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CHAPTER 6

VIRUS ADSORPTION-ELUTION (VIRADEL) CARTRIDGE FILTER PROCEDURES FOR RECOVERING VIRUSES FROM SEWAGES, EFFLUENTS, AND WATERS

In principle, the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedures described in this chapter are the same as Method 2 described in Chapter 5. The VIRADEL cartridge filter procedures require much greater volumes of elutant than Method 2 in Chapter 5 requires, but the cartridge filter procedures may be used for sample volumes greater than 200 liters and perhaps for volumes greater than 2000 liters.

Waters that contain chlorine and cannot be processed immediately must be dechlorinated immediately upon collection. Immediate dechlorination may be accomplished by placing into the collection vessel 0.8 mL of a 10 percent solution of sodium thiosulfate ($Na_2S_2O_3$) for each liter of water to be collected. That quantity of $Na_2S_2O_3$ is sufficient for neutralizing 15 mg of chlorine per liter.

Use aseptic techniques and sterile materials and apparatus only. Sterilize all contaminated materials before discarding them (see Chapters 2 and 3).

Provide physical support as necessary for equipment that is not free-standing.

1. ADSORPTION -- METHOD ONE

This procedure may be used for all waters that do not require

prefiltration.

1.1 Preparation

1.1.1 Apparatus and Materials. Install quick-disconnect connectors on ports of all apparatus except on additive pumps.

(a) Holder for 10-inch (size is given in inches when commercially designated only in that unit) cartridge filter (Fulflo, Model No. F15-10, Commercial Filter Division, Carborundum Co., or equivalent).

(b) Cartridge filter, pleated epoxy-fiberglass -- 10-inch, 0.45-micrometer pore size (DUO-FN 10-E-0.45 A ECIS, Filterite Corp., or equivalent).

(c) Plastic-coated drums -- 200-liter capacity, or other containers of size suitable to hold sample, if sample is not pumped directly from source.

(d) Sterilizable self-priming water pump that delivers approximately 25-50 liters per minute. Pump is not needed if sampled water is under pressure, e.g., tap water.

(e) Carboy, autoclavable plastic with nipple on bottom fitted with tubing clamped to a dispensing Y (clamp tubing closed between nipple and Y) -- 20-liter capacity. If the water at the sampling site is to be drawn directly from a pressurized source and is to be dechlorinated, then two similarly fitted carboys are needed. Otherwise, only one carboy is needed. Twice the number of carboys is needed under these conditions if water volumes greater than 400 liters are to be processed.

(f) Fluid proportioner consisting of fluid-driven motor with four additive pumps (Johanson and Son Machine Corp., Model M14Q with one P-562 and one P-750 additive pump affixed to each side of the fluid-driven motor, or equivalents). Assemble fluid proportioner, and connect tubing in accordance with manufacturer's instructions.

(g) Mixing chamber (Johanson and Son Machine Corp., C-SS, or equivalent).

(h) pH meter, measuring to an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode (Van London Co., or equivalent, for electrode only).

(i) Tee, stainless steel, with three female NPT (National Pipe Thread) ports. Center port equipped with pH electrode in-line adapter (Van London Co., or equivalent, for adapter only).

(j) Autoclavable inner-braided tubing fitted with metal quick-disconnect connectors for connecting tubing to equipment to be used under pressure. Quick-disconnect connectors can be used only after equipment has been properly adapted.

(k) Magnetic stirrer and stir bars.

(l) Water meter (Badger Meter Inc., or equivalent).

(m) Positive pressure source equipped with pressure gauge. Pressure source, if laboratory air line or pump, must be equipped with oil filter. If source is capable of producing high pressure, deliver to filter holder no more pressure than recommended by manufacturer.

1.1.2 Media and Reagents

(a) Hydrochloric acid (HCl) -- 0.12 and 12 M (concentrated) solutions. Prepare 100 to 500 mL of 0.12 M HCl. This solution may be stored for several months at room temperature.

(b) Sodium thiosulfate ($Na_2S_2O_3 - 5H_2O$) -- 40 percent (w/v) stock solution (with respect to $Na_2S_2O_3 - 5H_2O$). Prepare one liter of $Na_2S_2O_3$ solution by dissolving 400 g of $Na_2S_2O_3 - 5H_2O$ in 500 mL of deionized distilled water and bringing final volume of solution to one liter with deionized distilled water. This solution may be stored in dark, rubber-stoppered bottle for up to one month at room temperature. This solution is to be used to dechlorinate water that cannot be dechlorinated except immediately prior to test procedure (e.g., tap water tested directly at source). For dechlorinating all other waters, see beginning of chapter.

(c) Aluminum chloride ($AlCl_3 - 6H_2O$) -- 3 M stock solution. Prepare 100 mL of 3 M $AlCl_3$ for each 400 liters of water to be processed.

1.2 Procedure (see Figure 1 for flow diagram of procedure)
In this procedure an apparatus is described that can be used with clean waters, such as tap waters, where only a 0.45 micrometer pleated epoxy-fiberglass cartridge filter is needed. For waters that are sufficiently turbid so that the volume filtered will clog this filter, prefilters are required and Method 1 cannot be used. For turbid waters, use Method 2 described in Section 2. Experience usually dictates the method of choice. (CAUTION: Turbid water may clog the fluid proportioner and, if abrasive, may damage it).

1.2.1 Preparation and Implementation. It is usually convenient to sterilize each piece of apparatus and equipment one or more

days before it is used (see Chapter 3). It is convenient to sterilize apparatus in small units when sterilization is accomplished by steam or ethylene oxide. However, it is advisable to assemble and connect units of apparatus that are to be sterilized by chlorination. The interconnected apparatus can be disassembled after the chlorination procedure is completed, the ports covered with aluminum foil, and the units stored until used.

(a) Assembly of apparatus (see Figures 2 and 3). Use inner-braided tubing fitted with quick-disconnect connectors to make all connections for apparatus to be used under pressure.

(a.1) If sample is under pressure, connect water source A (e.g., tap water), to inlet port B2 of fluid proportioner B. If sample is not under pressure, connect water source to inlet port AA1 of self-priming water pump AA. Connect outlet port AA2 of water pump AA to inlet port B2 of fluid proportioner B.

(a.2) Connect outlet port B4 of fluid proportioner B to inlet port E1 of mixing chamber E.

(a.3) Connect outlet port E2 of mixing chamber E to one arm of pipe tee F.

(a.4) Lock pH electrode G1 into pH electrode in-line adapter G2. The same pH electrode (after sterilization) that is used to adjust pH in Step (c.4) may be used.

(a.5) Connect other arm of pipe tee F to inlet port H1 of cartridge filter holder H.

(a.6) Connect outlet port H3 of cartridge filter holder H to inlet port I1 of water meter I.

(a.7) Connect outlet port I2 of water meter I to discard.

(b) Preparation of salt supplement. Preparation of sufficient salt supplement for 400 liters of processed water is described below. If more or less water is to be processed, proportionately more or less salt supplement needs to be prepared. When more salt supplement is needed, prepare it in another carboy.

(b.1) Remove cover from 20-liter carboy C.

(b.2) Pour 10 liters of deionized distilled water into carboy C, and add 67 mL of 3 M $AlCl_3$ solution to the deionized distilled water.

(b.3) Replace cover loosely on carboy C.

(c) Preparation of acid for adjustment of pH

(c.l) Pour 380 mL of test water into a 600-mL beaker.

(c.2) Place stir bar into test water.

(c.3) Place beaker on magnetic stirrer, and stir at speed sufficient to develop vortex in test water.

(c.4) Place pH electrode into test water. pH meter must be standardized before it is used.

(c.5) Adjust pH of test water to 3.5 plus or minus 0.1 with 0.12 M HCl.

(c.6) Record volume of 0.12 M HCl used.

(c.7) Add to carboy C a volume of 12 M HCl equal to 11 times the quantity of 0.12 M HCl needed to reduce the pH in the 380 mL volume of test water to 3.5 plus or minus 0.1.

(c.8) Bring the volume of acid-salt solution to 20 liters with deionized distilled water, and mix solution well.

(d) Preparation of Na2S2O3 solution for dechlorination. Step (d) applies only to chlorinated waters processed directly from a source (e.g., tap water). All chlorinated test waters obtained from sources outside of the processing facility must be dechlorinated immediately when the samples are obtained (see beginning of chapter). Preparation of sufficient Na2S2O3 for dechlorinating 400 liters of processed water is described below. If more or less water is to be processed, proportionately more or less Na2S2O3 needs to be prepared. When more Na2S2O3 is needed, prepare it in another carboy.

(d.1) Remove cover from 20-liter carboy D.

(d.2)Pour 10 liters of deionized distilled water into carboy D.

(d.3) Add 186 mL of 40 percent Na2S2O3 solution to the deionized distilled water in carboy D to give final molarity of 0.03, and mix solution well.

(d.4) Replace cover loosely on carboy D.

(e) Fluid proportioner

(e.1) Connect a long length of tubing to each end of dispensing

Y on 20-liter carboy C that contains the acid-salt solution prepared in Step (c.8) above. Tubing is already in place if additive pumps are sterilized with chlorine (see Section 1.2.1). In this instance, disconnect tubing from bottoms of (larger) additive pumps B1a, and continue with Step (e.2).

(e.2) Remove cover from top of carboy C.

(e.3) Place free end of each tube into mouth of carboy C.

(e.4) Release pinch clamp, and allow acid-salt solution to flow into tubes.

(e.5) Remove tubes from mouth of carboy C, and insert tubes into the inlet (bottom) ports of (larger) additive pumps B1a. Allow acid-salt solution to flow freely into tubing, but manipulate tubes to prevent overflow.

(e.6) Replace cover loosely on carboy C.

(e.7) Adjust the calibration on the metering rod for each pump B1a to a 3.2 setting. This calibration equals delivery rate of 1 part of acid-salt solution to each 19 parts of test water. If dechlorination is not necessary, leave the ports of the two remaining (smaller) additive pumps B1b covered (see Section 1.2.1), and go to Step (e.15).

(e.8) Connect a long length of tubing to each end of dispensing Y on 20-liter carboy D that contains the 0.03 M $Na_2S_2O_3$ solution prepared in Steps (d.1-d.4) above. Tubing is already in place if pumps are sterilized with chlorine (see Section 1.2.1). In this instance, disconnect tubing from bottoms of additive pumps, and continue with Step (e.9).

(e.9) Remove cover from top of carboy D.

(e.10) Place free end of each tube into mouth of carboy D.

(e.11) Release pinch clamp, and allow $Na_2S_2O_3$ solution to flow into tubes.

(e.12) Remove tubes from mouth of carboy D, and insert tubes into the inlet (bottom) ports of (smaller) additive pumps B1b. Allow $Na_2S_2O_3$ solution to flow freely into tubes, but manipulate tubes to prevent overflow.

(e.13) Replace cover loosely on carboy D.

(e.14) Adjust the calibration on the metering rod for each additive pump B1b to a 1.3 setting. This calibration equals

delivery rate of one part of 0.03 M $Na_2S_2O_3$ solution to each 99 parts of test water.

(e.15) Disconnect tube from inlet port E1 of mixing chamber E, and connect tube to discard.

(e.16) To remove air from tubes, prime all additive pumps by hand-operating metering rods in a reciprocating motion.

(e.17) Reconnect tube from outlet port B4 of fluid proportioner B to inlet port E1 of mixing chamber E.

1.2.2 Filtration of Sample

(a) Unscrew base of cartridge filter holder H.

(b) Center 0.45-micrometer pleated epoxy-fiberglass cartridge filter into base of filter holder H.

(c) Screw base of cartridge filter holder H into its top section, and wrench-tighten to seal.

(d) Make initial reading on water meter I, and record reading.

(e) Open vent/relief valve H2 on top of cartridge filter holder H.

(f) Open source valve A or start water pump AA to provide maximum flow through system.

(g) Close vent/relief valve H2 on cartridge filter holder H as soon as water flows through valve.

(h) Wipe up spilled water with laboratory disinfectant.

(i) Read pH meter G to ascertain that proper pH is achieved. Read meter periodically to be certain that proper pH is maintained. If pH readjustment is necessary, appropriately alter settings on metering rods for (larger) additive pumps B1a.

(j) After required volume of water has been filtered, close source valve A or turn off water pump AA.

(k) Open vent/relief valve H2 on top of cartridge filter holder H to relieve pressure in system.

(l) Close vent/relief valve H2. Wipe up spills with disinfectant, as necessary.

