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Environmental Factors Influencing Isolation of Enteroviruses from Polluted Surface Waters

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The influence of water quality upon the concentration of virus on location was assessed in field studies conducted in the Houston ship channel, Galveston Bay, and Houston waste treatment plants. Clarification of polluted surface waters was accomplished with minimal loss of virus. Virus from clarified sewage effluents and saline waters was then adsorbed and concentrated on textile and membrane filter surfaces. Direct measurements of virus from large volumes of polluted surface waters under existing field conditions were then made using the virus concentrator equipment.

Equipment and methods for concentration of viruses added to large volumes of water have been reported by Wallis et al. (8) and by Sobsey et al. (5). Using tap water, clarification of samples without significant loss of virus was achieved. Clarification of samples was followed by injection of salts into the water under test to enhance adsorption of virus upon virus adsorbents. Virus was recovered off virus adsorbents by elution with basic buffers. Reconcentration of virus eluates with reduction of eluate volume to 10 ml was necessary before viruses could be detected in cell cultures. Excess hydrogen ions were not used in this study because these will only enhance virus adsorption to membrane filters in the absence of organics (5).

Adsorption of virus to reactive surfaces is known to be enhanced in the presence of several inorganic salts. Using Houston tapwater, $AlCl_3$ was shown to be at least 200 times more efficient than $MgCl_2$ for promoting enterovirus adsorption on cellulose membranes (6). In the same study, attention was directed to the logistic advantage realized through the use of $AlCl_3$. For every 1,000 gal of water examined for virus, 112.5 lb (about 51 kg) of $MgCl_2$ is required. Using $AlCl_3$, only 1 lb (453.6 g) is required for the same examination.

Existence of environmental factors potentially influencing the recovery of natural virus from polluted surface waters has been reported (7). Their role and significance have not been fully assessed in appropriate field studies. For example, will salt enhancement be required for virus adsorption in the presence of saline waters?

The primary purpose of the study was to subject the virus concentrator and methods to the most searching and critical field conditions possible. Two Houston waste treatment plants and waters of the Houston ship channel and Galveston Bay were selected as test areas most likely to provide severe and unique challenges. The waste treatment plants, Houston ship channel, and Galveston Bay exist in one of the most heavily industrialized areas in the world. The channel has been described as one of the most extensively polluted bodies of water in the United States. It receives domestic waste discharges from metropolitan Houston, and industrial discharges from more than 30 major industries situated along its banks. The channel flows into Galveston Bay, carrying its pollutants into estuarine waters harboring an extensive and varied marine fauna.

Study objectives were to evaluate the performance of equipment and methods in the field, and to assess the influence of environmental factors upon the processing of samples for direct isolation of virus.

MATERIALS AND METHODS

Viruses. Poliovirus types 1 and 2 (vaccine strains) and echovirus type 1 (Farouk) were added to the sewage effluents as indicated in the text and tables.

Surface waters. Artificial seawater was prepared by the method of Lyman and Fleming (3). Desired salinities were prepared by dilution using distilled water. Effluents from two Houston municipal waste treatment plants discharging approximately 70 and 38 million gallons/day (mgd), respectively, were used. Where chlorine residuals existed, 11 ml of 1 g % of

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sodium thiosulfate per gal was used to neutralize chlorine if virus was to be added exogenously. Houston ship channel and Galveston Bay samples were obtained with the help of boat support made available through the office of Sea Grant, Texas A & M University. Samples were either processed aboard ship by means of the virus concentrator (8) or water samples were collected and returned to the laboratory for use in experimental studies.

Virus concentrator. Equipment as described previously (7, 8) was used. Ten-inch (about 25.4 cm) orlon textile filters of 10 and 1 μm porosity plus a Tween-80-treated 10-inch cellulose acetate textile filter (7) were the clarifying filters. Ten-inch fiberglass filters (K-27), nominal porosity of 5 μm, and 293-mm Cox membrane filters (porosity indicated in the text), were used as virus adsorbents. Aluminum chloride prepared as a 0.05 M stock solution and diluted to a final concentration of 0.0005 M was used to enhance virus adsorption (6). Injection of salt under positive pressure was made after pH adjustment of a clarified sample to 3.5. Collection of samples was made at flow rates up to 2 gal/min, using two K-27 filters in series, and two membrane filters in parallel. Sample volumes collected varied from 5 to 160 gal (about 19 to 606 liters).

