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CHAPTER 5

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

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).

1. ADSORPTION -- METHOD ONE

This procedure may be used for volumes of 100 mL to 20 liters for all sewages and for all heavily polluted waters.

1.1 Preparation

1.1.1 Apparatus and Materials. Unless thumb-screw-drive-clamps are to be used to connect tubing to equipment, install quick-disconnect connectors on the ports of all apparatus.

(a) Disc filter holders -- 47, 90, 142, or 293 mm diameters (Millipore Corp., or equivalent). Use only pressure type filter holders. The diameter of the holder used depends upon

the volume and turbidity of the water tested. Experience with the clogging potential of the volumes of sewage, effluents, or other waters under study dictates the diameter of the filter holders used. See Sections 1.2 and 2 for further guidance.

(b) Virus-adsorbing disc filters -- 0.45-micrometer pore size (Millipore Corp. HA series, or equivalent). Select diameter of filter appropriate for the disc filter holder that is used.

(c) Fiberglass prefilters (Millipore Corp., AP15 and AP20, or equivalents).

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

(e) Positive air or nitrogen 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 pressure vessel and filter holder no more pressure than recommended by the filter manufacturer.

(f) Carboy, autoclavable plastic, or flask of a size sufficient to collect total volume of sample.

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

(h) Autoclavable inner-braided tubing with metal quick-disconnect connectors or with thumb-screw-drive-clamps for connecting tubing to equipment to be used under pressure. Quick-disconnects can be used only after equipment has been properly adapted.

(i) Magnetic stirrer and stir bars.

(j) Filling bell attached to inner-braided tubing.

1.1.2 Media and Reagents

(a) Hydrochloric acid (HC1) -- 1 M. Prepare 1 liter of 1 M hydrochloric acid solution. This solution may be stored at room temperature for several months.

(b) Sodium hydroxide (NaOH) -- 1 M. Prepare 100 mL of 1 M NaOH. This solution may be stored at room temperature for several months.

(c) Magnesium chloride (MgCl2 - 6H20) -- 1 M. Prepare 50 mL

of 1 M MgCl for each liter of sample.

1.2 Procedure (see Figure 1 for flow diagram of procedure)
Usually virus-adsorbing filters with diameters of 47 or 90 mm, coupled with prefilters of appropriate size, are adequate for raw sewage and primary effluents where volumes of 200 mL or less need to be filtered. Filters of larger diameter are required for the larger volumes of secondary and tertiary effluents that must be processed.

1.2.1 Assembly of Apparatus (see Figures 2 and 3)
Use inner-braided tubing to make all connections between apparatus to be used under pressure.

(a) Remove top of filter holder C.

(b) With two sets of forceps, place 0.45-micrometer virus-adsorbing filter onto support screen of holder.

(c) With two pairs of forceps, place AP 15 prefilter on top of 0.45-micrometer filter.

(d) With two pairs of forceps, place AP 20 prefilter on top of AP 15 prefilter.

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

(f) Connect positive pressure source A or AA to inlet port B1 of 20-liter pressure vessel B.

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

(h) Place filling bell D, with inner-braided tubing attached, over opening of flask or carboy (E) of a size sufficient to collect the total volume of sample.

(i) Connect free end of tube on the filling bell to outlet port C3 of filter holder C.

1.2.2 Salt Supplementation of Sample.

(a) Place stir bar into container holding sample.

(b) Place sample container on magnetic stirrer, and stir at speed sufficient to develop vortex.

(c) Add sufficient quantity of 1 M MgC12 to bring the concentration of MgCl2 in the sample to 0.05 M.

1.2.3 Adjustment of pH of Sample. Optimal conditions of pH vary for concentrating different viruses, especially viruses from different taxonomic groups. Conditions that favor recovery of enteroviruses are described below.

(a) Place pH electrode into salted water sample.

(b) Add sufficient 1 M HCl to bring pH of salted sample to 3.5 plus or minus 0.1. Rapid mixing of acid into sample is important because slow mixing may result in pH levels sufficiently low in parts of the sample to inactivate viruses.

(c) Turn off magnetic stirrer.

(d) Remove pH electrode from sample.

1.2.4 Filtration of Salted, pH-adjusted Sample (from Section 1.2.3, Step [b]).

(a) Remove top from pressure vessel B.

(b) Pour salted pH-adjusted sample into pressure vessel B. To prevent transfer of stir bar into pressure vessel, hold another stir bar or magnet underneath flask when decanting sample.

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

(d) Wrap vent/relief valve C2 on top of filter holder C with disinfectant-soaked gauze, and open valve about one-half turn.

(e) Apply pressure sufficient to purge trapped air from filter holder C.

(f) Close vent/relief valve C2 as soon as sample begins to flow from valve.

(g) Wipe up spilled sample with laboratory disinfectant.

(h) Increase pressure sufficiently to force sample through the filter (usually 0.4-1.5 kg/square cm).

(i) When all of sample has passed through filters, turn off pressure source A or AA.

(j) Wrap vent/relief valve B2 with disinfectant-soaked gauze, and open valve to relieve pressure in pressure vessel B.

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

(l) Discard filtrate.

(m) Elute viruses from filters immediately as described in Section 3.1.

2. ADSORPTION -- METHOD TWO

This method is recommended for volumes larger than 20 liters but not larger than 400 liters (e.g., tertiary effluents, surface waters, ground waters, and tap waters). The usefulness of this method is limited by the clarity of the water that is filtered. Prefilters must be replaced as they clog. More than ten changes of prefilters are generally impractical. Usually, 20 liters or less of river or ocean water clog a prefilter with a diameter of 293 mm. For chlorinated waters that contain sufficient solids to require elution, do not use this method. Instead, use the Viradel Cartridge Filter Procedure Method Two in Chapter 6.

2.1 Preparation

2.1.1 Apparatus and Materials. Unless thumb-screw-drive-clamps are to be used to connect tubing to equipment, install quick-disconnect connectors on the ports of all apparatus except on the additive pumps. Provide physical support as necessary for equipment that is not free-standing.

(a) Disc filter holders -- 142 and 293 mm diameter (Millipore Corp., or equivalent).

(b) Virus-adsorbing disc filters for 142 mm filter holder -- 0.45-micrometer pore size (Millipore Corp., HA series, or equivalent).

(c) Fiberglass prefilters for 293 mm filter holder (Millipore AP15 and AP20, or equivalents).

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

(e) 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 pressure vessel and filter holder no more pressure than recommended by the filter manufacturer.

(f) Plastic-coated drum(s) -- 200-liter capacity, or other

container of size suitable to hold sample if sample is not pumped directly from source.

(g) 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.

(h) Carboy, autoclavable plastic with nipple on bottom fitted with tube clamped to a dispensing Y (clamp tube 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.

(i) Fluid proportioner consisting of fluid-driven motor with four additive pumps (Johanson and Son Machine Corp., Model M 14 Q with one P-562 and one P-750 additive pump affixed to each side of the fluid-driven motor, or equivalent). Assemble fluid proportioner, and connect tubing in accordance with manufacturer's instructions.

(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 (National Pipe Thread) ports. Equip center port with pH electrode in-line adapter (Van London Co., or equivalent, for adapter only).

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

(n) Filling bell attached to inner-braided tubing.

(o) Magnetic stirrer and stir bars.

(p) Sterile aluminum foil.

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

2.1.2 Media and Reagents

(a) Hydrochloric acid (HC1) -- 0.12 M and 12 M (concentrated) solutions. Prepare 100 mL of 0.12 M HCl.