(m) Disconnect tubing from inlet port H1 of cartridge filter holder H. Disinfect spills at disconnect.

(n) Connect free end of tubing to discard.

(o) Elevate cartridge filter holder H, and invert to drain.

(p) Make final reading on water meter I, and record reading. Subtract initial reading from final reading to determine total volume filtered. Subtract volume of acid-salt solution used and volume of $Na_2S_2O_3$ solution, if used, from total volume filtered to determine volume of water sample filtered.

(q) Elute viruses from filter as described in Sections 3 and 4.

2. ADSORPTION -- METHOD TWO

This procedure may be used for waters that require prefiltration.

2.1 Preparation

2.1.1 Apparatus and Materials. Install quick-disconnect connectors on ports of all apparatus except on additive pumps.

(a) Cartridge filter, pleated epoxy-fiberglass -- 10-inch (size is given in inches when commercially designated only in that unit), 0.45-micrometer pore size (DUO-FN 10-E-0.45 N-ECIS, Filterite Corp., or equivalent).

(b) Cartridge filter, honeycomb-wound fiberglass yarn -- 10-inch, 1-micrometer and 5-micrometer pore sizes (K 27, 1-micrometer and K19, 5-micrometer, Commercial Filter Division, Carborundum Co., or equivalent), as needed. One or more fiberglass-wound filters needs to be used only when it is anticipated that the pleated filter will clog before the filtration procedure is complete. In the absence of experience, honeycomb-wound filters should be used for all waters except tap waters, but may be used for tap waters, if necessary.

(c) Holders for 10-inch cartridge filters (Fulflo, Model No. F15-10, Commercial Filter Division, Carborundum Co., or equivalent). One holder is needed for pleated filter. An additional holder is needed for each honeycomb-wound cartridge that is to be used.

(d) Plastic-coated drums -- 200-liter capacity, or other containers of size suitable to hold sample, if sample is not

pumped directly from source.

(e) Sterilizable self-priming water pump that delivers approximately 25-50 liters per minute. Pump is not needed if sample water is under pressure, e.g., tap water.

(f) Carboy, autoclavable plastic with nipple on bottom fitted with tubing clamped to a dispensing Y (clamp tubing closed between nipple and Y) -- 20-liter capacity. If the water at the sampling site is to be drawn directly from a pressurized source and is to be dechlorinated, then two similarly fitted carboys are needed. Otherwise, only one carboy is needed. Twice the number of carboys is needed, under these conditions, if water volumes greater than 400 liters are to be processed.

(g) Four stainless steel pipe plugs (Johanson and Son Machine Corp., A40, or equivalent).

(h) Hose adapter for fluid proportioner equipped with four hose fittings (quad system) (Johanson and Son Machine Corp., A34-Q and A33 or equivalent).

(i) Fluid proportioner consisting of fluid-driven motor with four additive pumps (Johanson and Son Machine Corp., Model M 14Q with one P-562 and one P-750 additive pump affixed to each side of the fluid-driven motor, or equivalents). Assemble fluid proportioner in accordance with the manufacturer's instructions except when otherwise indicated. If four tube fittings with attached tubing are connected to hose adapter H3, remove the tubing from the fittings, and replace the fittings with four stainless steel pipe plugs (see Figures 4 and 5 for location of hose adapters). Then, screw the four tube fittings into hose adapter B. Connect a 1.8 meter (6-foot) length of tubing to the top port on each additive pump. Connect the free end of each tube leading from the outlet (top) port of each of the four additive pumps to a tube fitting on hose adapter B.

(j) Mixing chamber (Johanson and Son Machine Corp., C-SS, or equivalent).

(k) pH meter, measuring to an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode (Van London Co., or equivalent, for electrode only).

(l) Tee, stainless steel, with three female NPT ports. Center port equipped with pH electrode in-line adapter (Van London Co., or equivalent, for electrode and adapter only).

(m) Autoclavable inner-braided tubing with metal quick-disconnect connectors for connecting tubing to equipment to be used under pressure. Quick-disconnect connectors can be used only after equipment has been properly adapted.

(n) Magnetic stirrer and stir bars.

(o) Water meter (Badger Meter Inc., or equivalent).

(p) Positive pressure source equipped with pressure gauge. Pressure source, if laboratory air line or pump, must be equipped with oil filter. If source is capable of producing high pressure, deliver to filter holder no more pressure than recommended by manufacturer.

2.1.2 Media and Reagents

(a) Hydrochloric acid (HCl) -- 0.12 and 12 M (concentrated) solutions. Prepare 100-500 mL of 0.12 M HCl. This solution may be stored for several months at room temperature.

(b) Sodium thiosulfate ($Na_2S_2O_3 - 5H_2O$) -- 40 percent (w/v) stock solution (with respect to $Na_2S_2O_3 - 5H_2O$). Prepare one liter of $Na_2S_2O_3$ solution by dissolving 400 g of $Na_2S_2O_3 - 5H_2O$ in 500 mL of deionized distilled water and bringing final volume of solution to one liter with deionized distilled water. Solution may be stored in dark, rubber-stoppered bottle for up to one month at room temperature. Solution is to be used for water that cannot be dechlorinated except immediately prior to test procedure (e.g., water tested directly at source). For dechlorinating all other waters, see beginning of chapter.

(c) Aluminum chloride ($AlCl_3 - 6H_2O$) -- 3 M stock solution. Prepare 100 mL of 3M $AlCl_3$ for each 400 liters of water to be processed.

2.2 Procedure (see Figure 6 for flow diagram of procedure)
In this procedure, an apparatus is described that can be used for waters so turbid that the volume filtered will clog a 0.45-micrometer pleated epoxy-fiberglass cartridge filter. This apparatus is similar to that described in Method One of this chapter except that honeycomb-wound fiberglass filters are installed in advance of the pleated filter to remove particulate matter in the water, and in-line placement of the equipment is modified to allow adjustment of the pH and salt concentration of the test waters before those waters are prefiltered. Experience usually dictates whether prefiltration is needed. In the absence of experience, use procedure in Section 1. ADSORPTION -- METHOD ONE for tap waters and for other waters of similar clarity. Use a 1 micrometer

honeycomb-wound fiberglass yarn cartridge filter preceding the 0.45-micrometer pleated filter for surface waters and for other waters of similar clarity, and add a 5-micrometer honeycomb-wound fiberglass yarn cartridge filter preceding the l-micrometer filter for secondary and tertiary effluents and for other waters of similar clarity.

2.2.1 Preparation and Implementation. It is usually convenient to sterilize each piece of apparatus and equipment one or more days before it is used (see Chapter 3). It is convenient to sterilize apparatus in small units when sterilization is accomplished by steam or ethylene oxide. It is convenient to assemble and connect all units of apparatus that are to be sterilized by chlorination. The interconnected apparatus can be disassembled after chlorination, the ports covered with aluminum foil and the units stored until used.

(a) Assembly of apparatus (see Figures 4 and 5). Use inner-braided tubing fitted with quick-disconnect connectors to make all connections for equipment under pressure.

(a.1) If sample is under pressure, connect water source A to either port of hose adapter B. If sample is not under pressure, connect water source to inlet port AA1 of self-priming water pump AA. Connect outlet port of water pump AA2 to either port of hose adapter B.

(a.2) Connect remaining port of hose adapter B to inlet port C1 of mixing chamber C.

(a.3) Connect outlet port C2 of mixing chamber C to one arm of pipe tee D.

(a.4) Lock pH electrode E1 into pH electrode in-line adapter E2. Same pH electrode (after sterilization) that is used to adjust pH in Step (c.4) may be used.

(a.5) Connect other arm of pipe tee D to inlet port F1 of cartridge holder F.

(a.6) Connect outlet port F3 of cartridge holder F to inlet port G1 of cartridge holder G.

(a.7) Connect outlet port G3 of cartridge holder G to inlet port H2 of fluid proportioner H.

(a.8) Connect outlet port H4 of fluid proportioner H to inlet port K1 of cartridge holder K.

(a.9) Connect outlet port K3 of cartridge holder K to inlet

port L1 of water meter L.

(a.10) Connect outlet port L2 of water meter L to discard.

(b) Preparation of salt supplement. Preparation of sufficient salt supplement for 400 liters of processed water is described below. If more or less water is to be processed, proportionately more or less salt supplement needs to be prepared. When more salt supplement is needed, prepare it in another carboy.

(b.1) Remove cover from 20-liter carboy I.

(b.2) Pour 10 liters of deionized distilled water into carboy I, and add 67 mL of 3 M AlCl3 solution to the deionized distilled water.

(b.3) Replace cover loosely on carboy I.

(c) Preparation of acid for adjustment of pH

(c.1) Pour 380 mL of test water into a 600-mL beaker.

(c.2) Place stir bar into test water.

(c.3) Place beaker on magnetic stirrer, and stir at speed sufficient to develop vortex in test water.

(c.4) Place pH electrode into test water. pH meter must be standardized before it is used.

(c.5) Adjust pH of test water to 3.5 plus or minus 0.1 with 0.12 M HCl.

(c.6) Record volume of 0.12 M HCl used.

(c.7) Add to carboy I a volume of 12 M HCl equal to 11 times the quantity of 0.12 M HCl needed to produce the required pH in the 380-mL volume of test water.

(c.8) Bring acid-salt solution to 20-liters with deionized distilled water, and mix solution well.

(d) Preparation of Na2S2O3 solution for dechlorination Step (d) applies only to chlorinated waters processed directly from a source. All chlorinated test waters obtained from sources outside of the processing facility must be dechlorinated immediately when the samples are obtained (see beginning of chapter). Preparation of sufficient Na2S2O3 for dechlorinating 400 liters of processed water is described

below. If more or less water is to be processed, proportionately more or less $Na_2S_2O_3$ needs to be prepared. When more $Na_2S_2O_3$ is needed, prepare it in another carboy.

(d.1) Remove cover from 20-liter carboy J.

(d.2) Pour 10 liters of deionized distilled water into carboy J.

(d.3) Add 186 mL of 40 percent $Na_2S_2O_3$ solution to the deionized distilled water in carboy J to give a final molarity of 0.03, and mix solution well.

(d.4) Replace cover loosely on carboy J.

(e) Fluid proportioner

(e.1) Connect a long length of tubing to each end of dispensing Y on 20-liter carboy I that contains the acid-salt solution prepared in Step (c.8) above. Tubing is already in place if additive pumps are sterilized with chlorine (see Section 2.2.1). In this instance, disconnect tubing from bottom of additive pumps H1a, and continue with Step (e.2).

(e.2) Remove cover from top of carboy I.

(e.3) Place free end of each tube into mouth of carboy I.

(e.4) Release pinch clamp, and allow acid-salt solution to flow into tubes.

(e.5) Remove tubes from mouth of carboy I, and insert tubes into inlet (bottom) ports of (larger) additive pumps H1a. Allow acid-salt solution to flow freely into tubing, but manipulate tubes to prevent overflow.

(e.6) Replace cover loosely on carboy I.

(e.7) Adjust the calibration on the metering rod for each pump H1a to a 3.2 setting. This calibration equals delivery rate of one part of acid-salt solution to each 19 parts of test water. If dechlorination is not necessary, leave the ports of the two remaining additive pumps H1b covered (see Section 2.2.1), and go to Step (e.15).

(e.8) Connect a long length of tubing to each end of dispensing Y on 20-liter carboy J that contains the 0.03 M $Na_2S_2O_3$ solution prepared in Steps (d.1-d.4) above. Tubing is already in place sterilized with chlorine (see Section 2.2.1). In this instance, disconnect tubing from bottoms of additive pumps, and

continue with Step (e.9).

(e.9) Remove cover from top of carboy J.

(e.10) Place free end of each tube into mouth of carboy J.

(e.11) Release pinch clamp, and allow $Na_2S_2O_3$ solution to flow into tubes.

(e.12) Remove tubes from mouth of carboy J, and insert tubes into inlet (bottom) ports of (smaller)additive pumps H1b. Allow $Na_2S_2O_3$ solution to flow freely into tubes, but manipulate tubes to prevent overflow.

(e.13) Replace cover loosely on carboy J.

(e.14) Adjust the calibration on the metering rod for each additive pump H1b to a 1.3 setting. This calibration equals delivery rate of one part of 0.03 M $Na_2S_2O_3$ solution to each 99 parts of test water.

(e.15) Disconnect tube from inlet port C1 of mixing chamber C, and connect tube to discard.

(e.16) To remove air from tubes, prime additive pumps by hand-operating metering rods in a reciprocating motion.