Elution of virus. The method for elution of virus off adsorbent filters has been described previously (6, 8). Textile filters including clarifying (orlon and cellulose acetate) and adsorbent (fiber glass) types were treated with 1,000 ml each of 0.05 M glycine, pH 11.5. Virus adsorbed on the 293-mm membrane filters was eluted with 600 ml of eluent. Elutions were carried out using a positive pressure of 5 to 10 lb/inch^2 for the textile filters and up to 20 lb/inch^2 for the membrane filters. Eluate pH values were checked immediately. The elution was considered satisfactory if a pH value of 10.5 to 11.0 was found. A pH value less than 10.5 was considered unsatisfactory, and the elution procedure was repeated using fresh eluent. Eluates were immediately neutralized using either 0.5 N HCl or equal volumes of 0.05 M glycine, pH 2.0. Contact times of pH 11.5 eluent with virus adsorbents were less than 5 min, because preliminary experiments revealed that numerous enteroviruses were resistant to this pH for periods less than 15 min.

Reconcentration of eluates. Neutralized eluates were reduced from their initial volume (600 to 2,000 ml) to a final volume of 10 ml. The reduction in volume was made through repeated adsorption and elution procedures designed to alternately adsorb virus to a membrane and then elute with an appropriately smaller volume of eluent (6). Depending upon the eluate volume to be filtered, Cox membrane filters of 293, 142, 90, and/or 47 mm were used. Membrane filters of 0.45 μm were used with a prefilter of either 1, 2, or 5 μm porosity. Eluent volumes used for the above filters were 600, 80, 40, and 10 ml, respectively. The final step in reconcentration was passage of the 10-ml eluate from a 47-mm filter through a sterile 25-mm Millex (Millipore Corp.), 0.2-μm filter assembly. Loss of virus upon the filter was minimized by pretreatment of the filter with 1 or 2 ml of a sterile solution of 10% fetal calf serum in borate buffer, pH 9 (9). The filtered eluate was then neutralized with 0.1 N HCl and made isotonic using 0.5 ml of 3 M NaCl.

Turbidity measurements. Water turbidity was determined as Jackson turbidity units, using a Hach model 2100 A turbidimeter.

Isolation of virus from samples. Reconcentrated samples were inoculated onto drained primary monkey kidney (MK) monolayers in 1-oz (about 28 ml) bottles, using 0.2 ml per bottle culture and 15 bottles per sample. After a 1-h adsorption period at 37 C, monolayers were overlaid with agar medium consisting of 1.5% agar (Difco), 1:54,000 neutral red, 1 $\times$ Eagle medium without phenol red, 100 U of penicillin, 100 μg of streptomycin, 23 mM $MgCl_2$, and 0.4% $NaHCO_3$. Bottles were examined periodically over a 14-day period for plaques. Developed plaques were plucked and passaged once in MK cells using techniques described previously (11).

Resin treatment of samples. A cation resin, C-249, manufactured by Ionac Corp., Birmingham, N.J., was used to treat samples for reduction or removal of membrane coating components (MCC) (9). Treatment was applied during reconcentration of eluates. Before use, the resin was washed two or three times with distilled water (until a clear supernatant was obtained).

Samples to be treated with resin were adjusted to a pH of 8.0 to 8.5, and then combined with resin. A sample to resin ratio of 4:1 was used for treatment during virus reconcentration steps. Resin treatment during virus reconcentration was carried out in a beaker with magnetic mixing of sample and resin for 30 min. A resin wash with 2 liters of 0.05 M glycine, pH 8.5, was used routinely to recover residual virus from resin suspensions after withdrawal of resin supernatant.

RESULTS

Virus removal from salt water by natural and synthetic textile filters. Artificial seawater was prepared and used to evaluate the capabilities of a number of natural and synthetic textile fibers to remove virus. A typical experiment involved addition of poliovirus to 5 gal of freshly prepared seawater, followed by passage of the sample through a test filter at a flow rate of 1 gal/min. Filtrates were collected and assayed for virus. The results of a series of experiments are summarized in Table 1. Orlon, polypropylene, and polyester filters removed the least virus. These filters allowed 88 to 92% of virus in seawater to pass into the filtrate. Fiber-glass and cellulose acetate filters allowed the least virus to pass into filtrates. Only 3 to 5% of virus appeared in filtrates. Dynel, viscose, and cotton filters were intermediate in virus removal characteristics.

