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Reconcentration of Poliovirus from Sewage

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Virus can be adsorbed from effluents of sewage treatment plants on large-surface membranes. Subsequent elution of virus requires large volumes, which in turn requires reconcentration of virus for assay. However, reconcentration of such viral eluates on small adsorbent surfaces is difficult because certain soluble sewage components are adsorbed along with the virus on the initial virus adsorbent and are removed along with the virus by the eluent. Upon acidification of the initial eluate to reconcentrate the virus on smaller membrane surfaces, flocs are formed that interfere with the reconcentration process. To circumvent this problem, the interfering sewage components can be removed by activated carbon and ion-exchange resins. The virus is then readily reconcentrated on small membranes.

We have previously reported on the detection of small numbers of viruses in large volumes of water that was relatively free of organics (5, 6). Reconcentration of these viruses, which were adsorbed to a large surface area, could be performed by elution of the virus off the virus adsorbent with a basic buffer and reconcentration of the eluted virus on a small surface membrane (5, 6). Under these conditions, hundreds of gallons of test sample could be reduced to a final eluate of 10 to 20 ml with 75 to 100% of the initial virus contained therein.

In the case of sewage we have reported concentrating viruses from 30-gallon (ca. 114-liter) amounts, but until recently we did not find it necessary to reconcentrate the adsorbed virus, since relatively large amounts of virus were already present in the eluate (2). However, interest in detecting small numbers of virus in recycled or reclaimed waters, as well as in determining the efficiency of different disinfection methods (e.g., chlorination, ozonation, etc.), now requires the detection of small numbers of virus in large volumes of organic-matter-laden water. Thus, reconcentration of eluates is required; such eluates contain heavy organic loads as well as precipitable materials that impede flow rates and efficient readsorption of viruses to small membrane surfaces. Therefore, this study was conducted to delineate the optimal parameters for reconcentration of viruses isolated from sewage on large-surface virus adsorbents.

MATERIALS AND METHODS

Virus. Type 1 poliovirus (strain LSc) was used as a model agent and added to sewage as described in the text and tables. The virus was grown in BSC-1 cells and assayed in these cells by the plaque-forming unit method as described in detail elsewhere (4).

Virus concentrator. Viruses added to sewage effluent were concentrated by a virus concentrator that was developed by Wallis et al. (7) and that is now available commercially from the Carborundum Co. (Niagara Falls, N.Y.) as the Aquella virus concentrator. In brief, effluent sewage is passed through a series of clarifiers to remove solids, and then the clarified effluent is treated in-line with acid and $AlCl_3$ to attain a pH of 3.5 and a final aluminum concentration of 0.0005 M. The treated fluids are then passed through virus adsorbents (a 10-inch [ca. 25.4-cm] fiberglass depth filter [K-7] and 293-mm Cox filters of 1- and 0.45-μm porosities). The effluent is discarded after passing these filters. Virus is then eluted off the virus adsorbents with 2 liters of pH 11.5 glycine buffer, and the eluate is collected in 2 liters of pH 2 glycine buffer, which immediately neutralizes the eluate.

pH meter and probes. The fluid used for eluting viruses off membranes and other reactive surfaces should be adjusted to pH 11.5. For this purpose, a qualified pH electrode must be used, in which the manufacturer states its range of efficiency. Commercially available buffers cannot be used to standardize the pH meter, since their stability and accuracy are not always adequate. A saturated solution of calcium hydroxide should be used to standardize the electrodes and pH meters, since this buffer remains pH 12.4 at 25°C as long as it is saturated. It should be stressed that if an electrode is exhausted or inadequate for measuring pH 11.5 accurately, one may be forcing a sluggish electrode to pH 11.5 with inadequate control standards. Although the pH meter may read 11.5, pH levels in fact may be in the 12 to 13 range and thus virucidal.

Charcoal. Pittsburgh charcoal (BPL grade, Calgon Corp., Pittsburgh, Pa.) of 50 mesh was used to remove organics from virus eluates. Charcoal beds

were conditioned with pH 10.0 glycine buffer until the effluent indicated the same pH as the input washing fluids, and the absorbance at 254 nm was approximately zero.

Ion-exchange resin. Dowex 1X-8 (chloride form, 20 to 50 mesh) was prepared for use by rinsing with 10 bed volumes of 1 M NaCl followed by pH 10.0 glycine buffer until the column effluent was pH 10.0 and the absorbance at 254 nm was reduced to approximately zero.