(e.17) Reconnect tube from outlet port of hose adapter B to inlet port C1 of mixing chamber C.

2.2.2 Filtration of Sample

(a) Unscrew base of cartridge filter holder F.

(b) Center 5-micrometer honeycomb-wound fiberglass yarn cartridge filter into base of filter holder F.

(c) Screw base of cartridge filter holder F back into its top section, and wrench-tighten to seal.

(d) Unscrew base of cartridge filter holder G.

(e) Center 1-micrometer honeycomb-wound fiberglass yarn cartridge filter into base of filter holder G.

(f) Screw base of cartridge filter holder G back into its top section, and wrench-tighten to seal.

(g) Unscrew base of cartridge filter holder K.

(h) Center 0.45-micrometer pleated epoxy-fiberglass cartridge filter into base of filter holder K.

(i) Screw base of cartridge filter holder K back into its top section, and wrench-tighten to seal.

(j) Make initial reading on water meter L, and record reading.

(k) Open vent/relief valves F2, G2, and K2 on top of cartridge filter holders F, G, and K.

(l) Open source valve A or start water pump AA to provide maximum flow through system.

(m) Close vent/relief valves F2, G2, and K2 on top of cartridge filter holders F, G, and K as soon as water flows through valves.

(n) Wipe up spilled water with laboratory disinfectant.

(o) Read pH meter E to ascertain that proper pH is achieved. Read meter periodically to be certain that proper pH is maintained. If pH readjustment is necessary, appropriately alter settings on metering rods for (larger) additive pumps H1a.

(p) After required volume of water has been filtered, close source valve A or turn off water pump AA.

(q) Open vent/relief valves F2, G2, and K2 on top of cartridge filter holders F, G, and K to relieve pressure in system.

(r) Close vent/relief valves F2, G2, and K2. Wipe up spills with disinfectant as necessary.

(s) Disconnect tubing from source A or from water pump outlet AA2. Disinfect spills at disconnect.

(t) Connect free end of tubing to discard.

(u) Elevate cartridge filter holders F, G, and K, and invert to drain.

(v) Take final reading on water meter, and record reading. Subtract initial reading from final reading to determine total volume filtered. Subtract volume of acid-salt solution used and, if used, volume of $Na_2S_2O_3$ solution from total volume filtered to determine volume of water sampled.

(w) Elute viruses from filters as described in Sections 3 and

3. ELUTION AND RECONCENTRATION -- METHOD ONE

This method may be used for eluting viruses not significantly inactivated at pH levels of about 10.5 in 15 minutes at ambient temperatures. To elute viruses that cannot be safely recovered by this procedure, see Section 4.

3.1 Procedure for Eluting Viruses from Cartridge Filters (see Figures 7.1 and 8.1 for flow diagrams of procedure)

3.1.1 Apparatus and Materials

(a) Positive pressure source equipped with a pressure gauge. Gauge necessary only if pressure source is capable of producing pressures exceeding tolerances of equipment. Pressure source, if laboratory air line or pump, must be equipped with an oil filter. If source is capable of producing high pressure, deliver to pressure vessel and filter holder no more pressure than recommended by manufacturer.

(b) Dispensing pressure vessel -- 4 liters (Millipore Corp., or equivalent).

(c) Beakers, graduated -- 2 liters. One beaker is needed for each filter that is eluted.

(d) pH meter, measuring to an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode.

(e) Autoclavable inner-braided tubing fitted with metal quick-disconnect connectors on one end and glass elbow on the other. Make glass elbow from a 13-cm length (approximate O.D. 6 mm) of glass tubing by making a 40 degree bend about 5 cm from one end. Connect tubing onto longer end of elbow. One elbow is needed for each filter that is eluted.

(f) Magnetic stirrer and stir bars.

3.1.2 Media and Reagents

a) Sodium hydroxide (NaOH) -- 10 M. Prepare 500 mL of 10 M NaOH.

b) Basic glycine solution -- 0.05 M glycine, adjusted to pH 10.5 plus or minus 0.1 with 10 M NaOH. Autoclave glycine solution before adjusting pH. Prepare 3 liters of basic 0.05 M glycine solution.

(c) Hydrochloric acid (HCl) -- 12 M (concentrated) HCl solution.

(d) Acid glycine solution -- 0.05 M glycine, adjusted to pH 2 with 12 M HCl. Autoclave glycine solution before adjusting pH. Prepare 3 liters of acid 0.05 M glycine solution.

3.1.3 Rearrangement of Apparatus

(a) Rearrangement for Method One (see Figures 2 and 3).

(a.1) Disconnect at pipe tee F, the tubing leading to inlet port H1 of filter holder H.

(a.2) Connect free end of tubing from inlet port H1 of filter holder H to outlet port of pressure vessel. Pressure vessel is not shown in Figures 2 and 3.

(a.3) Connect inlet port of pressure vessel to positive air pressure source.

(a.4) Disconnect tubing from outlet port H3 of filter holder H.

(a.5) Hook glass elbow with 40 degree bend onto pouring spout of a 2-liter glass beaker. Raise aluminum foil covering beaker enough to expose only the pouring spout.

(a.6) Connect free end of tubing from glass elbow to outlet port H3 of filter holder H.

(a.7) Crimp aluminum foil cover over glass elbow.

(a.8) Elute viruses from filter as described in Section 3.1.4 below.

(b) Rearrangement for Method Two (see Figures 4 and 5).

(b.1) Disconnect at pipe tee D, the tubing leading to the inlet port F1 of filter holder F.

(b.2) Connect free end of tubing from inlet port F1 of filter holder F to outlet port of pressure vessel. Pressure vessel is not shown in Figures 4 and 5.

(b.3) Connect inlet port of pressure vessel to positive pressure source.

(b.4) Disconnect tubing from outlet port F3 of filter holder F.

(b.5) Hook glass elbow with 40 degree bend onto pouring spout

of a 2-liter glass beaker. Raise aluminum foil covering beaker enough to expose only the pouring spout.

(b.6) Connect free end of tubing from glass elbow to outlet port F3 of filter holder F.

(b.7) Crimp aluminum foil cover over glass elbow.

(b.8) Elute viruses from filter as in Section 3.1.4 below.

(b.9) Disconnect tubing from outlet port of pressure vessel.

(b.10) Connect free end of tubing from inlet port G1 of filter holder G to outlet port of pressure vessel.

(b.11) Disconnect tubing from outlet port G3 of filter holder G.

(b.12) Hook glass elbow with 40 degree bend onto pouring spout of a 2-liter glass beaker. Raise aluminum foil covering beaker enough to expose only the pouring spout.

(b.13) Connect free end of tubing from glass elbow to outlet port G3 of filter holder G.

(b.14) Crimp aluminum foil cover over glass elbow.

(b.15) Elute viruses from filter as described in Section 3.1.4 below.

(b.16) Disconnect tubing from outlet port of pressure vessel.

(b.17) Disconnect at outlet port H4 of fluid proportioner H, the tubing leading to the inlet port K1 of filter holder K.

(b.18) Connect free end of tubing from inlet port K1 of filter holder K to outlet port of pressure vessel.

(b.19) Disconnect tubing from outlet port K3 of filter holder K.

(b.20) Hook glass elbow with 40 degree bend onto pouring spout of a 2-liter graduated glass beaker. Raise aluminum foil covering beaker enough to expose only the pouring spout.

(b.21) Connect free end of tubing from glass elbow to outlet port K3 of filter holder K.

(b.22) Crimp aluminum foil cover over glass elbow.

(b.23) Elute viruses from filter as described in Section 3.1.4 below.

3.1.4 Elution Procedure

(a) Remove top of pressure vessel.

(b) Pour into pressure vessel 1600 mL of basic glycine solution (pH 10.5 plus or minus 0.1).

(c) Replace top of pressure vessel.

(d) Close vent/relief valve on pressure vessel.

(e) Open vent/relief valve on cartridge filter holder.

(f) Apply pressure sufficient to purge trapped air from filter apparatus.

(g) Close vent/relief valve on cartridge filter holder as soon as basic glycine solution begins to flow from valve.

(h) Wipe up spilled liquid with laboratory disinfectant.

(i) Increase pressure to that sufficient to force basic glycine solution through the filter. Do not exceed a pressure of 0.4 kg/square cm so that basic glycine solution passes through cartridge filter slowly thereby maximizing elution contact period.

(j) Turn off pressure at source.

(k) Open vent/relief valve on pressure vessel.

(l) Check pH of eluate. If pH of eluate is below 9.5, repeat elution procedure with fresh elutant, combine eluates in graduated beaker, and reconcentrate. Instructions for reconcentrating viruses begin in Section 3.2. Reconcentration must begin immediately, because pH of eluate must be reduced immediately to prevent inactivation of viruses.

3.2 Reconcentration -- Method A. Membrane Disc Procedure (see Figure 7.2 for flow diagram of procedure). Where it can be used, the membrane disc procedure is the preferred method for reconcentrating viruses from the eluates resulting from the procedures described in the preceding section (Section 3.1.4). However, in some eluates, a precipitate is present that impedes filtration of the eluate through a membrane filter. Reconcentrate such eluates by the aluminum hydroxide-hydroextraction procedure described in Section 3.3. If, during

acidification in the membrane procedure, turbidity occurs in previously clear eluates, discontinue acidification and reconcentrate these eluates by the aluminum hydroxide-hydroextraction procedure. Optionally, for any given sample, all clear eluates may be pooled and all turbid eluates may be pooled for reconcentration.

3.2.1 Apparatus and Materials

(a) High pressure disc filter holders -- 47mm diameter (Millipore Corp., XX4504700, or equivalent).

(b) Virus-adsorbing disc filter, mixed esters of cellulose -- 0.45-micrometer pore size (Millipore HA, or equivalent).

(c) Dispensing pressure vessel -- 20-liter capacity (Millipore Corp., XX6700L20, or equivalent).

(d) Positive pressure source equipped with pressure gauge. Gauge necessary only if pressure source is capable of producing pressures exceeding tolerances of equipment. Pressure source, if laboratory air line or pump, must be equipped with oil filter. If source is capable of producing high pressure, deliver to pressure vessel and filter holder no more pressure than recommended by manufacturer.

(e) pH meter, measuring to an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode.

(f) Autoclavable inner-braided tubing with metal quick-disconnect connectors or with thumb-screw-drive-clamps for connecting tubing to equipment.

(g) Magnetic stirrer and stir bar.

(h) Filling bell connected to inner-braided tubing.

3.2.2 Media and Reagents

(a) Hydrochloric acid (HCl) -- 12 M (concentrated) HCl solution.

(b) Acid glycine solution, 0.05 M, adjusted to pH 2 with 12 M HCl. Autoclave glycine solution before adjusting pH.

(c) Sodium hydroxide (NaOH) -- 10M. Prepare 500 mL of 10 M NaOH.

(d) Basic glycine solution, 0.05 M, adjusted to pH 10.5 plus or minus 0.1 with 10 M NaOH. Prepare 3 liters of basic glycine

solution. Autoclave glycine solution before adjusting pH.

(e) Fetal calf serum.

3.2.3 Procedure

(a) Assembly of Apparatus (See Figures 9 and 10)

(a.1) Remove top of filter holder C.

(a.2) With forceps, lay 0.45-micrometer virus-adsorbing filter onto support screen of holder.

(a.3) Replace and tighten down top of filter holder C.

(a.4) Connect positive pressure source A or AA to inlet port B of pressure vessel B.

(a.5) Connect outlet port B3 of pressure vessel B to inlet port C1 of filter holder C.

(a.6) Place filling bell D, with inner-braided tubing attached, over opening of flask E, and connect free end of the tubing to the outlet port C2 of filter holder C.

(b) Adjustment of pH of eluates from Section 3.1.4, Step (l). Add with rapid, continuous stirring sufficient acid glycine solution to bring pH of eluate to 3.5 plus or minus 0.1. It is important to mix acid glycine solution into sample rapidly, because slow mixing may result in pH levels sufficiently low in parts of the sample to inactivate viruses. If eluate becomes turbid during or after acidification, terminate procedure and reconcentrate viruses by the aluminum hydroxide-hydroextraction procedure described in Section 3.3. Reconcentration with the aluminum hydroxide- hydroextraction procedure must begin immediately, because pH of eluate must be reduced immediately to prevent inactivation of viruses.

(c) Filtration of eluate.

(c.1) Remove top of pressure vessel B.

(c.2) Pour eluate into pressure vessel B.

(c.3) Replace and tighten down top of pressure vessel B.