The results of these experiments indicated that clarification of seawater should prove feasible with orlon, polypropylene, or polyester filters without significant loss of virus. Since fiber-glass and cellulose acetate filters allowed

TABLE 1. *Virus removal from artificial seawater by natural and synthetic textile filters*

Textile filters[a]	Seawater salinity (0/00)[c]	Virus found in filtrate (%)						
		1[b]	2	3	4	5	6	Avg
Orlon	22			87	89	91	97	91
Orlon	10			88	96	93	94	92
Orlon	34			85	88	97	96	91
Viscose	34	73	71					72
Cotton	21	18	21					19
Cotton	32	17	14					15
Fiber glass	34	2	3	1	5			3
Fiber glass	20		6	1		4		4
Polypropylene	34	95	83					89
Polyester	34	90	86					88
Cellulose acetate	30	3	4	2				3
Cellulose acetate	14	5	7	8	2			5
Dynel	34	10						10

[a] Filters (1 to 3 μm porosity) were washed with 30 gal of filtered tapwater prior to use. Poliovirus was added to artificial seawater in a final concentration of 1,000 PFU/0.1 ml. Five-gallon samples were passed through filters at a flow rate of 1 gal/min. Filtrates were rendered isotonic before assay.

[b] Experiment number.

[c] Parts per thousand.

only small numbers of virus to pass, these filters would be candidates for use as virus adsorbents.

Clarifying action of textile filters in polluted natural salt waters. A series of experiments was conducted to determine if natural marine and estuarine waters could be clarified without virus loss. Poliovirus type 1 and echovirus type 1 were added exogenously to artificial seawater and samples of natural salt waters collected from the Houston ship channel and Galveston Bay, and returned to the laboratory. In a series of tests, 5 to 50 gal of water was passed through different combinations of orlon and Tween-80-treated cellulose acetate clarifying filters. Virus removal in each test was determined by assay of filtrates. The results of this series of tests are shown in Table 2. The amount of virus passing clarifying filters when natural waters were examined varied from 83 to 94%. An assembly of three clarifying filters did not alter appreciably the virus removal experienced with one filter. This fact, combined with the improved degree of sample clarification achieved, led to the routine use of three clarifying filters in subsequent tests.

The ability of clarifying filters to promote clarification without loss of virus when larger volumes of polluted estuarine water are sampled was examined in a series of tests performed in Galveston Bay. Samples of 5 to 160 gal were pumped through filters, one series of filters for each sample. The filters were returned to the laboratory where each series was challenged with 1 gal of virus suspension at pH 3.5 with 0.0005 M $AlCl_3$. (To enhance virus adsorption, $AlCl_3$ at pH 3.5 was used as a diluent for virus challenge; viruses react more avidly with membrane sites which are not coated with MCC.) Replicate 1-gal virus suspensions were used for each filter series challenged. The results of a series of tests are shown in Table 3. Virus removal increased as sample volume increased. Under test conditions, deposition of sedimentary particulates occurred to the greatest extent on the 10-μm orlon filter. The 1-μm orlon filter was noticeably protected from deposits,

TABLE 2. *Passage through clarifying filters of virus added exogenously to seawater*[a]

Test	Sample (gal)	Virus	Clarification method[b]	Seawater salinity[c] (0/00)	Virus assay (PFU/0.1 ml)		Virus passage (%)
					Before filtration	After filtration	
1	5	Poliovirus 1	A	30	10.0	9.5	95.0
2	5	Poliovirus 1	B	30	744.0	744.0	100.0
3	50	Poliovirus 1	C	7	136.0	124.0	91.0
4	50	Poliovirus 1	C	20	334.0	286.0	85.0
5	50	Echovirus 1	C	10	243.0	229.0	94.0
6	50	Echovirus 1	C	20	191.0	159.0	83.0
7	50	Echovirus 1	C	21	71.0	62.0	87.0

[a] Filtration carried out at a flow rate of 1 gal/min. Natural virus was not detected in 50- or 75-gal samples collected at same time as test samples.

[b] Clarification methods: (A) 1-μm orlon filter; (B) 10- and 1-μm orlon filter; (C) 10- and 1-μm orlon filters, 1-μm Tween-80 (0.1%)-treated cellulose acetate filter.