Turbidity. Turbidity was measured on a turbidimeter (Hach Chemical Co., Ames, Iowa) and reported as Jackson turbidity units (JTU).

Virus adsorbents used for reconcentration of virus. Virus eluted off the initial virus adsorbents (293-mm Cox filter and K-27 fiberglass filter) was readsorbed to a series consisting of 3.0-, 0.45-, and 0.25-μm Filterite filters (Filterite Corp., Timonium, Md.) or to a 0.45-μm Cox filter (Cox Instrument Corp., Detroit, Mich.). The filters were contained in 47-mm holders.

Eluent. Fluid used for elution of virus off the final adsorbents described above was pH 10.5, 0.05 M glycine buffer.

Measurement of organics. Measurement of organics was accomplished by assaying absorbance at 254 nm on a dual-beam Varian spectrophotometer (model 635). The correlation between ultraviolet absorbance at 254 nm and total organic carbon has been shown to have high correlation coefficients (1).

Source of sewage. Secondary effluent from Houston sewage processing plants was used for most of this study. Additional samples of eluates were provided by the Pomona Research and Development Facility, County Sanitation District of Los Angeles. Initial eluates from the 293-mm/K-27 virus adsorbents were shipped to our laboratories frozen and were reconcentrated by the methods described above.

RESULTS

Effects of resin exchanger on the flow rate of eluates through membranes. Forty liters of effluent sewage seeded with poliovirus was processed through the virus concentrator as described above. After elution of the virus off the 293-mm/K-27 virus adsorbents with 2 liters of pH 11.5 glycine buffer and collection of the eluate in 2 liters of pH 2 glycine, assays indicated that approximately 85% of the virus could be recovered (based on initial input) in each of 10 consecutive experiments. However, reconcentration of this type of eluate onto a small-surface Cox membrane filter as previously described (5) proved difficult, since acidification of the eluate to pH 3.5 and addition of $AlCl_3$ resulted in formation of precipitates that clogged the smaller surface virus adsorbent. Less than 100 ml of the total 4-liter eluate could be processed before the membrane clogged. Treating the eluate with an anion-exchange resin (Dowex 1X-8) increased the flow rate through the smaller filter and increased the volume that could be processed before clogging without significant loss of virus (Fig. 1).

It was observed that the increase in flow rate paralleled a decrease in organic content of the eluates as measured by the absorbance at 254 nm. Therefore, different anion-exchange resins and organic adsorbents were tested for their ability to remove organic compounds from eluates. Carbon was the most effective organic adsorbent tested (Table 1). Eluates treated with carbon were similar to those treated with resin in that clogging components were reduced. However, when carbon columns were used to treat virus-containing eluates, a high percentage of virus was removed along with the organic compounds. It was observed that the pH of the eluate changed after carbon treatment and that a layer of gel often formed at the surface of the carbon column. These results suggested that the aluminum ions initially added to sewage effluents to enhance viral adsorption were concentrated along with the virus and organic compounds. Lowering of the pH of the eluate during carbon treatment could promote the formation of aluminum hydroxide flocs that adsorb virus and are retained on the carbon column. To determine if concentrated aluminum ions were a problem, the nature of the precipitates formed in the eluate was examined.

After a 40-liter run of effluent sewage, the eluate obtained after passing 2 liters of pH 11.5 glycine buffer through the virus adsorbents was recovered and immediately adjusted to pH 10.0. One liter of the pH 10 eluate was placed in a beaker and magnetically stirred while adjusting the pH from 10 to 3.5 and obtaining aliquots at various pH levels, as indicated in Table 2. When the pH 3.5 sample was removed, $AlCl_3$ at a final concentration of 0.0005 M was added to the pH 3.5 eluate in the beaker to simulate the procedure for reconcentrating viruses, and a sample was obtained. All samples were tested on the turbidimeter to determine at which pH level the precipitate was magnified. To determine if the major problem was due to recovery of aluminum ions concentrated on the initial virus adsorbents in the form of complexed metals or organics, a sample of the pH 11.5 eluate was treated with an excess of tetrasodium ethylenediaminetetraacetate (EDTA) and also tested on the turbidimeter. The results of this test are shown in Table 2.