(b) Sodium thiosulfate ($Na_2S_2O_3$-$5H_2O$) -- 40 percent stock solution (with respect to $Na_2S_2O_3$-$5H_2O$). Prepare 50 mL of 40 percent (w/v) stock solution for each 100 liters of water to be processed. 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. If lesser quantities of $Na_2S_2O_3$ are needed, lesser quantities may be prepared. Sodium thiosulfate is used for dechlorinating waters 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) Magnesium chloride ($MgCl_2$-$6H_2O$) -- 5 M stock solution. Prepare 1 liter of solution for each 100 liters of water to be processed.

(d) Tween 80 -- 0.1 percent (v/v) prepared in deionized distilled water. Prepare 6 liters of 0.1 percent Tween 80.

2.2 Procedure (see Figure 4 for flow diagram of procedure) Usually, prefilters with diameters of 293 mm and virus-adsorbing filters with diameters of l42 mm are appropriate for volumes greater than 20 liters.

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. 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 5 and 6). Use inner-braided tubing to make all connections for apparatus to be used under pressure. To simplify procedures and maintain sterility, the apparatus is totally assembled at this time although sections of the apparatus will need to be disassembled and reassembled later.

(a.1) If sample is under pressure (e.g., tap water), connect water source H to inlet port I1 of filter holder I (293 mm). If sample is not under pressure, connect sample source to inlet port HH1 of self-priming water pump HH, and connect outlet port HH2 of pump HH to inlet port I1 of filter holder I.

(a.2) Connect outlet port I3 of filter holder I to inlet port

J2 of fluid proportioner J.

(a.3) Connect outlet port J4 of fluid proportioner J to inlet port M1 of mixing chamber M. Mixing chamber must be supported to prevent it from falling.

(a.4) Connect outlet port M2 of mixing chamber M to one arm of pipe tee N. Support pipe tee N to protect electrode, if necessary.

(a.5) Lock pH electrode O1 into pH electrode in-line adapter O2 in center post of pipe tee N. Same pH electrode (after sterilization) that is used to adjust pH in Step (d.4) may be used.

(a.6) Connect other arm of pipe tee N to inlet port P1 of filter holder P.

(a.7) Connect outlet port P3 of filter holder P to inlet port Q1 of water meter Q.

(a.8) Connect outlet port Q2 of water meter Q to discard.

(b) Treatment of prefilters with Tween 80 to prevent adsorption of viruses (see Figures 7 and 8; also see Figures 5 and 6). Treat AP15 and AP20 prefilters separately. AP15 and AP20 prefilters cannot be readily distinguished one from the other.

(b.1) Remove top of filter holder I.

(b.2) With two sets of forceps, place AP15 prefilters onto support screen of filter holder I. Up to 10 prefilters may be stacked in filter holder I for treatment. The number of prefilters stacked is the number that experience suggests will be needed to filter the waters to be tested. In the absence of experience, treat five prefilters of each type for relatively clear waters and 10 prefilters of each type for more turbid waters. Unused Tween-treated prefilters may be stored aseptically at 4 degrees C for up to two weeks.

(b.3) Replace and tighten down top of filter holder I.

(b.4) Open vent/relief valve I2.

(b.5) Disconnect tube from inlet port I1 of filter holder I. Protect sterility of exposed tube.

(b.6) With a new length of tubing, connect inlet port I1 of filter holder I to outlet port U3 of 20-liter pressure vessel U.

(b.7) Connect pressure source T or TT to inlet port U1 of pressure vessel U.

(b.8) Remove top of pressure vessel U.

(b.9) Pour 2 liters of 0.1 percent Tween 80 into pressure vessel U.

(b.l0) Replace top on pressure vessel U and tighten down. Check vent/relief valve U2 on pressure vessel U to be certain it is closed.

(b.11) Disconnect tube at inlet port J2 of fluid proportioner J, and place end of tube into 6-liter flask V.

(b.12) Cover inlet port J2 of fluid proportioner J with sterile aluminum foil.

(b.13) Apply pressure (T or TT) (about 0.4 kg/square cm) sufficient to force Tween 80 through prefilters.

(b.14) Close vent/relief valve I2 on filter holder I as soon as Tween 80 flows through vent, and allow all of the Tween 80 to flow through the prefilters.

(b.15) Turn off pressure source (T or TT).

(b.16) Relieve pressure in pressure vessel U by opening vent/relief valve U2.

(b.17) Remove tube from flask V, discard Tween 80, and return tube to flask V.

(b.18) Remove top of pressure vessel U.

(b.19) Pour 4 liters of deionized distilled water into pressure vessel U.

(b.20) Replace and tighten down top of pressure vessel U.

(b.21) Close vent/relief valve U2.

(b.22) Open vent/relief valve I2 on filter holder I.

(b.23) Apply pressure (about 0.4 kg/square cm) sufficient to force water through prefilters (prefilter rinse).

(b.24) Close vent/relief valve I2 on filter holder I as soon as water flows through vent, and allow all of the deionized distilled water to flow through the prefilters.

(b.25) Turn off pressure source (T or TT).

(b.26) Relieve pressure in pressure vessel U by opening vent/relief valve U2.

(b.27) Discard rinse water, and replace tube outlet port I3 of filter holder I into same flask.

(b.28) Remove top of filter holder I.

(b.29) With two sets of forceps, remove the AP15 prefilters from filter holder I, and place the prefilters on aluminum foil.

(b.30) Cover the stack of prefilters with another piece of foil.

(b.31) Repeat steps (b.2) through (b.10) and (b.13) through (b.30) with AP20 prefilters.

(b.32) Remove aluminum foil from inlet port J2 of fluid proportioner J.

(b.33) Remove tube from 6-liter flask, and connect to inlet port J2 of fluid proportioner J.

(b.34) With two sets of forceps, remove top AP15 prefilter from stack.

(b.35) Place the AP15 prefilter onto support screen of filter holder I.

(b.36) With two sets of forceps, remove top AP20 prefilter from stack, and lay the AP20 prefilter on top of AP15 prefilter.

(b.37) Replace and tighten down top of filter holder I.

(b.38) Disconnect tube from outlet port U3 of pressure vessel U, and cover tube end with aluminum foil.

(b.39) Disconnect tube from inlet port I1 on filter holder I.

(b.40) Reconnect tube from pressure source H or HH2 to inlet port I1 on filter holder I.

(c) Preparation of salt supplement. Preparation of sufficient salt supplement for 400 liters of processed water is described below. If less water is to be processed, proportionately less salt supplement needs to be prepared.

(c.1) Remove cover from 20-liter carboy K.

(c.2) Pour 8 liters of deionized distilled water into carboy K.

(c.3) Add 4 liters of 5 M $MgCl_2$ solution to the deionized distilled water in carboy K.

(c.4) Replace cover loosely on carboy K.

(d) Preparation of acid for adjustment of pH

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

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

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

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

(d.5) Add sufficient 0.12 M HCl to test water to obtain pH 3.5 plus or minus 0.1.

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

(d.7) Add to salt solution from Step (c.3) above 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.

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

(e) Preparation of $Na_2S_2O_3$ solution for dechlorination Step (e) 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 $Na_2S_2O_3$ for dechlorinating 400 liters of processed water is described below. If less water is to be processed, proportionately less $Na_2S_2O_3$ needs to be prepared.

(e.1) Remove cover from 20-liter carboy L.

(e.2) Pour 10 liters of deionized distilled water into carboy L.

(e.3) Add 186 mL of 40 percent $Na_2S_2O_3$ solution to the deionized distilled water in carboy L to give a final molarity

of 0.03, and mix solution well.

(e.4) Replace cover loosely on carboy L.

(f) Fluid proportioner

(f.1) Connect a long length of tubing to each end of dispensing Y on 20-liter carboy K that contains the acid-salt solution prepared in Step (d.7) 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 pump J1a, and continue with Step (f.2).

(f.2) Remove cover from top of carboy K.

(f.3) Place free end of each tube into mouth of carboy K.