[c] Seawater: 30 0/00, artificial seawater; 7 and 10 0/00, Houston ship channel water; 20 and 21 0/00, Galveston Bay water.

TABLE 3. *Virus passage through clarifying filters following filtration of large volumes of estuarine water*[a]

Water passing clarifying filters[b] (gal)	Virus in filtrates	
	PFU/0.1 ml	Virus in filtrate (%)
0 (untreated filter assembly)	115	100.0
5	118	102.7
10	95	82.7
20	90	87.6
40	84	73.1
80	87	75.8
160	77	67.0

[a] Salinities varied from 17 to 18 0/00. Turbidities varied from 4 to 6.5 Jackson turbidity units.

[b] Clarifying filters: 10- and 1-μm orlon filters, 1-μm Tween-80 (0.1%)-treated cellulose acetate filter. Virus challenge: 128 PFU/0.1 ml.

whereas virtually no deposits could be seen on the 1-μm cellulose acetate filter. We have previously reported that metallic and other solids deposited on clarifying filters removed virus as the particulate load on filter surfaces increased (7). In subsequent tests, replacement of 10-μm orlon filters visibly loaded with marine deposits prevented undue loss of virus during sample clarification.

Virus adsorption to textile filters from polluted waters. The virus adsorption capacity of fiber-glass and untreated cellulose acetate filters was tested in the Houston ship channel to determine whether the mixture of industrial and domestic wastes present would interfere with virus adsorption to these filters. After a preliminary clarification step, samples of 5 to 160 gal were pumped through sets of the two filter types, one filter per type per sample. All filters were returned to the laboratory where each was challenged as described above. Replicate 1-gal virus suspensions were used for challenge of each set of filters. A similar test was performed in Galveston Bay. The results of the two sets of tests in the two locales are shown in Table 4. The increasingly greater passage of virus through cellulose acetate filters as sample volume of ship channel water increased indicated serious interference with virus adsorption. The degrees of interference found in the tests suggested the presence of MCC at both test locations which affected virus adsorption to both filters. Test results showed virus loss could be reduced sharply in heavily polluted waters by using fiber-glass filters for collection of virus. Virus penetration through filters in less heavily polluted estuarine waters could be minimized through the use of a fresh fiber-glass filter after collection of a 10- to 20-gal sample. In subsequent tests, fiber-glass filters were used to concentrate virus in order to minimize the effects of MCC.

Comparisons of the effectiveness of salt enhancement on virus recovery from textile adsorbents were made using Houston ship channel water. Water samples were collected and returned to the laboratory. The experimental procedure involved the addition of virus to a large volume of water, followed by separation of the water into two or three equal portions. Portions without salt were compared with those containing either $AlCl_3$ or $MgCl_2$. All portions were processed through the virus concentrator and compared on the basis of virus recovered after elution off the virus adsorbent. The results of a series of tests are shown in Table 5. The importance of salt enhancement to virus recovery was documented in both Houston ship channel and Galveston Bay waters. The presence of natural salts in both bodies of water did not replace either magnesium or aluminum salts as virus-adsorbent enhancers. The superior virus recoveries obtained with $AlCl_3$ led to its adoption for routine salt enhancement of virus adsorption in subsequent studies.

Influence of resin treatment of eluates upon virus reconcentration. Recent work at this laboratory has shown that MCC contained

TABLE 4. *Virus passage through adsorbent filters after filtration of large volumes of polluted salt waters*

Water passed through filters (gal)	Virus in filtrates (PFU/0.1 ml)			
	Houston ship channel[a]		Galveston Bay[b]	
	Fiber glass[c]	Cellulose acetate[c]	Fiber glass	Cellulose acetate
0 (untreated filter)	14.0	12.0	25.0	5.0
5	19.0	32.0	43.0	29.0
10	23.0	56.0	43.0	37.0
20	20.5	66.5	54.0	43.0
40	16.0	111.0	66.0	43.5
80	23.5	113.0	67.5	44.0
160			68.0	45.0

[a] Houston ship channel samples: salinity, 7.2 0/00; temperature, 26.6 C; turbidity, 1.5 Jackson turbidity units. Virus challenge: 116 PFU/0.1 ml. As a preliminary clarification step, raw ship channel waters were passed through Tween 80-treated, 1-μm cellulose acetate filters.