At pH 9.0 or higher, a range at which aluminum and ferric hydroxide are soluble, the turbidity was minimal. As the eluate was acidified, maximal turbidity was manifest at pH 4 to 6, the range at which aluminum hydroxide gels

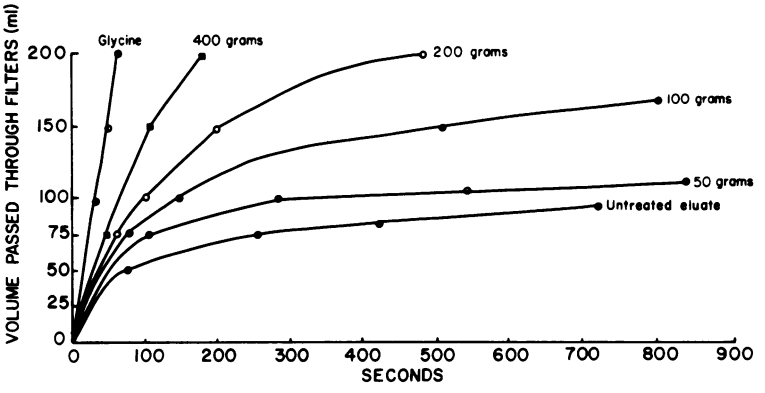

FIG. 1. *Influence of resin treatment on flow rates of eluates through membrane filters. Eluates were obtained as described in Table 1. A 100-ml sample of eluate at pH 8.0 was passed through the indicated amount of resin contained in 3-cm-diameter columns. The columns were washed with 100 ml of glycine, pH 8.0. The treated eluates and the wash were pooled, adjusted to pH 3.5 and 0.0005 M aluminum chloride, and passed through 0.45-μm Cox filters under 30 lb/in² of pressure.*

form. Continued acidification of the eluate to pH 3.5 solubilized some of the gel, but it was evident that once gels were formed it was difficult to solubilize them at acid levels. The eluate sample at pH 11.5 that was treated with EDTA and then acidified was sparkling clear and had the same reading on the turbidimeter at pH 11.5 as at pH 3.5.

Titration of trivalent ions eluted off virus adsorbents. Since sewage processed and eluted by different workers contains different amounts of gel-forming trivalent ions, titration of trivalent ions present in eluates was conducted. Based on the results shown in Table 2, a sample of the pH 11.5 eluate was adjusted to pH 5 with 1 N HCl to form a precipitate. After 30 min the precipitate matured. Numerous samples were treated with different amounts of EDTA, and turbidity was scored accordingly. When a JTU reading was attained that did not decrease further in value, the end point of EDTA was assumed to have been reached. The results of this test are shown in Table 3. About 0.004 to 0.005 M EDTA appears to be the optimal concentration to dissolve the precipitates.

To determine the amount of trivalent ions present in the eluate as evaluated in Table 3, a known constant amount of $AlCl_3$ was titrated against different amounts of EDTA. Sodium carbonate was added to form a floc at pH 5.0. The lowest level of EDTA that prevented flocculation determined an assay for the presence of known amounts of $AlCl_3$ or other trivalent ions that may be eluted off virus adsorbents. From the results shown in Table 4, it is evident that the amount of $AlCl_3$ or other trivalent ion present in the eluate studied in Tables 2 and 3 was 0.0025 M. EDTA (0.008 M) was required to solubilize 0.005 M $AlCl_3$. Thus, for each 0.001 M EDTA used for solubilization of an unknown, 0.00063 M trivalent ions may be present.

TABLE 1. *Removal of organic compounds in filter eluates by anion-exchange resins and organic adsorbers*[a]

Adsorbing material[b]	Amt (g)	Column effluent	
		Absorbance at 254 nm	% of influent absorbance[c]
Duolite ES-161[d] (anion exchanger)	10	0.463	66
Duolite ES-863 (organic adsorbent)	10	0.675	96
Duolite A-109 (anion exchanger)	10	0.449	64
Dowex 1X-8 (anion exchanger)	10	0.390	55
BPL carbon	5	0.239	34

[a] Forty liters of sewage effluent was processed through the virus concentrator using a K-27/293-mm Cox filter series. The filters were then treated with 2,000 ml of pH 11.5 glycine, and the resulting eluate was adjusted to pH 8.0 with pH 2 glycine before treatment with resin.