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

(f.5) Remove tubes from mouth of carboy K, and insert tubes into the inlet (bottom) ports of larger additive pumps J1a. Allow acid-salt solution to flow freely into tubing, but manipulate tubes to prevent overflow.

(f.6) Replace cover loosely on carboy K.

(f.7) Adjust the calibration on the metering rod for each pump J1a to a setting of 3.2. 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 additive pumps J1b covered (see Section 2.2.1), and go to Step (f.15). If pressurized source is used, water should first be run for a length of time sufficient to cleanse spigot.

(f.8) Connect a long length of tubing to each end of dispensing Y on 20-liter carboy L that contains the 0.03 M $Na_2S_2O_3$ solution prepared in Step (e) above. Tubing may already be in place if pumps are sterilized with chlorine (see Section 2.2.1). In this instance, disconnect tubing from bottom of additive pumps, and continue with Step (f.9).

(f.9) Remove cover from top of carboy L.

(f.10) Place free end of each tube into mouth of carboy L.

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

(f.12) Remove the tubes from mouth of carboy L, and insert

tubes into the inlet (bottom) ports of smaller additive pumps J1b. Allow $Na_2S_2O_3$ solution to flow freely into tubes, but manipulate tubes to prevent overflow.

(f.13)Replace cover loosely on carboy L.

(f.14) Adjust the calibration on the metering rod for each additive pump J1b to a 1.3 setting. This calibration equals delivery rate of 1 part of 0.03 M $Na_2S_2O_3$ solution to each 99 parts of test water.

(f.15) Disconnect tube from inlet port M1 of mixing chamber M, and connect tube to discard.

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

(f.17) Reconnect tube from outlet port J4 of fluid proportioner J to inlet port M1 of mixing chamber M.

2.2.2 Filtration of Sample

(a) Make initial reading on water meter Q, and record reading.

(b) Remove top of filter holder P.

(c) With two sets of forceps, place 0.45 micrometer virus-adsorbing filter onto support screen of holder.

(d) Replace and tighten down top of filter holder P.

(e) Open vent/relief valves I2 and P2 on filter holders I and P.

(f) Open pressurized water source H or start water pump HH and purge trapped air from filter holders I and P.

(g) Close vent/relief valves I2 and P2 on filter holders I and P as soon as sample begins to flow from valves.

(h) Wipe up spilled sample with laboratory disinfectant.

(i) Read pH meter O to ascertain that proper pH is achieved. Check meter periodically to be certain that proper pH is maintained. If pH readjustment is necessary, appropriately alter settings on metering rods for additive pumps P-750.

(j) When appropriate volume has been filtered, or if flow rate becomes significantly reduced, turn off pressure either at pressurized source H or at water pump HH.

(k) Open vent/relief valves I2 and P2 on filter holders I and P.

(l) Disconnect tube from pressurized source H or water pump HH, and connect free end of tube to positive air or nitrogen pressure source.

(m) Close vent/relief valve I2 on filter holder I.

(n) Apply pressure sufficient to force remaining sample water from filter holder I.

(o) Turn off pressure at positive air or nitrogen pressure source.

(p) Open vent/relief valve I2 on filter holder I.

(q) Disconnect hose from positive pressure source, and reconnect to pressurized source H or water pump HH.

(r) Remove top of filter holder I.

(s) Replace clogged prefilters with new prefilters as described in Steps (b.34) through (b.37). If appropriate volume of sample has been filtered, do not insert new filter into filter holder.

(t) Place each set of clogged prefilters on aluminum foil, and cover. See Section 3.2 for processing solids on clogged prefilters. If appropriate volume of sample has been filtered, proceed to Step (cc).

(u) Close vent/relief valve I2 on filter holder I.

(v) Continue filtration procedure. Bleed air from both filter holders I and P at vent/relief valves I2 and P2 each time apparatus is opened to replace prefilters. As many changes of prefilters should be made as are necessary to process entire sample. Steps (q) through (t) may be completed for each set of prefilters as filtration procedure continues.

(w) Uncover one set of prefilters.

(x) With spatula, scrape solids from top prefilter (AP20).

(y) Place solids in a tared beaker, and cover mouth of beaker with aluminum foil.

(z) Maintain beaker at 4 degrees C. See Section 3.2 for processing solids.

(aa) After required volume of water has been filtered, turn off pressure either at pressurized source H or at water pump HH.

(bb) Open vent/relief valves I2 and P2 on filter holders I and P.

(cc) Disconnect at pipe tee N the tube leading to inlet port P1 of filter holder P, and connect free end of tube to positive pressure source.

(dd) Close vent/relief valve P2 on filter holder P.

(ee) Apply pressure sufficient to force remaining sample water from filter holder P.

(ff) Make final reading on water meter. Subtract initial reading from final reading to determine total volume filtered. Subtract volume of acid-salt solution and, if used, volume of Na2S2O3 solution from total volume filtered to determine volume of water sampled.

(gg) Turn off pressure at positive pressure source.

(hh) Open vent/relief valve P2 on filter holder P.

(ii) Disconnect tube from outlet port P3 of filter holder P, and replace with tube connected to filling bell.

(jj) Elute viruses from virus-adsorbing filter as described in Section 3.

3. ELUTION AND RECONCENTRATION

3.1 Procedure for Eluting Viruses from Filters (see Figures 2 and 3,and Figures 5 and 6)

3.1.1 Apparatus and Materials

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

(c) Autoclavable inner-braided tubing fitted with metal

quick-disconnect connectors or with thumb-screw-drive-clamps for connecting tubing to equipment.

(d) Magnetic stirrer and stir bars.

3.1.2 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 gm of beef extract powder and 7.5 g of glycine (final glycine concentration = 0.05 M) in 2 liters of deionized distilled water. Autoclave beef extract solution, and adjust pH to 9 with 1 M NaOH.

3.1.3 Procedure

(a) Place filling bell attached to outlet port of filter holder C (Method 1, Figures 2 and 3) or P (Method 2, Figures 5 and 6) on receiving flask. To prevent toppling, it may be necessary to support flask.

(b) Disconnect tube from inlet port of filter holder C (Method 1, Figures 2 and 3) or P (Method 2, Figures 5 and 6).

(c) Open vent/relief valve on filter holder.

(d) Pour into inlet port of filter holder 0.45 mL of beef extract (pH 9) for each square cm of effective filter area. Determine total effective filter area from manufacturer's specifications. Volume of beef extract thus needed for 142 mm filter is 44 mL.

(e) Close vent/relief valve on top of filter holder.

(f) Connect tube to inlet port of filter holder.

(g) Allow beef extract to remain in contact with filter(s) for 30 minutes.

(h) Apply pressure sufficient to force beef extract through filter(s). Lower receiving flask and tilt filter holder to permit complete evacuation of buffered 3 percent beef extract from filter(s).

(i) Turn off pressure at source.

(j) Open vent/relief valve on filter holder.

(k) Unless beef extract eluate is reconcentrated or assayed for viruses immediately, refrigerate eluate immediately at 4 degrees C, and maintain at that temperature until eluate is reconcentrated or is assayed for viruses. If reconcentration or assay for viruses cannot be undertaken within eight hours, store eluate immediately at -70 degrees C. The number of cell cultures necessary for the viral assay may be reduced by reconcentrating the viruses in the beef extract by the organic flocculation procedure of Katzenelson (see Section 3.3).

3.2 Procedure for Processing Solids. Often more viruses are recovered from the solids in waters than from the waters from which the solids are obtained.

3.2.1 Apparatus and Materials

(a) Magnetic stirrer and stir bars.

(b) Membrane filter apparatus for sterilization -- 47 mm diameter filter holder with 30-mL slip tip syringe (Millipore Corp., Swinnex filter No. SX0004700, or equivalent for filter holder only).