(c.4) Apply pressure from source A or AA sufficient to force sample through the filter (usually 0.4-1.5 kg/square cm).

(c.5) Turn off pressure at source A or AA.

(c.6) Open vent/relief valve B2 of pressure vessel B.

(c.7) When pressure is relieved, close vent/relief valve B2.

(d) Elution of viruses from filter.

(d.1) Disconnect tubing from outlet port C2 of filter holder C.

(d.2) Place 100-mL beaker under outlet port C2 of filter holder C.

(d.3) Disconnect tubing from inlet port C1 of filter holder C.

(d.4) Pour 5 mL of basic glycine solution into inlet port C1 of filter holder C.

(d.5) Reconnect tubing to inlet port C1 of filter holder C.

(d.6) Apply sufficient pressure from source A or AA to force basic glycine solution through filter.

(d.7) Turn off pressure at source A or AA.

(d.8) Open vent/relief valve B2 on pressure vessel B.

(d.9) When pressure is relieved, close vent/relief valve B2.

(d.10) Disconnect tubing from inlet port C1 of filter holder C.

(d.11) Pour another 5 mL of basic glycine solution into inlet port C1 of filter holder C.

(d.12) Repeat steps (d.5-d.8), collecting total 10 mL of eluates in the same beaker. Check pH of combined eluates. If pH is below 9.5, repeat steps (d.9-d.12) with fresh basic glycine solution.

(d.13) Adjust pH of combined eluates to 7.0-7.5 with acid glycine solution.

(d.14) Measure total volume of neutralized eluate.

(d.15) Add sufficient fetal calf serum to neutralized eluate to yield a final serum concentration of 2 percent.

(d.16) Refrigerate neutralized eluate at 4 degrees C immediately, and maintain at that temperature until eluate is assayed for viruses. If assay for viruses cannot be undertaken within eight hours, store eluate immediately at -70 degrees C.

3.3 Reconcentration -- Method B. Aluminum Hydroxide-Hydroextraction Procedure (see Figure 8.2 for flow diagram of procedure). Use the aluminum hydroxide-hydroextraction procedure to reconcentrate viruses from turbid eluates that result from the procedures described in Section 3.1.4 and from eluates that become turbid upon acidification during the membrane disc procedure described in Section 3.2.3, Step (b).

3.3.1 Apparatus and Materials

(a) Magnetic stirrer and stir bars.

(b) pH meter, measuring to an accuracy of at least 0.1 pH unit, equipped with combination-type electrode.

(c) Instrument tray, stainless steel -- overall dimensions 43 cm x 10 cm x 5 cm (Vollrath Co., No. 83170, or equivalent).

(d) Dialyzer tubing, molecular weight cutoff 12,000, 3-cm diameter (Arthur H. Thomas Co., No. 3787-D42, or equivalent).

(e) Clamps, dialyzer tubing (Arthur H. Thomas Co., No. 3787-N30, or equivalent).

(f) Centrifuge tubes, screw-capped, round-bottom -- 50-100 mL.

3.3.2 Media and Reagents

(a) Hydrochloric acid (HCl) -- 12 M (concentrated) HCl solution.

(b) Sodium hydroxide (NaOH) -- 10 M. Prepare 500 mL of 10 M NaOH.

(c) Aluminum chloride (AlCl3) -- 0.3 M. Prepare 500 ml of 0.3 M AlCl3.

(d) Sodium carbonate (Na2CO3) -- 1 M. Prepare 100 mL of 1 M Na2CO3.

(e) Glycine.

(f) Acid glycine solution -- 1 M glycine adjusted to pH 2 with 12 M HCl. Prepare 2 liters of acid glycine solution.

(g) Basic fetal calf serum (BFCS) with glycine -- 1 M glycine in fetal calf serum (FCS) adjusted to pH 11.5 plus or minus 0.1 with 10 M NaOH. Prepare 100 mL of BFCS with glycine. To prepare BFCS with glycine, autoclave glycine powder in a

covered vessel, add FCS, and adjust pH.

(h) Phosphate-buffered saline -- Solution A: Sodium chloride (NaCl), 40 g; potassium chloride (KCl), l g; calcium chloride (CaCl2), 0.5 g; magnesium chloride (MgCl2 - 6H2O), 0.5 g; deionized distilled water to 4 liters. Solution B: Sodium phosphate, dibasic (Na2HPO4), 1 g; potassium phosphate, monobasic, (KH2PO4), 1 g; deionized distilled water to 1 liter. Prepare solutions A and B separately, then mix them together in a ratio of 1:1.

(i) Polyethylene glycol -- 20,000 MW. Three kg of polyethylene glycol are required.

(j) Antibiotics. Prepare as indicated for medium in Chapter 9, Section 7.18.

3.3.3 Procedure

(a) Preparation of dialysis bag.

(a.1) Soak a 40-cm length of dialyzer tubing in deionized distilled water for five minutes.

(a.2) Fold one end of the tubing over itself to form a 2-cm overlap.

(a.3) Center dialyzer tubing clamp over overlap, and lock clamp in place to form dialysis bag.

(a.4) Grasp unclamped end of dialysis bag between thumb and forefinger, and rub the facing surfaces against each other. This procedure separates the facing surfaces and opens the dialysis bag.

(a.5) Fill dialysis bag two-thirds full with deionized distilled water.

(a.6) Fold free end of dialysis bag over itself to form a 2-cm overlap.

(a.7) Center dialyzer tubing clamp over overlap, and lock clamp in place.

(a.8) Squeeze dialysis bag gently to force water against clamped ends. If water leaks through either clamped end, remove clamp from faulty seal and repeat Steps (a.6-a.8).

(a.9) Squeeze dialysis bag near clamps, exerting sufficient pressure to test the integrity of the tubing. If water leaks

through tubing, discard tubing and repeat Steps (a.1-a.9).

(a.10) Sterilize dialysis bag by the procedure described in Chapter 3.

(a.11) After sterilization, recheck dialysis bag for leaks by repeating Steps (a.8-a.9).

(a.12) Store dialysis bag in deionized distilled water at 4 degrees C.

(b) Flocculation and hydroextraction.

(b.1) Measure volume of eluate against graduations on beaker (from Section 3.1.4, Step [l]).

(b.2) Place stir bar into eluate.

(b.3) Place beaker on magnetic stirrer, and stir at speed sufficient to develop vortex in eluate.

(b.4) Add sufficient volume of 0.3 M AlCl3 to eluate to obtain a final AlCl3 concentration of 0.003 M.

(b.5) Place pH electrode into eluate.

(b.6) Adjust pH of eluate to 7 plus or minus 0.1 with 1 M Na2CO3. An Al(OH)3 floc forms as Na2CO3 is added.

(b.7) After pH 7 plus or minus 0.1 is obtained, stir for five minutes.

(b.8) Remove pH electrode.

(b.9) Turn off magnetic stirrer.

(b.10) Allow floc to settle for 30 minutes.

(b.11) Aspirate liquid to 2-3 cm above level of floc.

(b.12) Discard aspirated liquid.

(b.13) Resuspend settled floc in remaining liquid. Resuspend floc by swirling beaker or by placing beaker on magnetic stirrer and activating stirrer.

(b.14) Pour suspended floc into round bottom centrifuge tube(s). Screw-capped centrifuge tubes with a capacity of 50-100 mL are usually adequate. To prevent transfer of stir bar into centrifuge tube, hold another stir bar or magnet

underneath beaker when decanting contents.

(b.15) Centrifuge tube(s) at 1000 x g for three minutes.

(b.16) Decant or aspirate supernate.

(b.17) Discard supernate.

(b.18) Measure approximate volume of Al(OH)3 residue. To a centrifuge tube similar to that containing the Al(OH)3 residue, add water to a level equal to the height of the residue, and estimate volume of residue by measuring the volume of water in a graduated cylinder or pipette.

(b.19) Add to each volume of Al(OH)3 residue, three volumes of BFCS-glycine, pH 11.5 plus or minus 0.1.

(b.20) Shake centrifuge tube(s) for five seconds to mix contents.

(b.21) Centrifuge each Al(OH)3 -- BFCS-glycine suspension for three minutes at 1000 x g.

(b.22) Pour BFCS-glycine supernate(s) into beaker.

(b.23) Place stir bar into beaker.

(b.24) Place beaker on magnetic stirrer, and stir contents of beaker at a speed sufficient to develop vortex.

(b.25) Place pH electrode into BFCS-glycine.

(b.26) Adjust pH of BFCS-glycine to 7 plus or minus 0.1 with 1 M acid glycine solution.

(b.27) Turn off magnetic stirrer.

(b.28) Remove pH electrode.

(b.29) Discard residue from Step (b.21).

(b.30) Remove dialysis bag from storage, and wipe exterior of bag with towel.

(b.31) Remove clamp from one end of dialysis bag.

(b.32) With sterile scissors, remove end of dialysis bag by cutting across the bag through center of clamp impression. This procedure removes inside edge of bag that had been exposed to contamination.

(b.33) Discard water from dialysis bag.

(b.34) Pour neutralized BFCS-glycine eluate into dialysis bag.

(b.35) Fold open end of the bag over itself to form a 2-cm overlap.

(b.36) Center dialyzer tubing clamp over overlap, and lock clamp in place.

(b.37) Place a 1-cm layer of polyethylene glycol into instrument tray.

(b.38) Place dialysis bag on polyethylene glycol.

(b.39) Add sufficient polyethylene glycol to cover dialysis bag.

(b.40) Place cover on instrument tray.

(b.41) Maintain instrument tray overnight at 4 degrees C. Hydroextract until approximately 10 to 20 mL of concentrated neutralized BFCS-glycine remain in bag.

(b.42) Rinse polyethylene glycol from outside surface of dialysis bag with deionized distilled water.

(b.43) Pour 900 mL of chilled (4-10 degrees C) phosphate-buffered saline into a 1-liter beaker. Maintain phosphate-buffered saline at 4-10 degrees C; carry out Steps (b.44-b.47) in cold room or in cold by other means available.

(b.44) Place beaker of phosphate-buffered saline on magnetic stirrer.

(b.45) Place stir bar into phosphate-buffered saline, and stir at a speed sufficient to develop vortex.

(b.46) Immerse dialysis bag into phosphate-buffered saline. Care must be taken not to puncture bag with stir bar.

(b.47) Stir for one hour.

(b.48) Remove dialysis bag from phosphate-buffered saline and wipe exterior of bag with towel.

(b.49) Remove clamp from one end of dialysis bag.

(b.50) With sterile scissors, remove end of dialysis bag by cutting across the bag through center of clamp impression.

This procedure removes inside edge of bag that had been exposed to contamination.

(b.51) Pour concentrate into 100-mL graduated beaker.

(b.52) Hold dialysis bag in fully inverted position over beaker.

(b.53) Place upper end of bag between forefinger and middle finger in a scissors grip.

(b.54) Squeeze bag between fingers in scissors grip.

(b.55) Pull fingers down over length of bag to remove remaining concentrate. Take care that fingers do not contaminate concentrate.

(b.56) Determine volume of concentrate in beaker.

(b.57) Add antibiotics to concentrate in accordance with instructions given for medium in Chapter 9, Section 7.18.

(b.58) Refrigerate concentrate immediately at 4 degrees C, and maintain at that temperature until concentrate is assayed for viruses. If assay for viruses cannot be undertaken within eight hours, store concentrate immediately at -70 degrees C.

4. ELUTION AND RECONCENTRATION -- METHOD TWO

This method may be used for eluting viruses from filters that cannot be safely eluted with Method One (this method should be as effective as Method One for eluting viruses sensitive to pH 10.5).

4.1 Procedure for Eluting Viruses from Filters (see Figure 11.1 for flow diagram of procedure)

4.1.1 Apparatus and Materials

(a) Positive pressure source equipped with a pressure gauge. Gauge necessary only if pressure source is capable of producing pressures exceeding tolerances of equipment. Pressure source, if laboratory air line or pump, must be equipped with an oil filter. If source is capable of producing high pressure, deliver to pressure vessel and filter holder no more pressure than recommended by manufacturer.

(b) Dispensing pressure vessel -- 4 liters (Millipore Corp., or equivalent).

(c) Beaker, graduated -- 2 liters.

(d) pH meter, measuring to an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode.

(e) Autoclavable inner-braided tubing fitted with metal quick-disconnect connectors on one end and glass elbow on the other. Make glass elbow from a 13-cm length (approximate O.D., 6 mm) of glass tubing by making a 40 degree bend about 5 cm from one end. Connect tubing onto longer end of elbow.