[b] The indicated amount of adsorbing material was placed in a column (1.5 by 14 cm). Anion exchangers were charged with Cl^- ions.

[c] A 20-ml portion of influent with an absorbance at 254 nm of 0.703 was passed through each column.

[d] Obtained from Diamond Shamrock Co., Redwood City, Calif.

Virus eluates obtained from the initial virus adsorbents and treated with EDTA, acidified, and treated again with $AlCl_3$ and sufficient $CaCl_2$ to deplete the activity of residual EDTA could now be filtered through 47-mm virus adsorbents without significant decrease in flow rates. However, experiments indicated that the organics present in the eluate were coating the

TABLE 2. *Nature of precipitates present in virus eluates*

Sample[a]	JTU
Eluate from virus adsorbents (using pH 11.5 glycine buffer as an eluent)	0.6
After adjustment of pH 11.5 eluate to pH:	
10.0	0.6
9.0	0.6
8.0	1.5
7.0	4.5
6.0	6.5
5.0	7.5
4.0	7.0
3.5	5.0
3.5 + $AlCl_3$ (0.0005 M)	5.8
Original pH 11.5 eluate with 0.005 M EDTA adjusted to pH 3.5	0.6
0.0005 M $AlCl_3$ added to the above pH 3.5 EDTA sample	0.6

[a] Forty liters of sewage effluent was processed through the virus concentrator. The K-27/293-mm virus adsorbents were treated with 2 liters of pH 11.5 glycine buffer to elute virus. This eluent also elutes organic compounds. The pH 11.5 eluate was then adjusted to the levels indicated with 1 N HCl, and 50-ml samples were obtained. All samples were allowed to stand for 30 min before measuring turbidity. To indicate that the precipitates formed by acidification of the eluate were due to trivalent ions, a sample of the pH 11.5 eluate was treated with EDTA at a final concentration of 0.005 M and then adjusted to pH 3.5. A portion of this sample was then treated with 0.0005 M $AlCl_3$ to simulate the requirements for reconcentration of virus on a membrane surface.

47-mm virus adsorbent and allowing the virus to pass into the filtrate instead of being concentrated on the membrane. Therefore, removal of the organics was still required. Virus loss on the carbon columns was reduced by the addition of EDTA to eluates at a final concentration of 0.01 M. When eluates seeded with virus were passed through charcoal beds without solubilizing the precipitates with EDTA as described above, virus was removed (from 10 to 60%). When the pH of the eluate containing virus was adjusted to pH 10 and EDTA at a final concentration of 0.01 M was added, no significant loss of virus occurred after passing through the charcoal bed. Therefore, the capacity of charcoal to remove the organics present in virus eluates as measured at 254 nm was determined.

A 50-g amount of 50-mesh BPL-grade charcoal was loaded into a column (4 by 100 cm) and washed with pH 10.0 glycine buffer until the effluent measured pH 10. The virus eluate described above at pH 10 containing 0.005 M EDTA was passed through the charcoal, and serial effluent samples were monitored at 254 nm for organic levels and assayed for virus. The capacity of this charcoal to remove organic compounds in the eluate is shown in Table 5. Since an organic breakthrough occurred between 600 and 700 ml, a formula may be derived as follows. For each 0.200 absorbance unit of eluates at 254 nm, 50 g of charcoal is required per 700-ml sample, or volume (milliliter) × absorbance at 254 nm × 0.36 = grams of carbon required.

Since the EDTA added to the eluates can chelate the metal ions required to enhance viral adsorption to small membranes, it must be removed or its effects must be negated. Although the effects of EDTA can be reduced by the addition of 0.1 M calcium chloride, some virus in treated eluates at pH 3.5 and 0.0005 M aluminum chloride are not adsorbed to small filters. It is possible to remove EDTA with an anion-exchange resin such as Dowex 1X-8. Resin treatment has the possible added advantage of re-

TABLE 3. *Titration of trivalent ions in virus eluates*

Final concn (M) of EDTA added to eluate[a]	JTU
None (control)	7.3
0.001	4.5
0.002	1.7
0.003	0.8
0.004	0.6
0.005	0.6
0.006	0.6

[a] The pH 11.5 eluate described in Table 2 was adjusted to pH 5.0 to attain a maximum precipitate, as described in Table 2. The JTU of this control sample was recorded. Numerous samples of this precipitated eluate were then treated with final concentrations of EDTA as indicated, and the JTU was scored accordingly.