(c) Membrane filters, 47 mm diameter -- 5-, 1.2-, 0.65-, and 0.45-micrometer pore sizes (Millipore Corp., HA series, or equivalent). Place filter with 0.45-micrometer pore size on support screen of Swinnex filter holder, and stack the remaining filters on top in order of increasing pore size. Filters stacked in tandem as described tend to clog more slowly when turbid material is filtered through them.

(d) Refrigerated centrifuge capable of attaining 2,500 x g.

3.2.2 Media and Reagents

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

(b) Citric acid.

(c) Beef extract powder (Gibco, or equivalent). Prepare buffered (pH 7.0) 10 percent beef extract by dissolving 10 g beef extract powder, 1.34 g $Na_2HPO_4-7H_2O$, and 0.12 g citric acid in 100 mL of deionized distilled water.

3.2.3 Procedure

(a) Weigh beaker that contains solids scraped from prefilters (from Section 2.2.2, Step [z]). Calculate weight of solids by subtracting tare weight of beaker from weight of beaker with solids.

(b) Place stir bar into beaker.

(c) Measure into beaker 3 mL of 10 percent buffered beef extract for every gram of solids.

(d) Place beaker on magnetic stirrer, and stir for 30 minutes. Viruses elute from solids.

(e) Pour suspension of solids and buffered beef extract eluate into 250-mL centrifuge bottle. Glass centrifuge bottles may not be able to withstand g force that will be applied. To prevent transfer of stir bar into centrifuge bottle, hold another stir bar or magnet underneath beaker when decanting solids.

(f) Centrifuge suspension for 30 minutes at approximately 2,500 x g.

(g) Decant buffered beef extract eluate into beaker of appropriate size, and discard solids. The number of cell cultures necessary for the viral assay may be reduced by reconcentrating the viruses in the beef extract by the organic flocculation procedure of Katzenelson. If viruses in eluate are to be reconcentrated, proceed to Step (h). If reconcentration is not required, proceed to Step (i).

(h) Add 7 mL of deionized distilled water to each 3 mL of eluate if reconcentration is required, and proceed according to Section 3.3.

(i) Load eluate into 30-mL syringe.

(j) Place tip of syringe into filter holder, and place filter holder on a 125-mL receiving flask.

(k) Force eluate through filters into 125-mL receiving flask. Take care not to put pressure on receiving flask. If filter clogs, invert filter, draw remaining fluid from top of clogged filter into syringe, and replace filter holder and filters. Steps (i) thru (k) may be repeated as often as necessary to filter entire volume of eluate.

(l) Refrigerate eluate immediately at 4 degrees C 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 Organic Flocculation Concentration Procedure of Katzenelson (see Figure 9 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.

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 a combination-type electrode.

(c) Refrigerated centrifuge capable of attaining 2,500 x g.

3.3.2 Media and Reagents

(a) Disodium hydrogen phosphate (Na2HPO4-7H2O) -- 0.15 M.

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

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

3.3.3 Procedure

(a) If concentration of beef extract in eluate is 10 percent, reduce it to 3 percent with deionized distilled water; if volume of beef extract eluate is less than 100 mL, add sufficient 3 percent beef extract to bring total volume to 100 mL.

(b) Place stir bar in flask that contains beef extract eluate.

(c) Place flask that contains beef extract eluate 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.

(d) Insert pH electrode into beef extract eluate.

(e) 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, if possible, reducing pH below 3.4 because some inactivation of viruses may occur.

(f) Continue to stir for 30 minutes more, and maintain pH at 3.5 plus or minus 0.1.

(g) Remove pH electrode from beef extract.

(h) Remove cap from 250-mL screw-capped centrifuge bottle. Glass centrifuge bottles may not be able to withstand g force that will be applied.

(i) Pour contents of flask into 250-mL screw-capped centrifuge bottle. To prevent transfer of stir bar into centrifuge bottle, hold another stir bar or magnet against bottom of flask when decanting contents.

(j) Replace and tighten down cap on screw-capped centrifuge bottle.

(k) Centrifuge precipitated beef extract suspension in refrigerated centrifuge (4 degrees C) for 15 minutes at 2,500 x g.

(l) Remove cap from screw-capped centrifuge bottle.

(m) Pour supernate into graduated cylinder, and record volume.

(n) Discard supernate.

(o) Place a stir bar into centrifuge bottle containing the precipitate.

(p) Add to the precipitate 5 mL of 0.15 M Na2HPO4 for each 100 mL of supernate decanted.

(q) Replace and tighten down cap on screw-capped centrifuge bottle.

(r) Place the centrifuge bottle on a magnetic stirrer, and stir slowly until precipitate 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.

(s) Remove cap from screw-capped centrifuge bottle.

(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 and tighten down cap on screw-cap centrifuge bottle.

(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.

4. BIBLIOGRAPHY

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FIGURES

Figure 1. Flow Diagram of Method for Recovering Viruses from Small Volumes (100 mL to 20 Liters) of Water, Sewage, or Effluent.

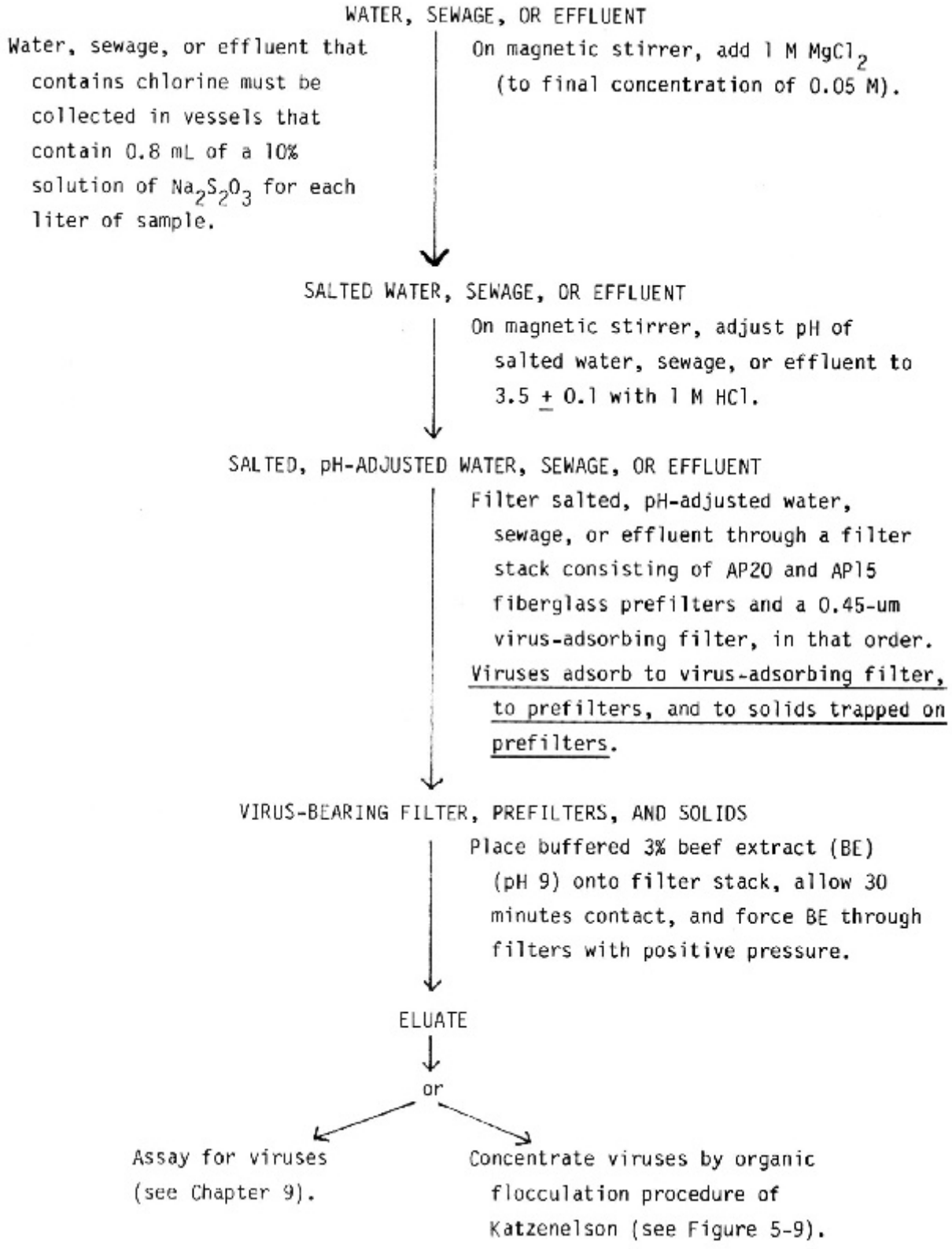


Figure 2. Schematic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Small Volume Filtrations.