(f) Magnetic stirrer and stir bars.

4.1.2 Media and Reagents

(a) Sodium hydroxide (NaOH) -- 1 M. Prepare 500 mL of 1 M NaOH. This solution may be stored for several months at room temperature.

(b) Glycine.

(c) Beef extract powder (Grand Island Biological Co., or equivalent). Prepare buffered 3 percent beef extract by dissolving 60 g of beef extract powder and 7.5 g of glycine (final concentration = 0.05 M) in 2 liters of distilled water. Autoclave beef extract solution, and adjust pH to 9 plus or minus 0.1 with 1 M NaOH.

4.1.3 Rearrangement of Apparatus

(a) Rearrangement for Method One (see Figures 2 and 3).

(a.1) Disconnect at pipe tee F, the tubing leading to inlet port H1 of filter holder H.

(a.2) Connect free end of tubing from inlet port H1 of filter holder H to outlet port of pressure vessel. Pressure vessel is not shown in Figures 2 and 3.

(a.3) Connect inlet port of pressure vessel to positive air pressure source.

(a.4) Disconnect tubing from outlet port H3 of filter holder H.

(a.5) Hook glass elbow with 40 degree bend onto pouring spout of a 2-liter glass beaker. Raise aluminum foil covering beaker enough to expose only the pouring spout.

(a.6) Connect free end of tubing from glass elbow to outlet port H3 of filter holder H.

(a.7) Crimp aluminum foil cover over glass elbow.

(a.8) Elute viruses from filter as described in Section 4.1.4 below.

(b) Rearrangement for Method Two (see Figures 4 and 5).

(b.1) Disconnect at pipe tee D, the tubing leading to the inlet port F1 of filter holder F.

(b.2) Connect free end of tubing from inlet port F1 of filter holder F to outlet port of pressure vessel. Pressure vessel is not shown in Figures 4 and 5.

(b.3) Connect inlet port of pressure vessel to positive pressure source.

(b.4) Disconnect tubing from outlet port G3 of filter holder G.

(b.5) Disconnect at outlet port H4 of fluid proportioner H, the tubing leading to the inlet port K1 of filter holder K.

(b.6) Connect free end of tubing from inlet port K1 of filter holder K to outlet port G3 of filter holder G.

(b.7) Disconnect tubing from outlet port K3 of filter holder K.

(b.8) Hook glass elbow with 40 degree bend onto pouring spout of a 2-liter graduated glass beaker. Raise aluminum foil covering beaker enough to expose only the pouring spout.

(b.9) Connect free end of tubing from glass elbow to outlet port K3 of filter holder K.

(b.10) Crimp aluminum foil cover over glass elbow.

(b.11) Elute viruses from filter as described in Section 4.1.4 below.

4.1.4 Elution Procedure

(a) Remove top of pressure vessel.

(b) Pour into pressure vessel 1600 mL of buffered 3 percent beef extract (pH 9).

(c) Replace top of pressure vessel.

(d) Close vent/relief valve on pressure vessel.

(e) Open vent/relief valve on cartridge filter holder. If more than one cartridge filter is used, open valves on all holders.

(f) Apply pressure sufficient to purge trapped air from filter apparatus.

(g) Close vent/relief valve on (each) cartridge filter holder as soon as buffered 3 percent beef extract solution begins to flow from valve.

(h) Wipe up spilled liquid with laboratory disinfectant.

(i) Increase pressure to that sufficient to force buffered 3 percent beef extract solution through the filter(s). Do not exceed a pressure of 0.4 kg/square cm so that buffered 3 percent beef extract solution passes through cartridge filter(s) slowly thereby maximizing elution contact period. When air enters line from pressure vessel, elevate and invert filter holder(s) to permit complete evacuation of buffered 3 percent beef extract from filters.

(j) Turn off pressure at source.

(k) Open vent/relief valve on pressure vessel.

(l) Proceed to Section 4.2 immediately. If concentration of viruses cannot be undertaken immediately, eluate may be stored for up to eight hours at 4 degrees C before reconcentration. If reconcentration cannot be undertaken within eight hours, store eluate immediately at -70 degrees C. Instructions for reconcentrating viruses begin in Section 4.2.

4.2 Organic Flocculation Concentration Procedure of Katzenelson (see Figure 11.2 for flow diagram of procedure). It is preferable to assay eluted viruses in the beef extract eluate without further concentrating them because some loss of viruses may occur in concentration. However, the numbers of cell cultures needed for assays may be reduced by further concentrating the viruses.

4.2.1 Apparatus and Materials

(a) Magnetic stirrer and stir bars.

(b) pH meter, measuring to an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode.

(c) Refrigerated centrifuge capable of obtaining 2500 x g. Each sample centrifuged at 2500 x g will consist of about 1600 mL.

4.2.2 Media and Reagents

(a) Disodium hydrogen phosphate ($Na_2HPO_4 - 7H_2O$) -- 0.15 M.

(b) Hydrochloric acid (HCl) -- 1 M.

(c) Sodium hydroxide (NaOH) -- 1 M.

4.2.3 Procedure

(a) Place stir bar into graduated beaker containing buffered 3 percent beef extract eluate from 4.1.4 (l).

(b) Place beaker that contains the beef extract on magnetic stirrer, and stir at a speed sufficient to develop vortex. To minimize foaming (which may inactivate viruses), do not mix faster than necessary to develop vortex.

(c) Insert pH electrode into beef extract eluate.

(d) Add 1 M HCl to flask slowly until pH of beef extract reaches 3.5 plus or minus 0.1. A precipitate will form. If pH is accidentally reduced below 3.4, add 1 M NaOH until pH is 3.5 plus or minus 0.1. Avoid reducing pH below 3.4 because some inactivation of viruses may occur.

(e) Remove pH electrode from beaker, and continue to stir for 30 minutes more.

(f) Remove caps from screw-capped centrifuge bottles. Glass centrifuge bottles may not be able to withstand g force that will be applied.

(g) Pour contents of beaker into centrifuge bottles. To prevent transfer of stir bar into centrifuge bottle, hold another stir bar or magnet against bottom of beaker when decanting contents.

(h) Replace and tighten down caps on centrifuge bottles.

(i) Centrifuge precipitated beef extract suspension in refrigerated centrifuge (4 degrees C) for 15 minutes at 2500 x g.

(j) Remove caps from screw-capped centrifuge bottles.

(k) Pour supernates into graduate cylinder, and record volumes.

(l) Discard supernates.

(m) Place a stir bar into each centrifuge bottle that contains precipitate.

(n) To each precipitate, add 5 mL of 0.15 M Na2HPO4 - 7H2O for each 100 mL of supernate decanted.

(o) Replace and tighten down caps on centrifuge bottles.

(p) Place each centrifuge bottle on a magnetic stirrer, and stir each precipitate slowly until it has dissolved completely. Support bottles as necessary to prevent toppling. Avoid foaming which may inactivate or aerosolize viruses. Precipitate may be partially dissipated with spatula before or during stirring procedure.

(q) Remove caps from screw-capped centrifuge bottles.

(r) Remove foil cover from 250-ml beaker.

(s) Combine the dissolved precipitates in beaker. To prevent transfer of stir bar into beaker, hold another stir bar or magnet against the bottom of the centrifuge bottle when decanting concentrate.

(t) Measure pH of concentrate (dissolved precipitate). If pH is above or below 7.0-7.5, adjust to that range with either 1 M HCl or 1 M NaOH.

(u) Replace foil cover securely on beaker.

(v) Refrigerate concentrate immediately at 4 degrees C, and maintain at that temperature until assay for viruses is undertaken. If assay for viruses cannot be undertaken within eight hours, store concentrate immediately at -70 degrees C.

(w) Assay for viruses in accordance with instructions given in Chapter 9.

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FIGURES

Figure 1. Flow Diagram of Method One for Concentrating Viruses from Large Volumes (More than 200 Liters) of Clean Waters.

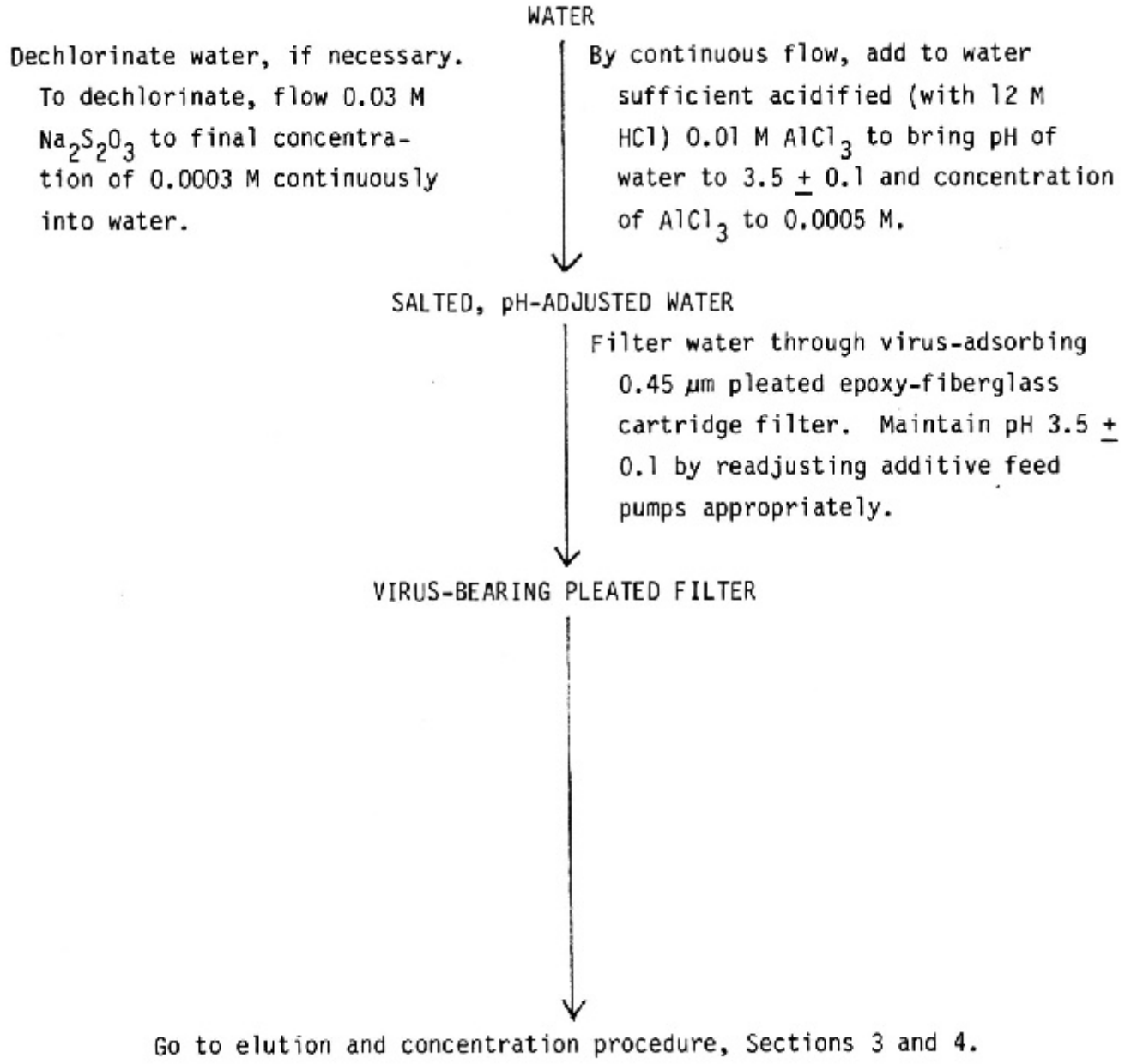


Figure 2. Schematic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedure for Large Volume Filtrations of Clean (Non-turbid) Waters.