TABLE 4. *Titration of $AlCl_3$ against EDTA*

Final concn (M) of EDTA[a]	JTU
None (control)	60
0.002	30
0.004	5
0.006	2
0.008	0.9
0.01	0.85
0.03	0.8

[a] Samples (22.5-ml) of 0.005 M $AlCl_3$ in distilled water were placed in numerous containers, and 2.5-ml amounts of different concentrations of EDTA were added to give the final EDTA levels indicated. Each sample was then treated with sufficient Na_2CO_3 until pH 5 was attained, the range at which a massive precipitate forms with $AlCl_3$ (EDTA free). Samples were allowed to stand for 30 min, mixed well, and read on the turbidimeter.

moving organic compounds that were not adsorbed by the carbon. EDTA and carbon-treated eluates were passed through a column of Dowex 1X-8 until the absorbance at 254 nm began to increase to determine the capacity of the resin. Using this procedure, the following formula for determining the amount of Dowex 1X-8 was derived: volume (milliliter) × absorbance at 254 nm × 0.125 = grams of resin required. Since water quality may vary and different materials may be concentrated in the eluates, the absorbance of the treated eluates should be checked. If the absorbance at 254 nm is less than 0.04, the eluate should be adjusted to pH 3.5 by the addition of 0.1 N HCl to the sample during continued mixing, mixed with aluminum chloride to produce a final concentration of 0.0005 M, and passed through a filter series consisting of 3.0-, 0.45-, and 0.25-μm Filterite filters in a 47-mm holder at 200 ml/min or less. This series was found to be comparable to Cox filters in virus-adsorbing properties but was more resistant to clogging. If the absorbance is greater than 0.04, sufficient organics are present to interfere with virus adsorption to the smaller filters and additional treatment with anion-exchange resin is required until the absorbance is below 0.04. The eluate can then be adjusted and passed through filters as described above.

Virus is eluted from the filters by treatment with 25 ml of pH 10.5 glycine buffer. The eluate is neutralized with pH 2 glycine.

Based on the above, we developed the following procedure for concentration of virus in sewage effluents.

(i) Sewage effluent is passed through the virus concentrator as previously described (7).

(ii) Adsorbed virus is eluted from the K-27/293-mm Cox filters using 2,000 ml of pH 11.5 glycine. The eluate is immediately neutralized by collection in pH 2 glycine.

(iii) While the neutralized eluate is being continuously stirred, 0.1 N NaOH is slowly added until pH 10 is reached. The absorbance at 254 nm is measured, and the amount of carbon required is determined. The carbon is washed with pH 10.0 glycine until the pH of the effluent reaches 10.0.

(iv) EDTA is added to produce a final concentration of 0.01 M. The eluate is then passed through a carbon column at 200 ml/min. After passage of the eluate the column is washed with a volume (milliliter) of glycine equal to the weight of the carbon (gram) used, and the wash is added to the treated eluate.

(v) The absorbance of the treated eluate is measured and the amount of resin required is determined. The resin is washed with pH 10.0 glycine until the absorbance at 254 nm is near zero and the pH is 10.0. The carbon-treated eluate is then passed through the column at 200 ml/min. The column is washed with a volume (milliliter) of glycine equal to the weight (gram) of resin used. At this point the absorbance at 254 nm should be less than 0.04. If the absorbance is greater, additional resin treatment is required until the 0.04 reading is attained.

(vi) The treated eluate is adjusted to pH 3.5 by the addition of 0.1 N HCl. To minimize viral loss, the eluate is continuously mixed and the HCl is added dropwise. Aluminum chloride is added to the eluate at pH 3.5 to produce a final concentration of 0.0005 M. The adjusted eluate is passed through a 3.0-, 0.45-, and 0.25-μm Filterite filter series in a 47-mm holder at 200 ml/min. Adsorbed virus is then eluted with 25 ml of pH 10.5 glycine. The eluate is neutralized with pH 2 glycine and is ready to be assayed. If the sample is to be assayed without dilution, 2 M NaCl is added to attain an isotonic level of 0.12 M NaCl. This procedure can concentrate virus in initial eluates by a factor of 120 with an average recovery of 40% (Table 6).