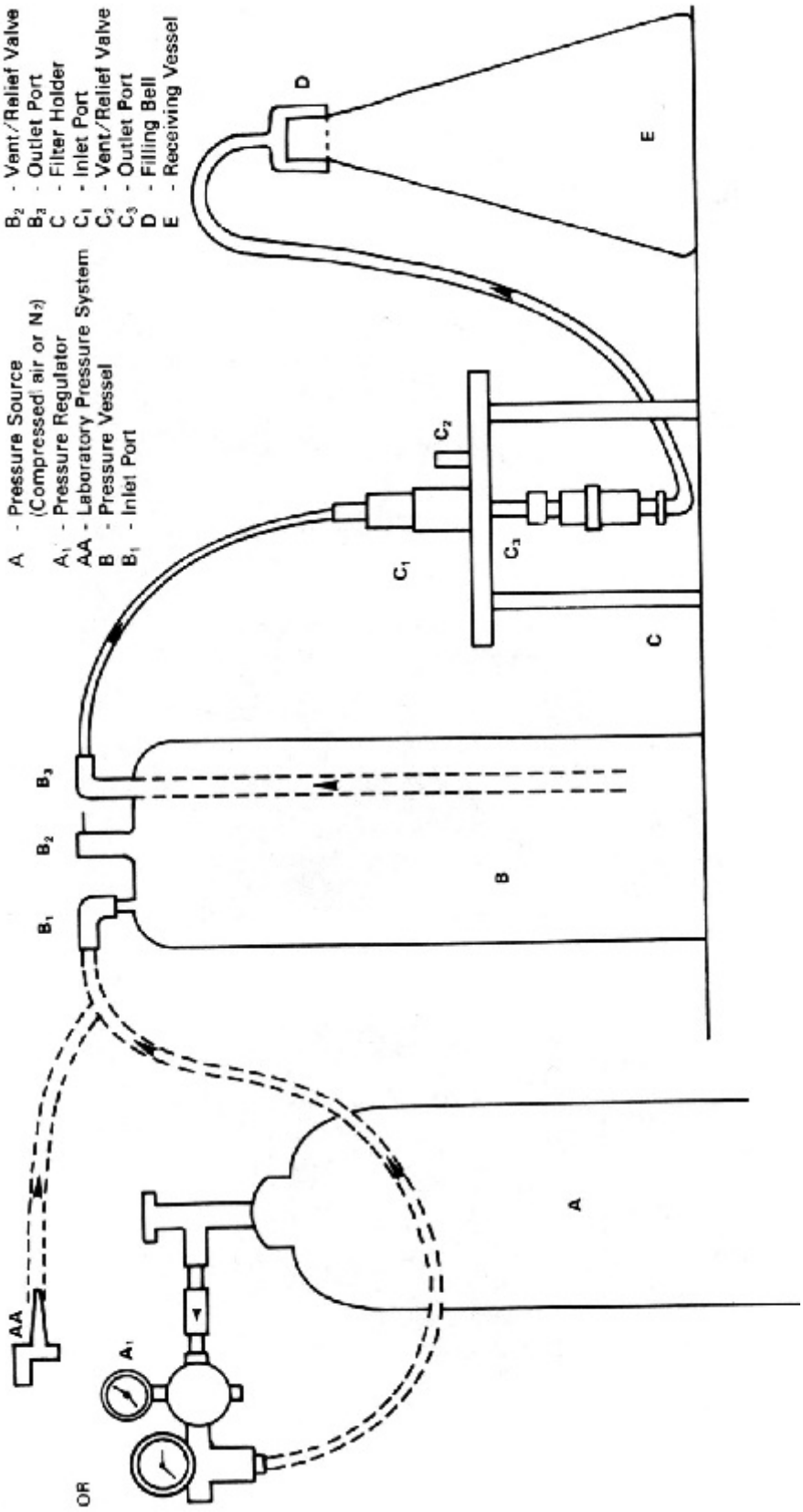


Figure 3. Photographic Representation of Apparatus for Recovering Viruses by the Virus-Adsorption-Elution (VIRADEL) Disc Filter Procedure for Small Volume Filtrations.

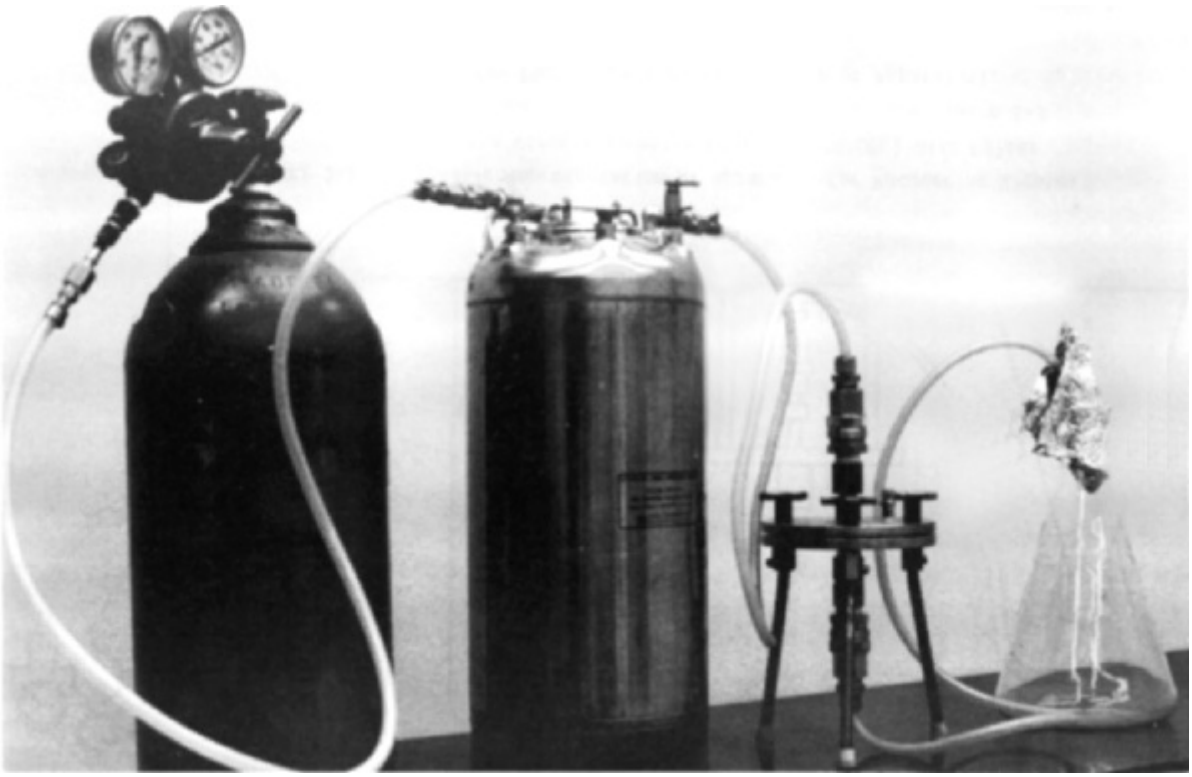


Figure 4. Flow Diagram of Method for Recovering Viruses from Large Volumes (More than 20 Liters) of Water, Sewage, or Effluents.