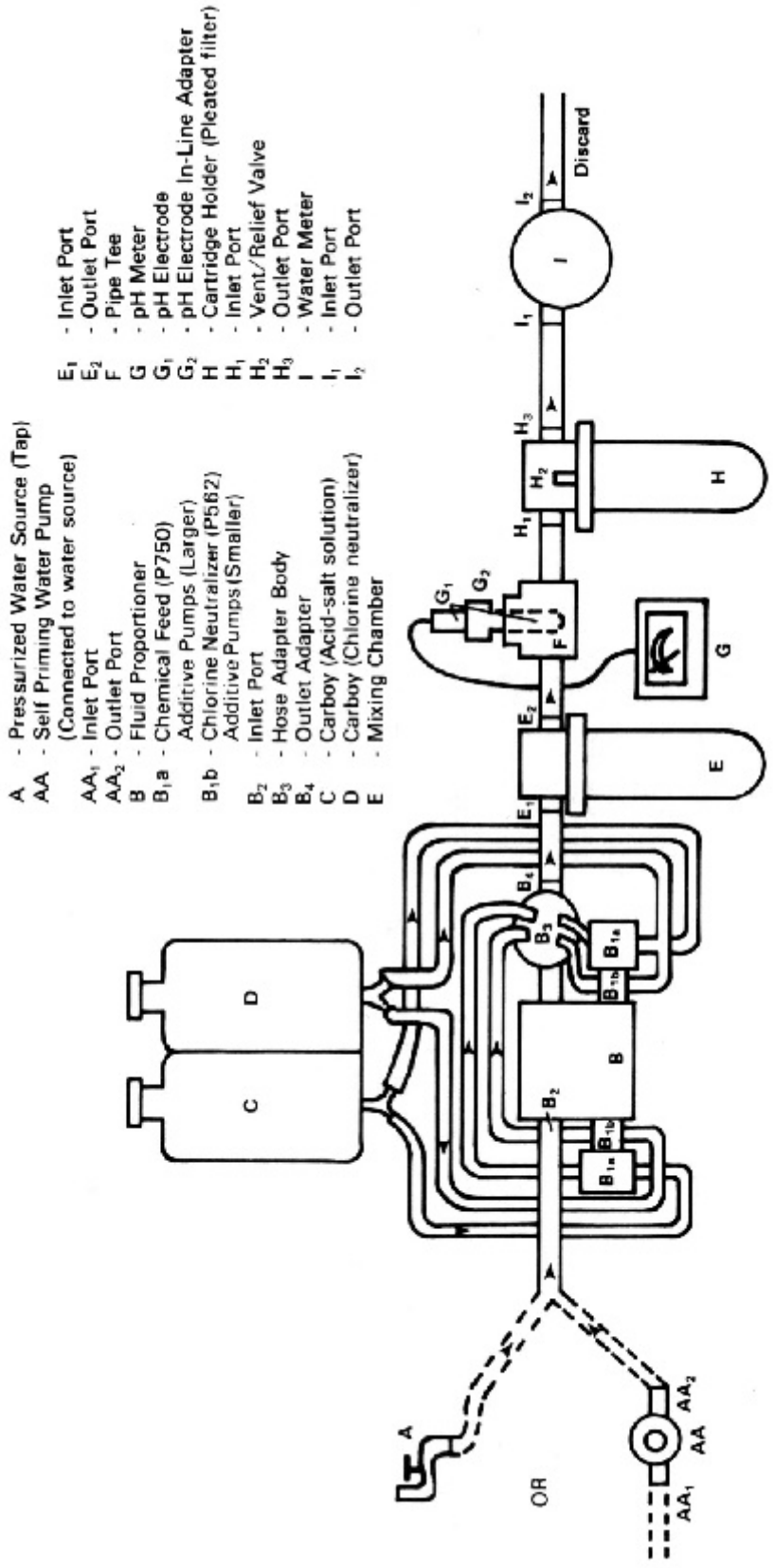


Figure 3. Photographic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedure for Large Volume Filtrations of Clean (Non-turbid) Waters.

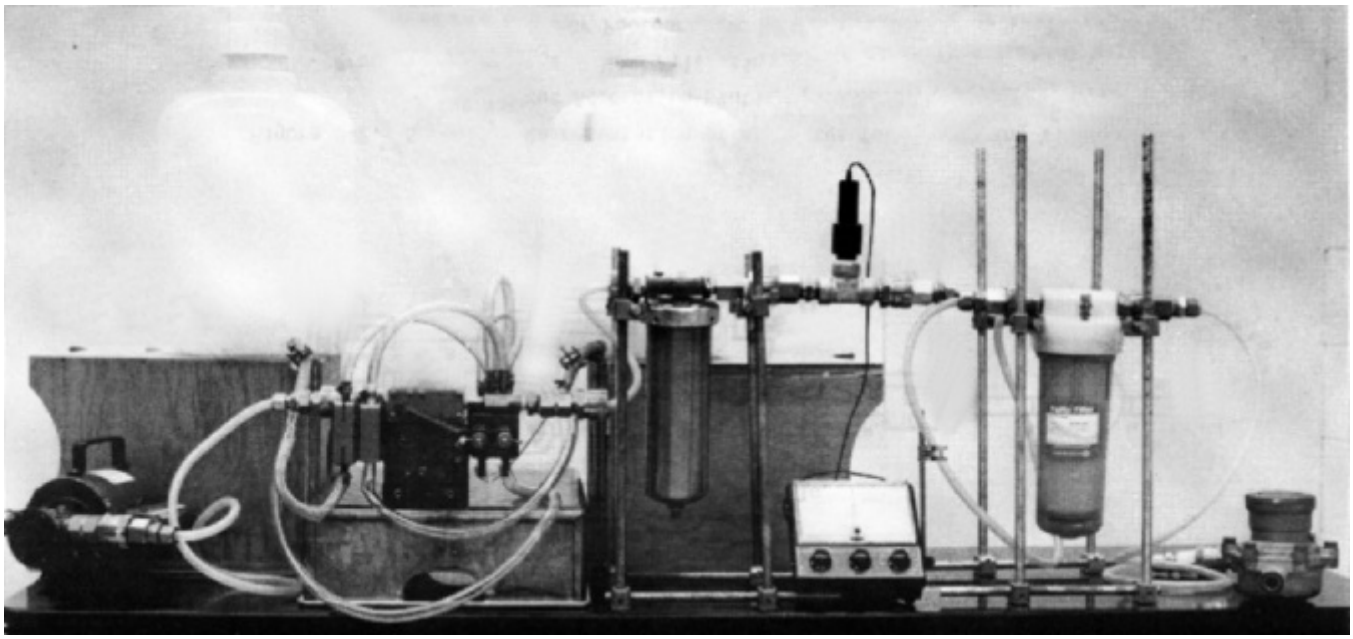


Figure 4. Schematic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedure for Large Volume Filtrations of Turbid Waters.

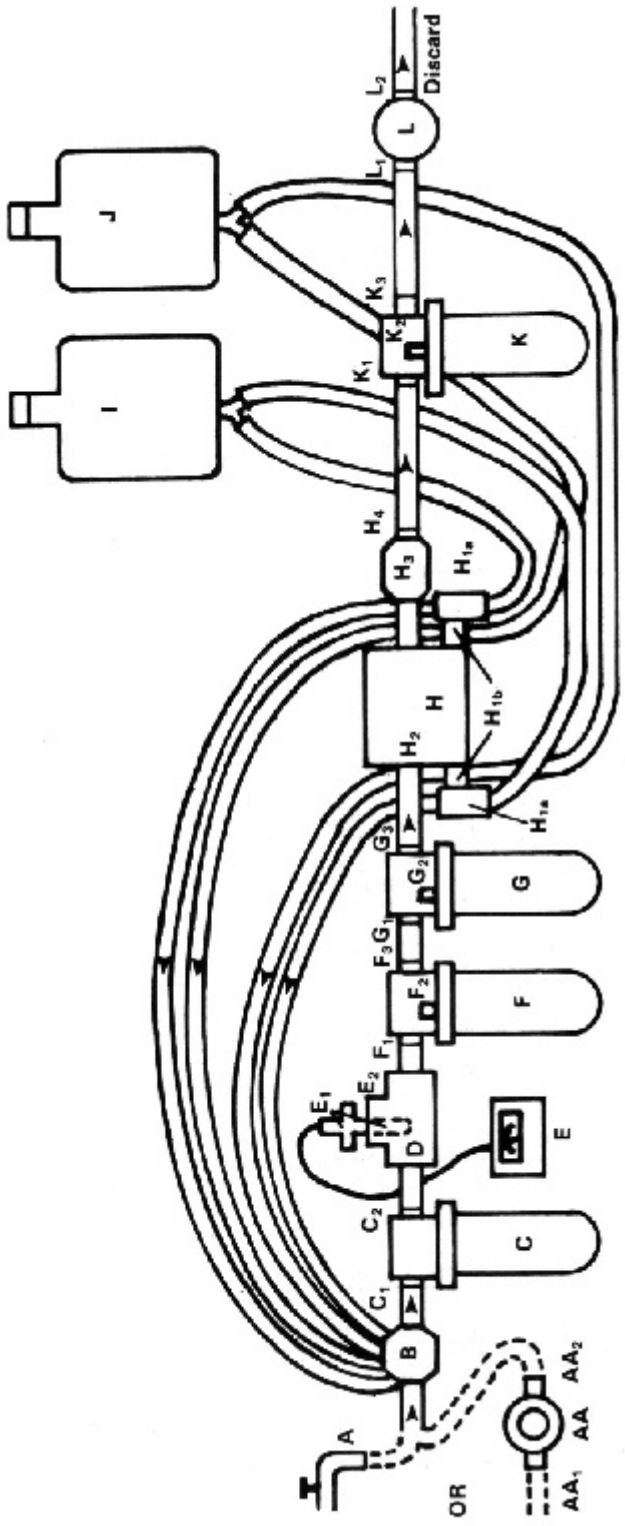


Figure 5. Photographic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedure for Large Volume Filtrations of Turbid Waters.

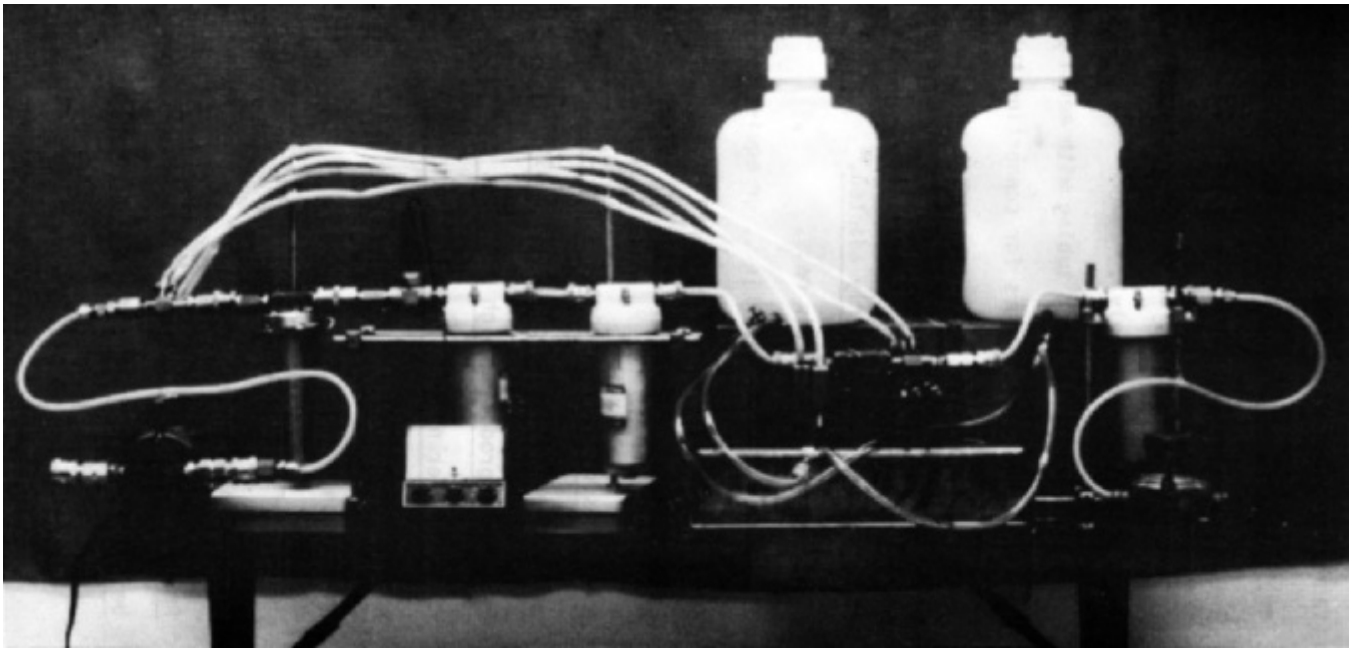


Figure 6. Flow Diagram of Method Two for Concentrating Viruses from Large Volumes (More than 200 Liters) of Turbid Waters.