TABLE 5. *Removal of organics from virus eluates*

Fraction (ml)[a]	Absorbance at 254 nm	PFU/ml
Control eluate	0.209	2,500
After addition of EDTA	0.210	3,100
200	0.010	175
400	0.030	3,000
600	0.032	3,050
800	0.045	3,200
1,000	0.072	3,000
1,200	0.095	3,150

[a] Forty liters of effluent sewage seeded with poliovirus was processed through the virus concentrator. Elution of the virus off the 293-mm Cox filter/K-27 depth filter indicated a recovery of 94% of the input virus (control eluate at pH 10.0). The control eluate was then treated with EDTA at a final concentration of 0.005 M, and the eluate was passed through a 50-g charcoal bed contained in a column (6 by 20 cm) at a flow rate of 300 ml/min. Samples (200-ml) were serially collected and assayed for organic matter at 254 nm and for virus by treating the control eluate and the charcoal filtrates with 0.005 M $CaCl_2$ to inactivate the residual EDTA and diluting all samples threefold in Tris-buffered saline for plating. Since EDTA absorbs at 254 nm, the reference beam on the spectrophotometer was loaded with the same final concentration of EDTA in glycine buffer as was added to the eluate. Thus, the value of EDTA was automatically subtracted from the test sample. When EDTA was not present in the test sample, glycine buffer was used in the reference beam.

TABLE 6. *Reconcentration of virus in eluates from filters used to process sewage effluents using membrane filters*[a]

Expt	Initial eluate			Organic adsorbents		Treated eluate			25-mm eluate		
	Vol (ml)	Absorbance at 254 nm	Virus (PFU)	Carbon (g)	Dowex 1X-8 (g)	Vol (ml)	Absorbance at 254 nm	Virus (PFU)	Vol (ml)	Virus (PFU)	% of initial eluate[b]
1	6,400	0.101	7.0×10^8	400	300	7,200	0.004	4.0×10^8	45	4.1×10^8	59
2	4,700	0.234	3.2×10^8	600	800	6,100	0.042	2.3×10^8	45	1.0×10^8	31
3	6,000	0.195	7.9×10^8	600	600	7,600	0.020	3.9×10^8	38	1.9×10^8	24
4	6,100	0.295	6.9×10^8	600	800	7,100	0.040	6.0×10^8	41	3.2×10^8	46

[a] A 390-liter amount of sewage effluent was processed through the virus concentrator using a 39-R clarifier and a K-27/293-mm Cox filter series. The filters were treated with 3,000 ml of pH 11.5 glycine, and the resultant eluate was neutralized with pH 2 glycine. The neutralized eluate was seeded with virus and adjusted to pH 10.0, and the absorbance at 254 nm was determined. EDTA was added to produce a final concentration of 0.01 M, and the eluate was passed through the carbon column. The absorbance at 254 nm was determined, and the eluate was passed through the Dowex 1X-8 column. The amounts of carbon and Dowex were determined using the formulas described in the text. The treated eluates were adjusted to pH 3.5 and 0.0005 M aluminum chloride and then passed through a series consisting of 3.0-, 0.45-, and 0.25-μm Filterite filters in 47-mm holders. Virus was eluted from the filters with 25 ml of pH 10.5 glycine, and the eluate was neutralized with pH 2 glycine. PFU, Plaque-forming units.

[b] Mean, 40%.

DISCUSSION

During concentration of virus from water using the Aquella virus concentrator, organic compounds and metal ions may also be concentrated. When relatively clean water is processed, the virus eluted from the primary virus adsorbents can be reconcentrated onto smaller membranes with no difficulty (3, 5, 6). However, reconcentration of eluates from filters used to process sewage effluents may pose a problem. The organic compounds and metal ions form flocs at low pH and rapidly clog small filters used for reconcentration. Removal of the flocculation problem with EDTA produces a problem of interference with virus adsorption to the smaller filters. However, removal of the organic compounds by treatment with carbon and ion-exchange resin permits reconcentration on smaller filters. This procedure yields a final volume of approximately 40 ml with a mean recovery of 40% of the virus initially present in the eluate.

Since most of the organic compounds in the eluate are removed by the carbon and anion-exchange resin, a relatively clean final concentrate is obtained. This is a further advantage if components that are toxic to the cells used for viral assays or that interfere with virus adsorption to the assay cells are concentrated with the virus during the initial adsorption and elution steps.

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