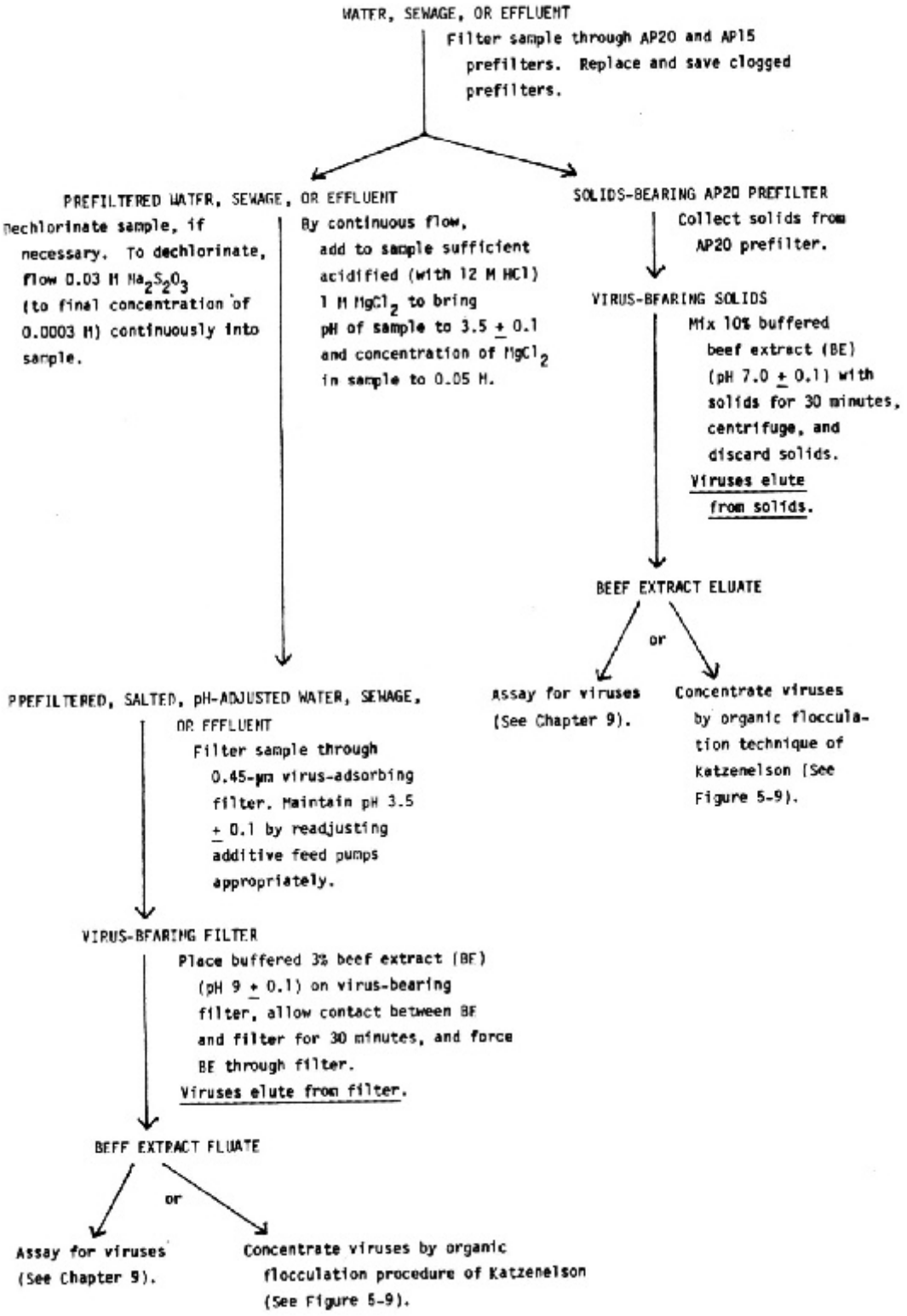


Figure 5. Schematic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Large Volume Filtrations.

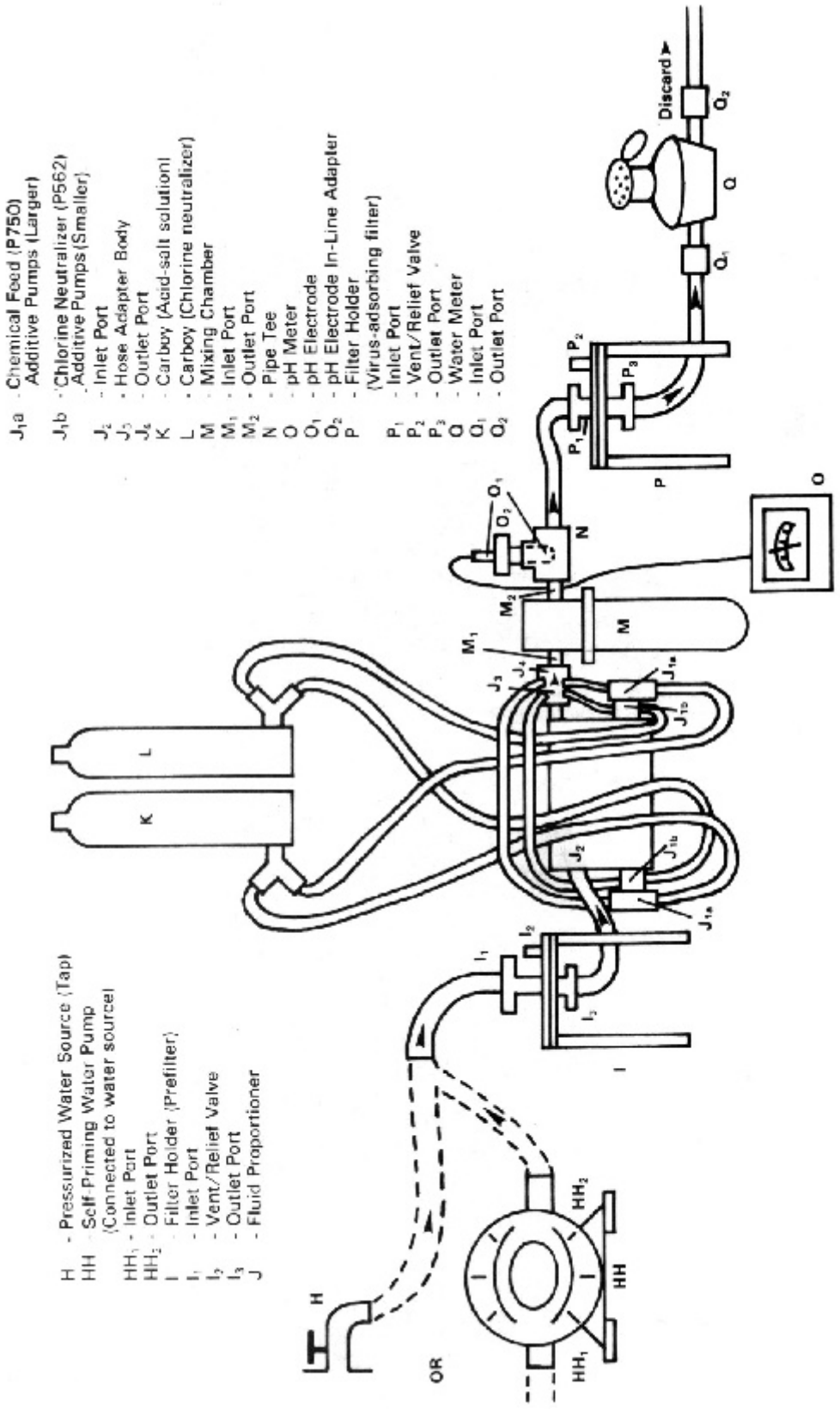


Figure 6. Photographic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Large Volume Filtrations.