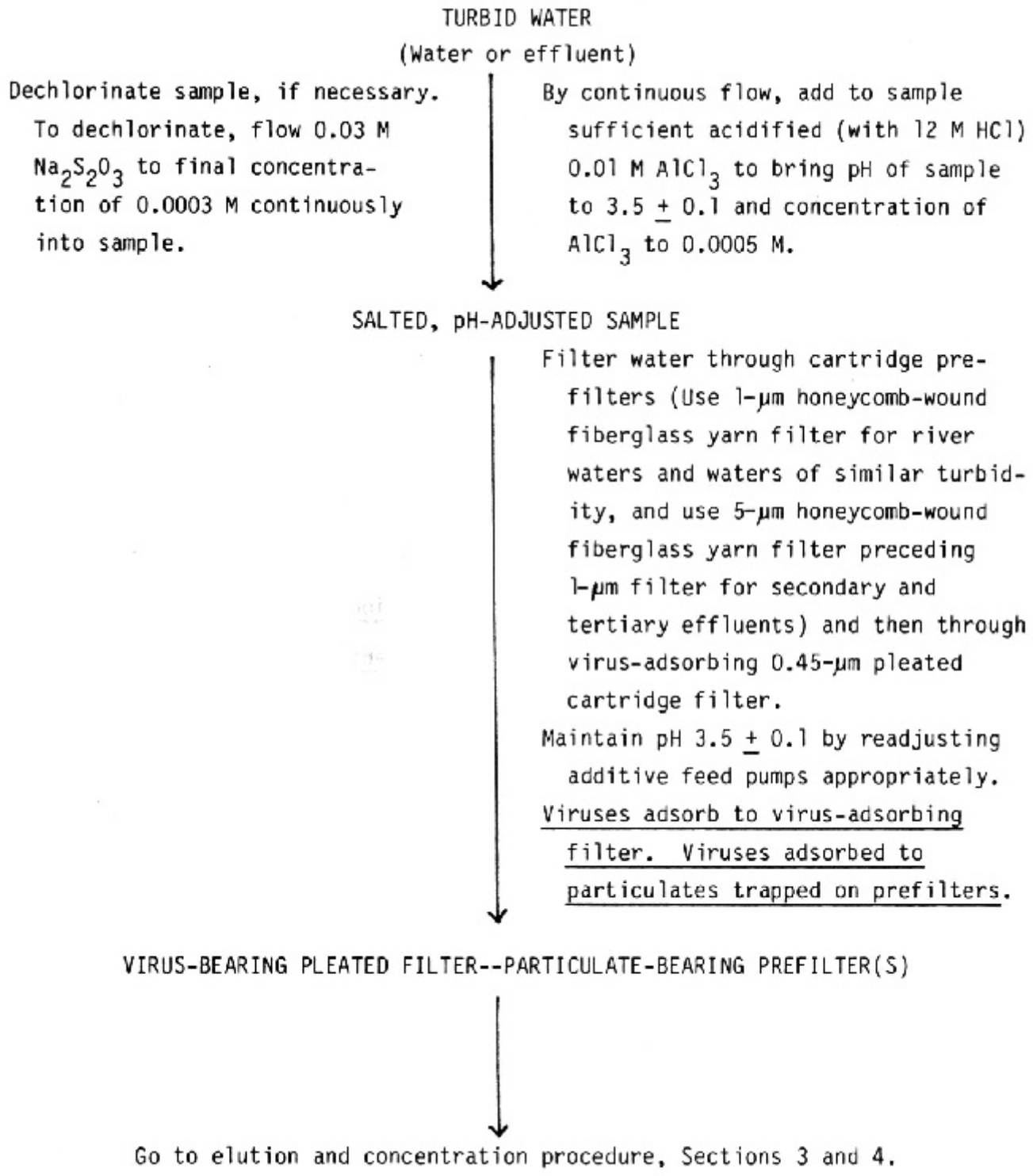


Figure 7. Flow Diagram of High pH Procedure (Basic Glycine, pH 10.5) for Eluting Viruses from Cartridge Filters and for Reconcentrating Viruses from Clear Eluates by the Membrane Filter Procedure.

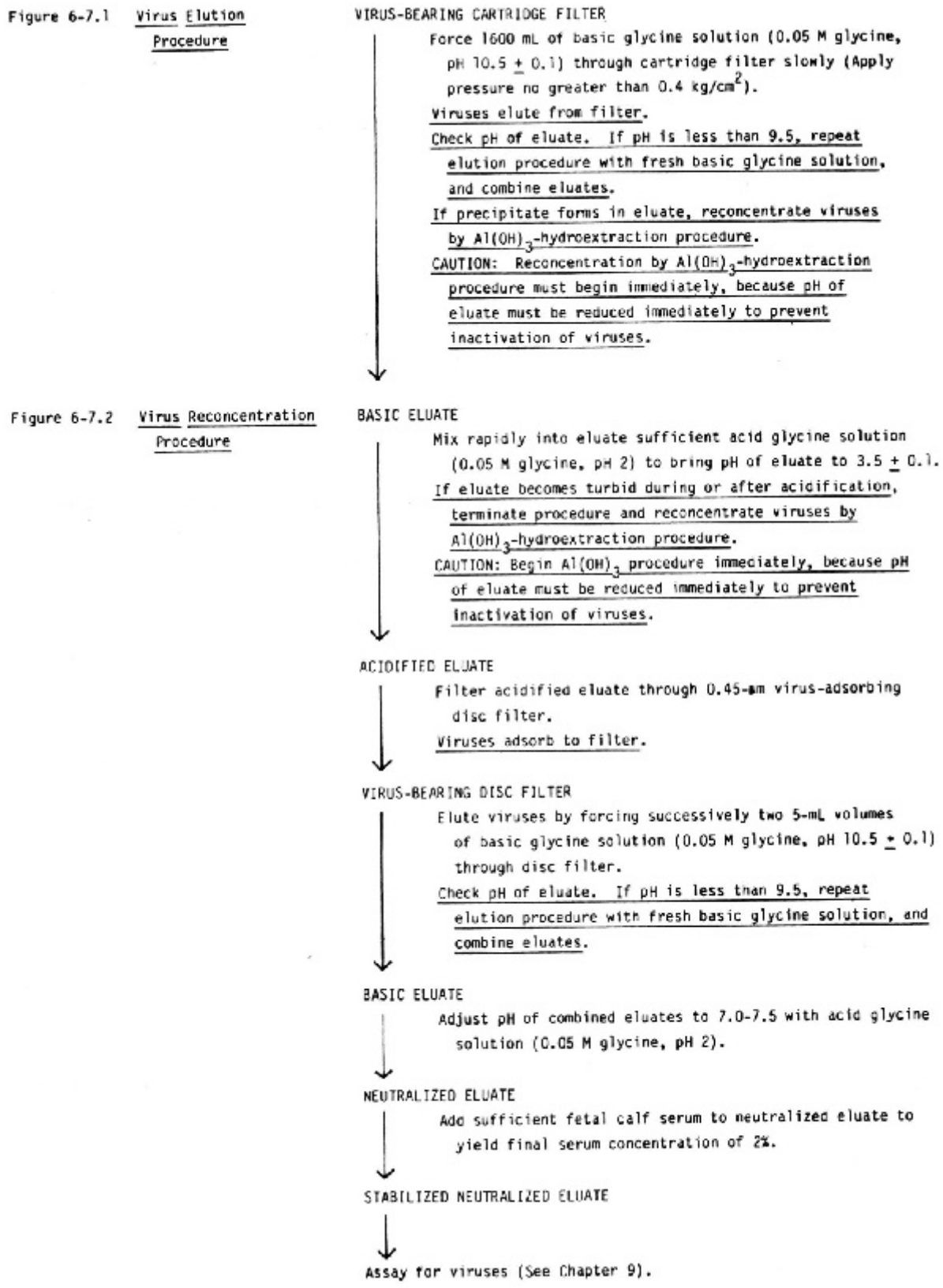


Figure 8. Flow Diagram of High pH Procedure (Basic Glycine, pH 10.5) for Eluting Viruses from Cartridge Filters and for Reconcentrating Viruses from Turbid Eluates by the Al(OH)3-Hydroextraction Procedure.

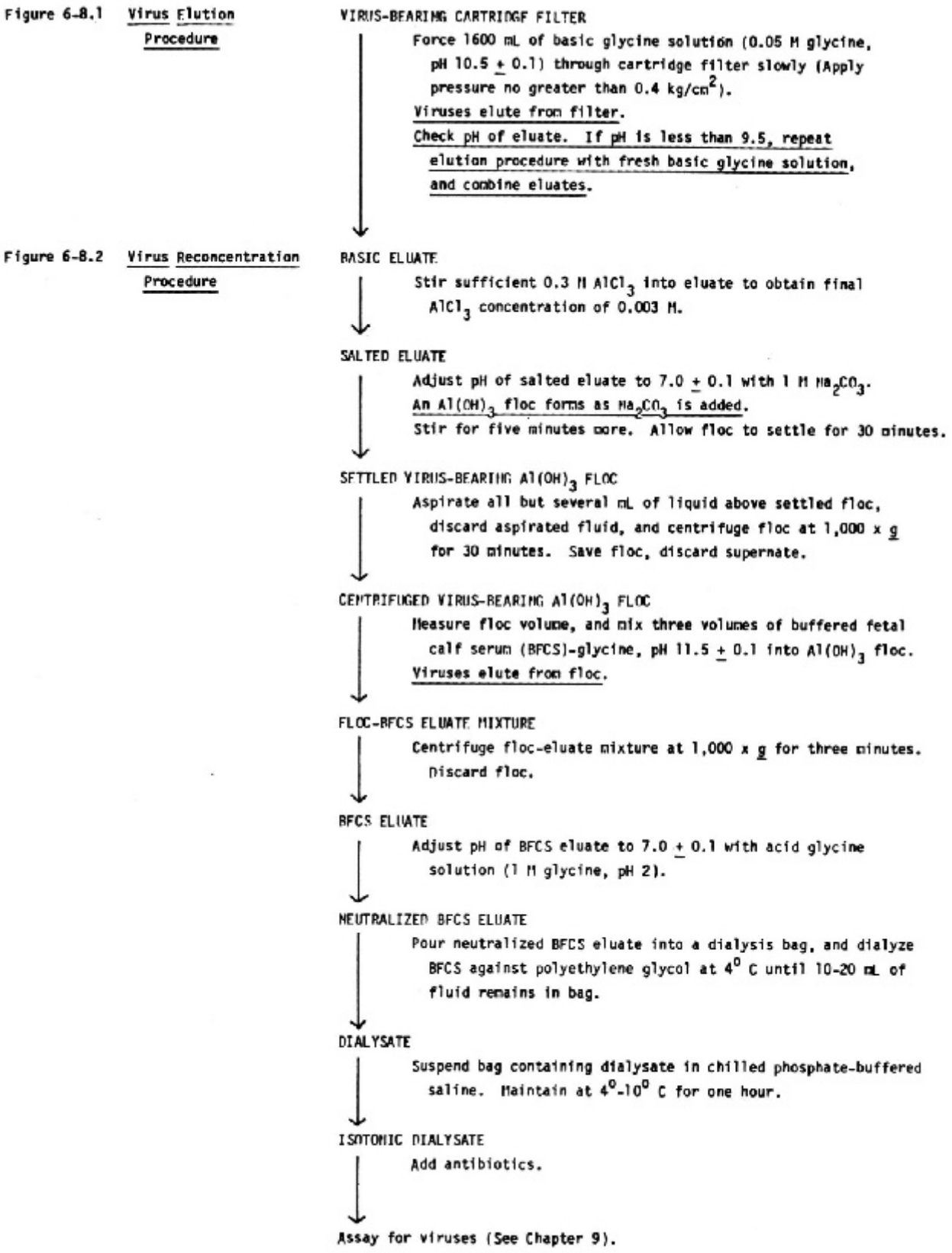


Figure 9. Schematic Representation of Apparatus for Reconcentration -- Method A, a Membrane Disc Procedure for Reconcentrating Viruses from Glycine Eluates.