[b] Galveston Bay samples: salinity, 24 0/00; temperature, 26 C; turbidity, 4.5 Jackson turbidity units. Virus challenge: 224 PFU/0.1 ml.

[c] Filter porosities: 1 μm.

TABLE 5. *Influence of different salts on enhanced recovery of poliovirus added exogenously to salt waters*[a, b]

Sample (gal)	Salinity[c] (0/00)	Virus added (PFU/gal)	Test volumes (gal)	Virus recovered (PFU/gal)		
				Without added salt	With $AlCl_3$[d] (0.0005 M)	With $MgCl_2$[d] (0.05 M)
75	7	4,600	25	1,748	2,944	2,116
50	10	140	25		100	67
75	8	34	25	11	19	12
75	14	3,100	25	1,270	1,990	1,660
75	24	1,560	25	523	955	733

[a] Fiber glass filters were used for virus adsorption.
[b] Natural virus was not detected in 50- or 75-gal samples collected at the same time as test samples.
[c] Samples with salinities of 7, 8, and 10 obtained from the Houston ship channel. Samples with salinities of 14 and 24 obtained from Galveston Bay.
[d] Final concentration of salt in sample.

in virus tissue culture harvests could be selectively adsorbed to certain cation resin exchangers (C. Wallis, M. Henderson, and J. L. Melnick, manuscript in preparation). These resins (C-249, Na^+ charged, Ionac Corp., Birmingham, N.J.) were used to remove MCC from virus eluted off virus adsorbents so that the viruses present therein could be more efficiently reconcentrated on membranes of smaller surfaces.

Samples collected from polluted surface waters were returned to the laboratory, and, after elution off adsorbent filters, eluates were divided into two equal portions. One was treated with resin, whereas the other received no resin treatment. The results of this series of tests are shown in Table 6. Resin treatment led to more isolations in all the samples examined. The most pronounced differences in virus recoveries

TABLE 6. *Virus recoveries from eluates of polluted surface waters with and without resin treatment*

Expt	Sample (site)	Sample volume (gal)	Virus recoveries	
			Without resin	With resin[a]
1	Ship channel	75	2	3
2	Galveston Bay	155	0	2
3	Ship channel	40	2	5
4	Sewage plant #1	30	7	14
5	Sewage plant #1	30	12	16
6	Sewage plant #2	34	4	8
7	Ship channel	72	1	3

[a] Cationic resin, C-249, Ionac Corp., N. J.

were obtained with waste treatment plant effluents. Since resin treatment removes MCC, it was apparent that all samples contained interfering substances of this type to varying degrees.

Virus stability to acid and alkaline adjustments. Satisfactory enterovirus pH stability for methods involving adsorption and elution of virus using membrane filters or polyelectrolytes has been reported previously (9, 10). To determine whether loss of natural virus could be expected from pH adjustments made during sample collection, elution, or reconcentration phases of virus concentrator methods, poliovirus stability to the acid and alkaline pH adjustments necessary was examined.

Virus stability to pH 11.5 adjustment during elution was examined first. Fifty milliliters of 0.05 M glycine solution, pH 11.5, was added to 0.5 ml of poliovirus suspension, pH 7.2. Final pH of the mixture was 11.5. Samples for virus assay were removed at 10-min intervals during a 30-min test period. The samples were diluted upon collection in a buffered diluent and 0.1 ml of inocula was immediately inoculated onto cell culture monolayers. No virus loss was detected at 10 min. At 20 min a 5% loss occurred, and at 30 min a 10% loss was found. The results showed no reason to expect natural virus loss during elution procedures since maintenance of pH 11.5 does not extend beyond 10 min.

Tests were also conducted to determine virus stability to a pH change from 7.0 to 11.0. Sodium hydroxide solutions of 1.0, 0.5, 0.1, 0.05, and 0.01 normalities, respectively, were used to adjust identical 50-ml portions of a 0.05 M glycine solution containing poliovirus from pH 7.0 to 11.0. Each suspension was stirred continuously by magnetic mixer, and alkali addition was made dropwise. Virus losses of 18% were suffered with 1.0 N NaOH and 15% with both 0.5 N and 0.1 N NaOH. No loss was found with 0.05 N or 0.01 N NaOH. These data showed alkaline pH adjustments could be made only with dilute alkali if virus loss was to be avoided.