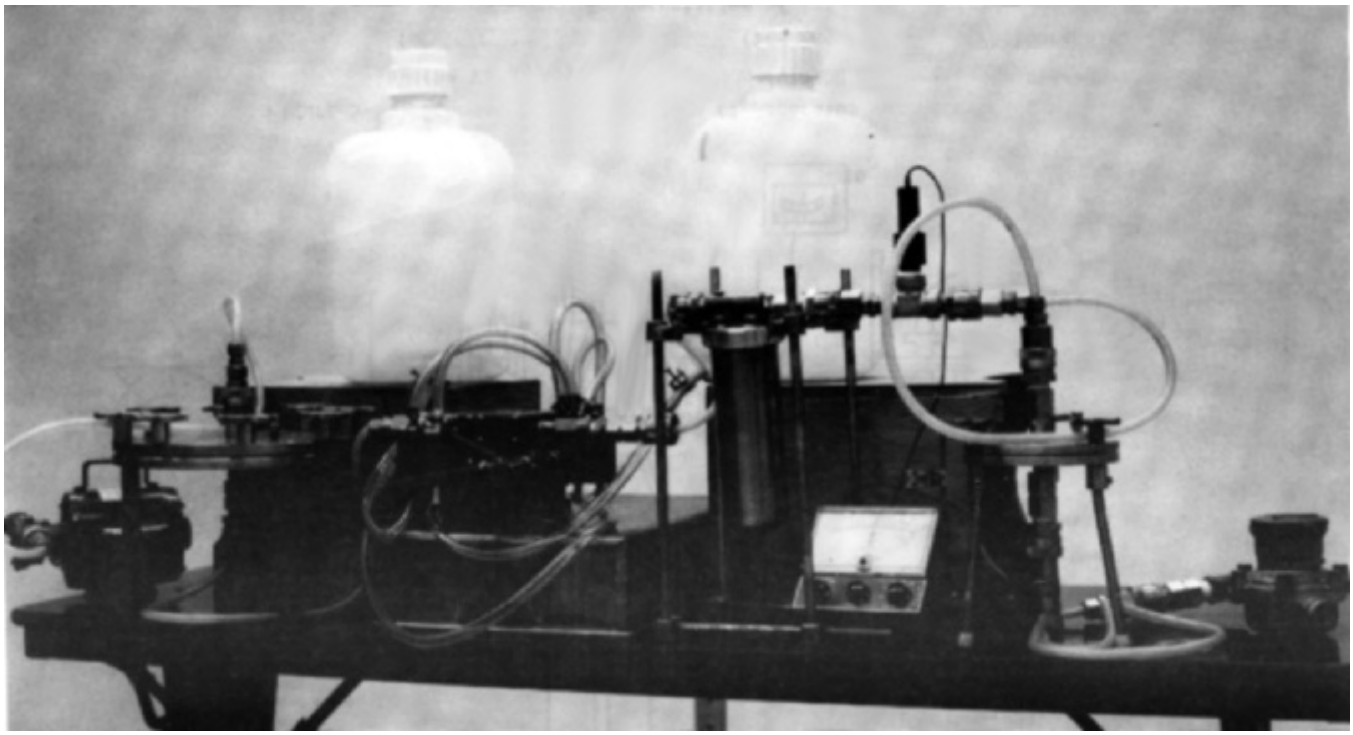


Figure 7. Schematic Representation of Apparatus for Treatment of Prefilters with Tween 80 to Prevent Adsorption of Viruses to the Prefilters in the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Large Volume Filtrations.

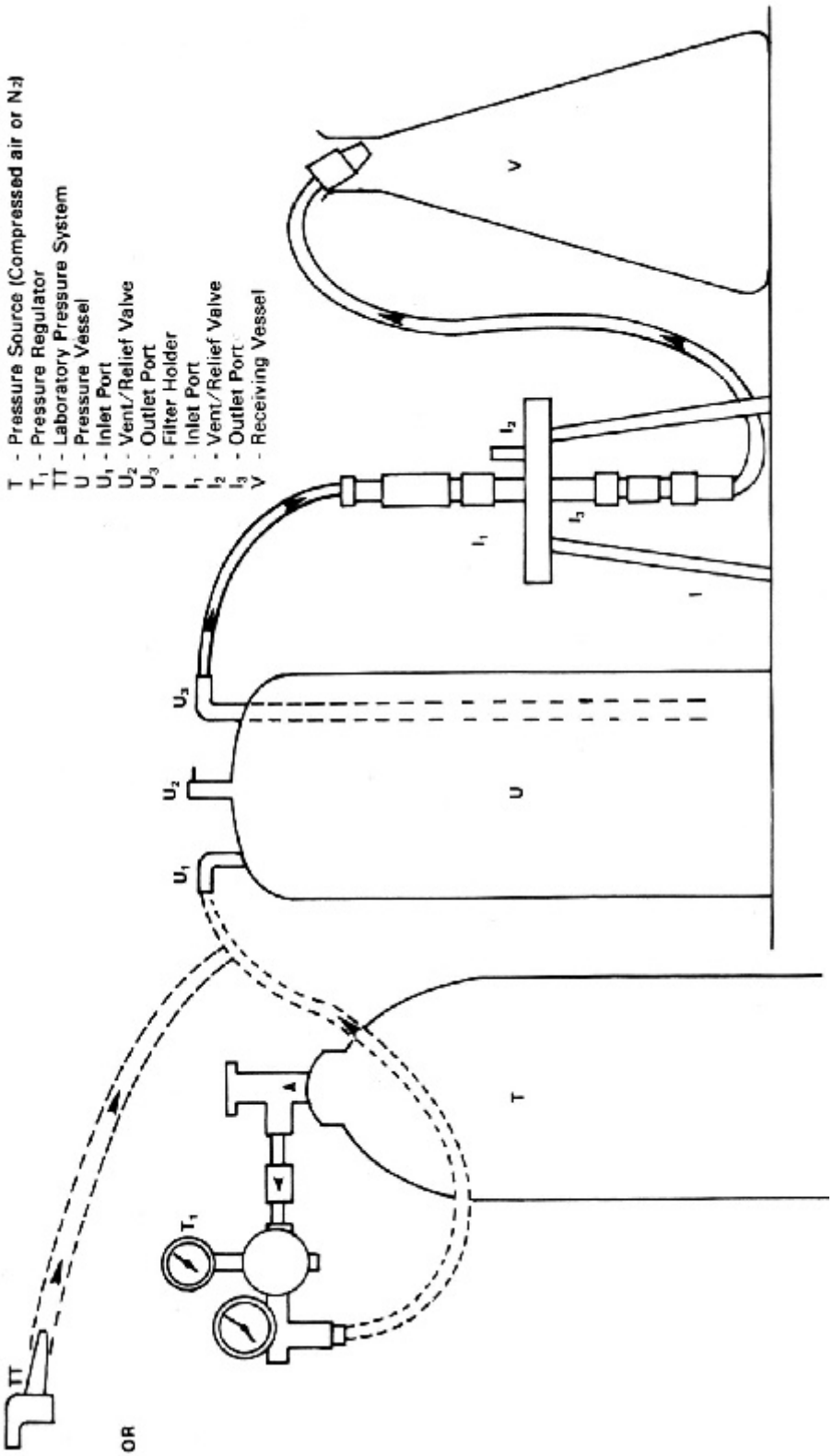


Figure 8. Photographic Representation of Apparatus for Treatment of Prefilters with Tween 80 to Prevent Adsorption of Viruses to the Prefilters in the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Large Volume Filtrations.