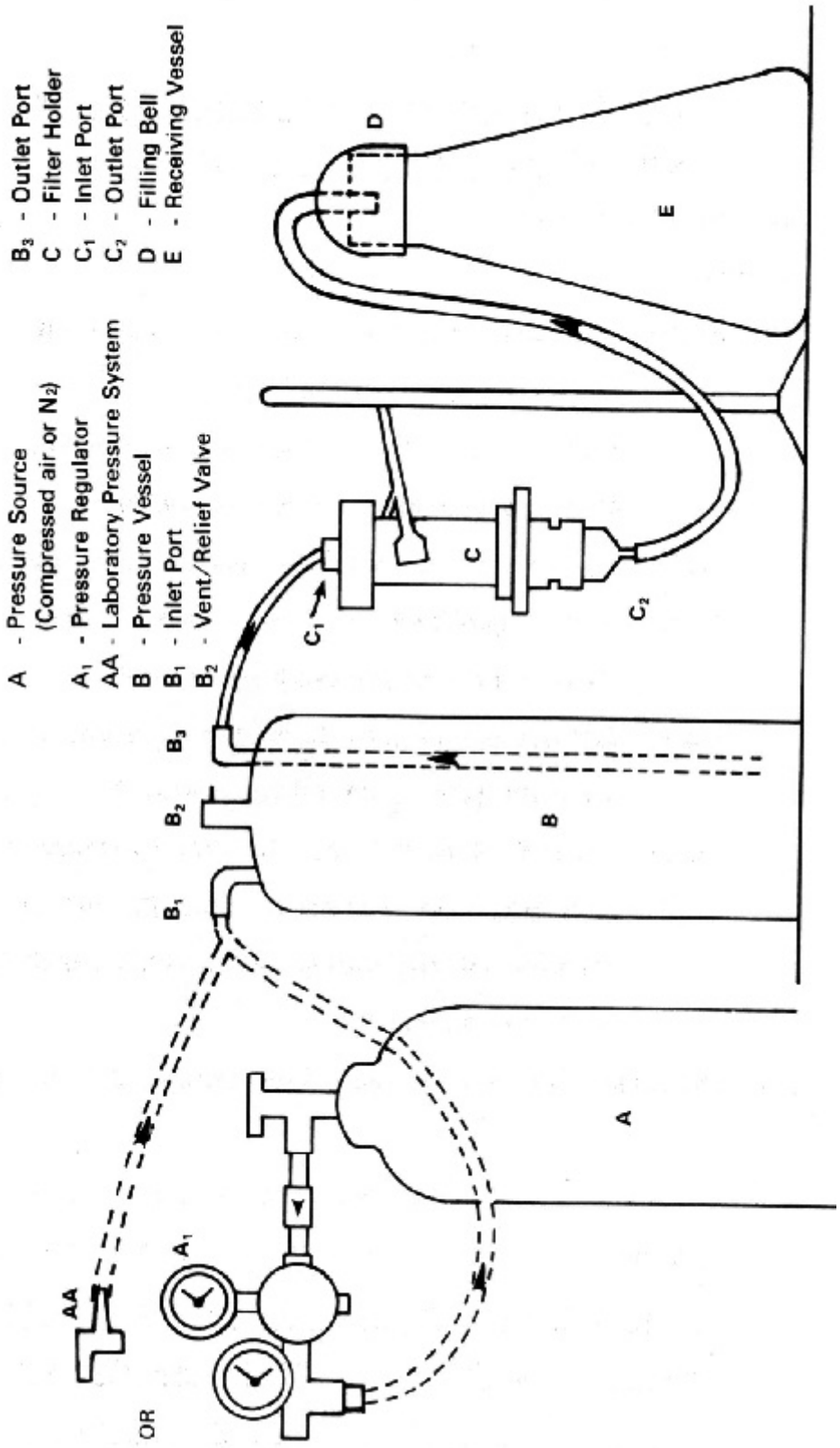


Figure 10. Photographic Representation of Apparatus for Reconcentration -- Method A, a Membrane Disc Procedure for Reconcentrating Viruses from Glycine Eluates.

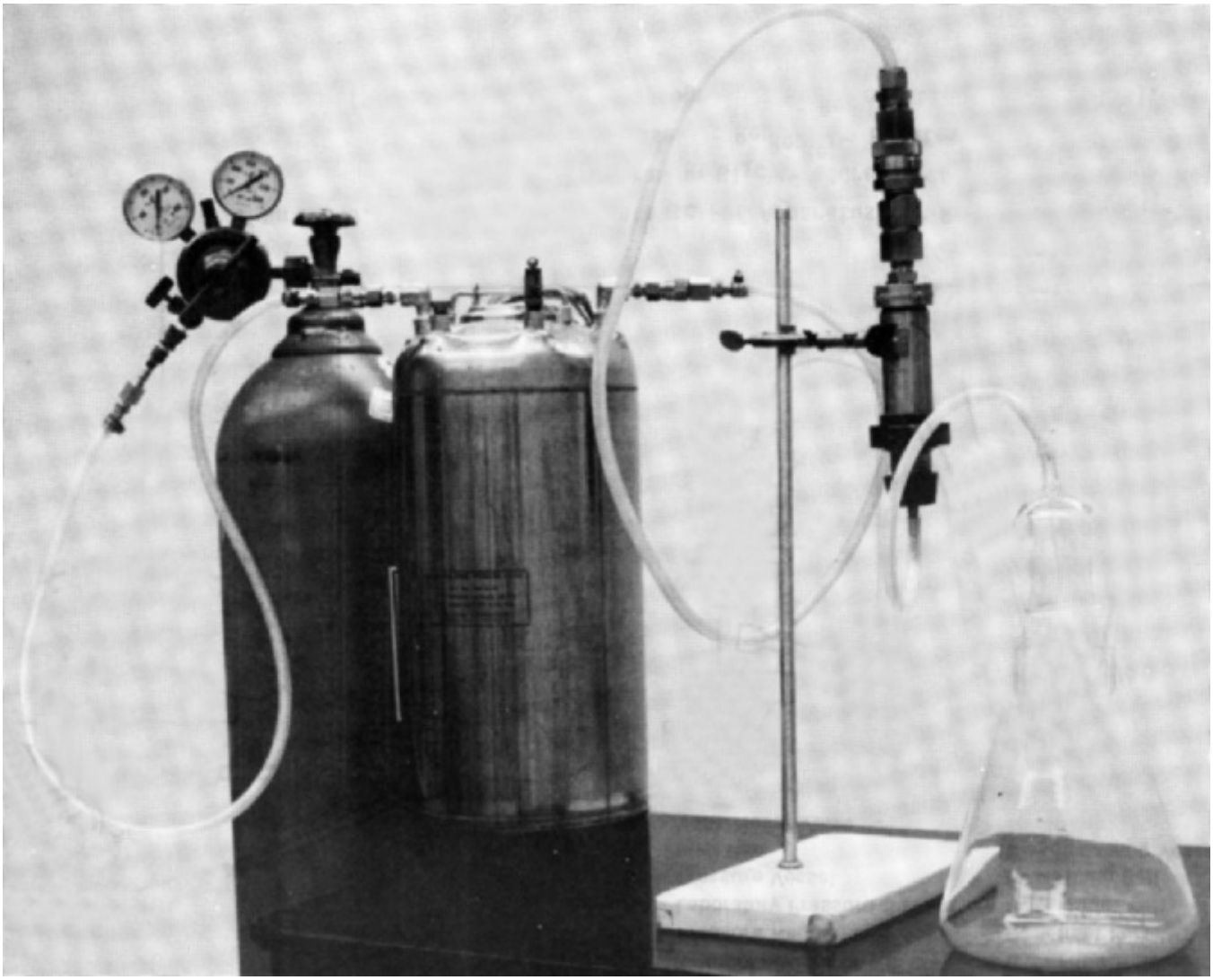
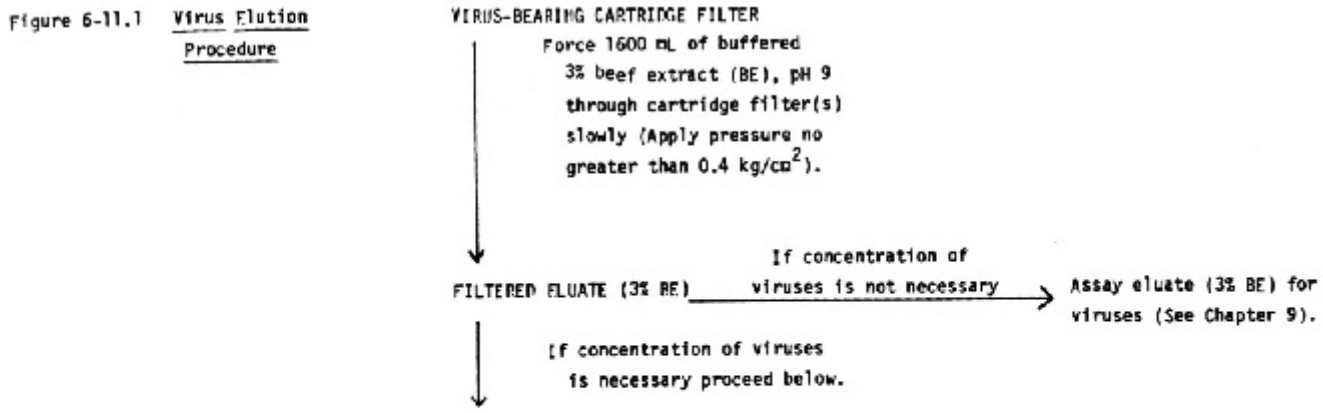
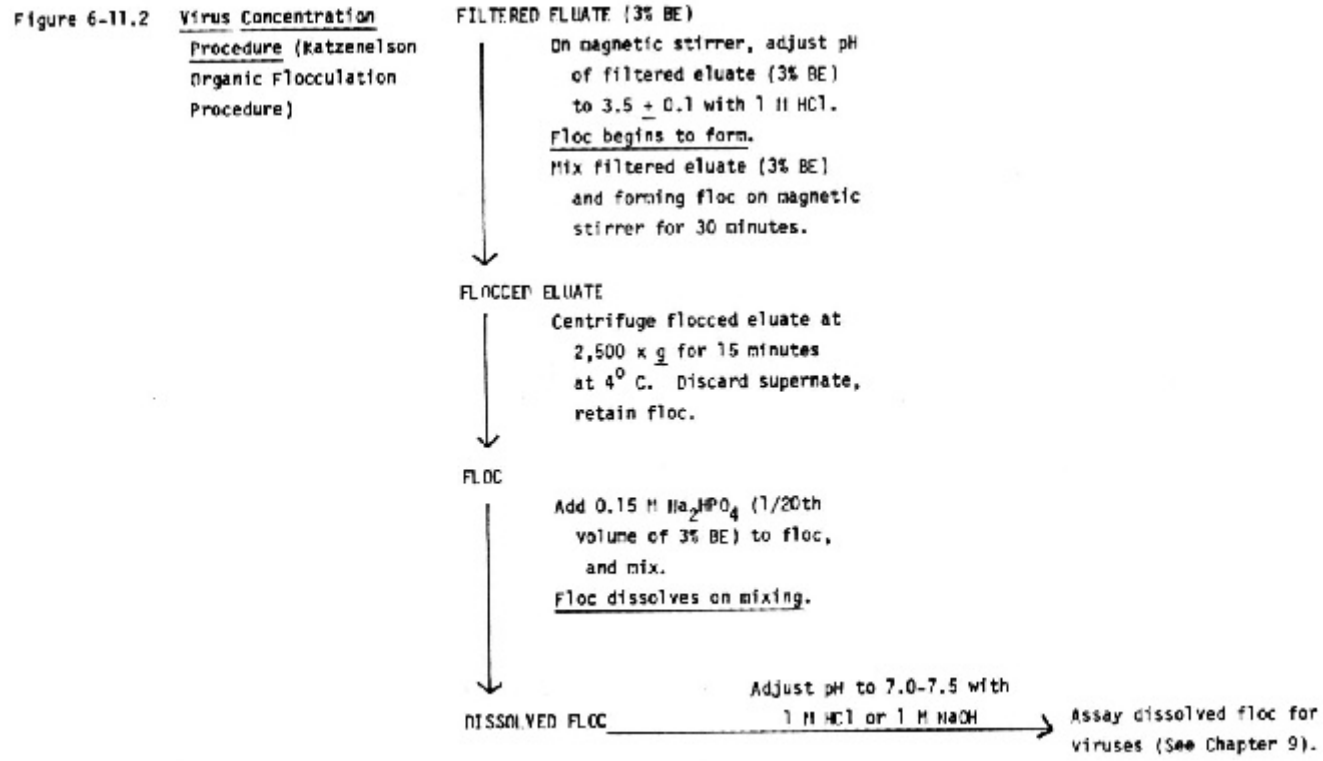


Figure 11. Flow Diagram of Beef Extract Method for Eluting Viruses from Cartridge Filters with Buffered 3% Beef Extract and for Concentrating Eluted Viruses by the Katzenelson Organic Flocculation Procedure.





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