During processing of a water sample, acidification to pH 3.5 is necessary prior to the addition of $AlCl_3$. Tests were made to determine virus stability to a pH change from about 7.8 to 3.5. Using Houston ship channel water to which poliovirus type 2 was added (this virus was not found in water used), the sequence of virus concentrating procedures from post-clarification to post-$AlCl_3$ injection only was examined. Virus stability to acid adjustment was determined by comparison of assay results obtained from pre-acid and post-$AlCl_3$ injection samples. Virus losses of 13 to 21% were found with flow

rates of 0.5 to 1.5 gal/min.

Examination of the entire sequence of virus concentrator procedures from beginning to end was made using field samples to which marker virus had been added. Results of a series of tests are shown in Table 7. The data shown include initial, post-clarification, and post-acid adjustment virus assays only. Virus losses averaged about 5%.

The results showed that acid adjustment of a sample led to an immediate loss of virus of at least 13%. An average loss of 5% was found during virus collection and represented the actual approximate loss of natural virus that might be expected.

DISCUSSION

Recovery of viruses from large volumes of surface water depends upon water quality and factors present which influence the virus recovery process. Chief among recognized factors are particulates, organic matter, and salts (1). The virus concentrator represents a unique extension of the flow-through principle in which the water being sampled is forced under pressure through a clarifying and adsorbing surface. In all such systems, the collecting surface is subject to clogging from deposition of particulates from the water being sampled. A common practice is the use of prefilters for removal of particulates and protection of adsorbent surface. Special filters of the cartridge type have been used effectively for recovery of low multiplicities of virus in large volumes of water. Hill et al. (2) reported recovery of 9 plaque-forming units (PFU) of poliovirus from 100 gal of estuarine water containing initially 24 PFU of virus. Studies of this type should be repeated in the field under exacting environmental conditions to determine their effectiveness under "real world" conditions. Virus loss to particulates during filtration was minimal in the study despite heavy loads of suspended solids in waters being sampled. The discrepancies in virus adsorption shown in Tables 1 and 4 are due to the waters under test. In Table 1, artificial seawater was used; it was free of organics, and thus no MCC were present, which permitted more efficient virus adsorption to the textile filters. In Table 4, natural water which contains MCC was used, and thus less efficient virus concentration ensued. The textile filters proved effective at clarifying marine and estuarine surface water without undue virus loss (4). This loss could be reduced further by exchange of one or more clarifying filters during collection of a sample. Flow rates not in excess of 2 gal/min were maintained during sampling. These rates are considered optimal for virus collection.

TABLE 7. *Virus concentration before and after acid adjustment of samples*

Expt	Sample (gal)	Virus assay (PFU/0.1 ml)			Loss from acid adjustment (%)
		Initial virus	Pre-acid	Post-acid	
1	12	139	138	127	8
2	45	200	193	187	3
3	50	81	74	72	3
4	50	97	94	90	4
5	50	106	103	98	5
6	50	77	75	69	8
7	50	122	120	113	6

The waters sampled during the study contained MCC which contributed to virus passage through adsorbent filters. The use of a fiberglass virus adsorber in place of a cellulose acetate virus adsorber reduced this effect. Virus loss during concentration procedures was minimized by optimizing the appropriate combination of acid adjustment, $AlCl_3$ addition, and membrane filter selection. The importance of resin treatment to natural virus isolation was shown by the nearly twice as many isolates found in treated eluates compared with nontreated samples.

The salt content of saline waters did not replace the need for salt enhancement for virus adsorption from these waters. The superiority of $AlCl_3$ over $MgCl_2$ was considered to result from greater amplification of electrostatic forces developed at adsorbent surfaces at acid pH levels.

Virus losses associated with pH changes were found to result from local inactivation effects attributable to interaction of virus with strong acid or base. When virus was introduced to pH 11.0 or 3.5, virtually no losses occurred. Any loss experienced always accompanied addition of strong acid or base solutions to a virus suspension, and could be avoided through use of weaker solutions.

The studies demonstrated the ability of the virus concentrator to recover virus from samples (100 gal or more) of surface water under harsh environmental conditions. All factors known to interfere with virus collection were examined and found capable of satisfactory resolution.

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