried out, point to the superiority of the organic flocculation method even with polluted surface water. Organic flocculation would therefore seem to be the most suitable second-step concentration method for the detection of virsues in tap water.

TABLE 3. *Recovery of seeded poliovirus I by organic flocculation versus glycine buffer elution*

Virus input/500 liter	% Recovery	
	Glycine buffer	Organic flocculation
1.8×10^3	30	72.5
1.5×10^3	31	66.0
2.2×10^3	31	91.0
1.7×10^3	42	76.0
1.5×10	47	80.0
1.5×10	33	60.0
1.2×10	33	75.0
Mean	35	74.4

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