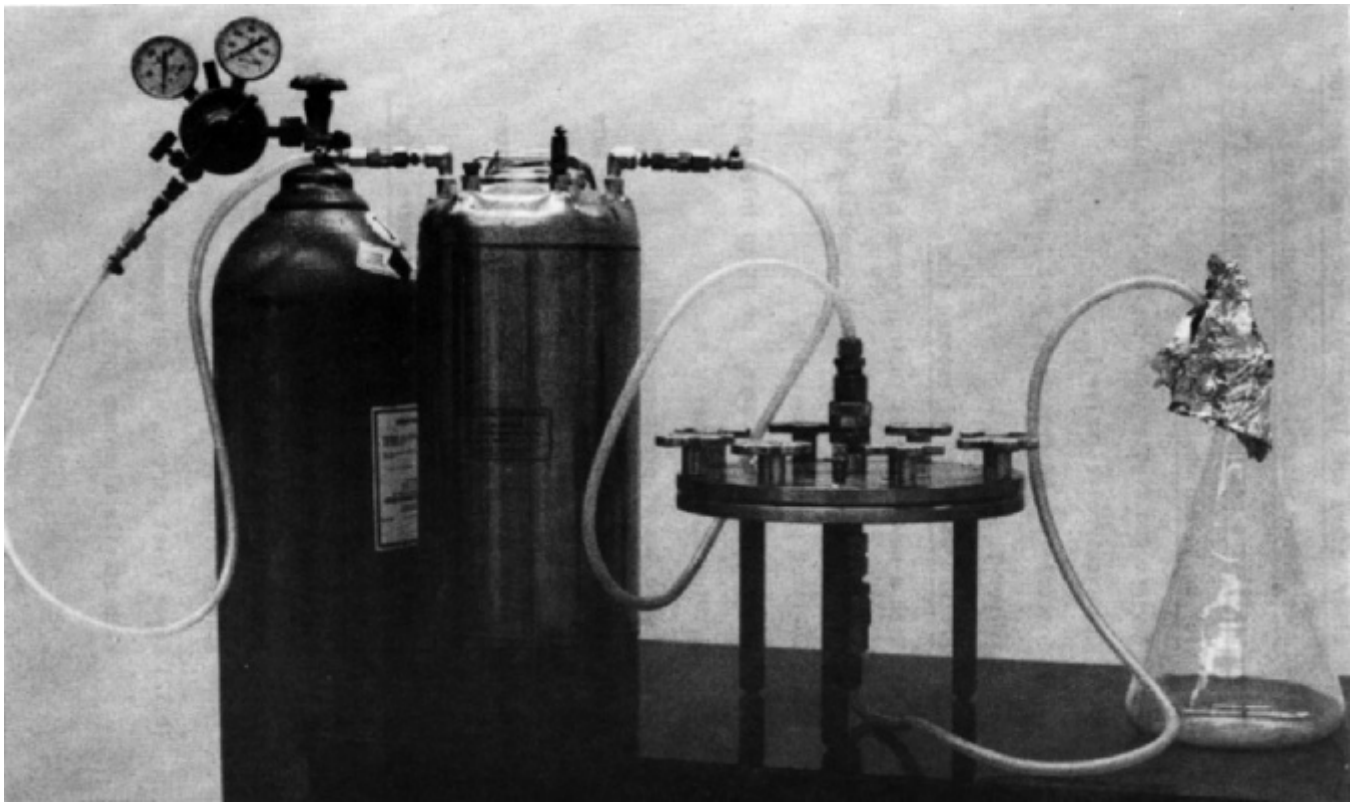
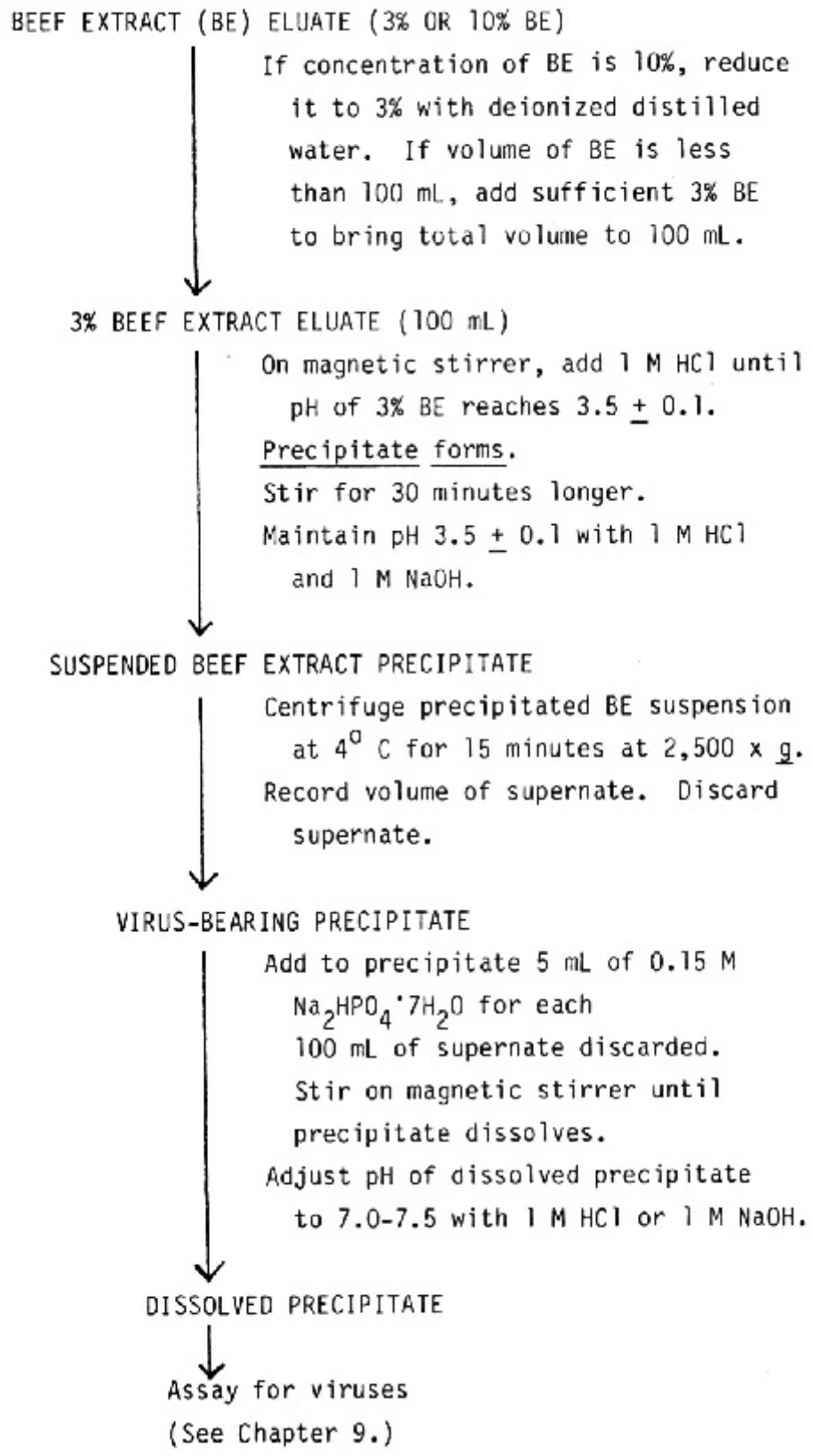


Figure 9. Flow Diagram of Reconcentration Procedure (Organic Flocculation Procedure of Katzenelson).



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