United States
Environmental Protection
Agency

Office of Research and
Development
Washington DC 20460

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ICR Microbial Laboratory Manual

PART 1 — SAMPLE COLLECTION PROCEDURE

APPARATUS AND MATERIALS

Several configurations are given below for the assembly of the filter apparatus. The standard filter apparatus will be used for all sampling, except where a prefilter, dechlorination or pH adjustment are required.

1. Standard filter apparatus (see **Figure VIII-1**).

   a. Parts needed (letters in bold print represent the origin of the abbreviations used to identify parts in the figures):

      i. One BR — **B**ackflow **R**egulator (Watts Regulator[1] Product Series 8 — ¾" Hose Connection Vacuum Breaker).

      ii. One SF — **S**wivel **F**emale insert with garden hose threads (United States Plastic Product No. 63003).

      iii. Three sections of BT — **B**raided **T**ubing, ½" clear (Cole-Parmer Product No. G-06401-03).

      iv. Six HC1 — **H**ose **C**lamps (Cole-Parmer Product No. G-06403-20).

      v. One HF1 — **H**ose **F**itting, nylon, ⅜" male NPT × ½" tubing ID (United States Plastic Product No. 61141).

      vi. One PR — **P**ressure **R**egulator (Watts Regulator Product No. ⅜" 26A (or 263A), Suffix B).

      vii. One PN — **P**VC **N**ipple, ⅜" male NPT (Ryan Herco Product No. 3861-057; not required with the 263A regulator).

      viii. One TE — PVC **TE**E with ⅜" female NPT ports (Ryan Herco Product No. 3805-003; not required with the 263A regulator).

      ix. One RB1 — **R**educing **B**ushing, ⅜" NPT(M) × ¼" NPT(F) (Cole-Parmer Product No. G-06349-32; not required with the 263A regulator).

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[1]See **Part 7** for addresses of the vendors listed. The vendors listed in this protocol represent one possible source for required products. Other vendors may supply the same or equivalent products.

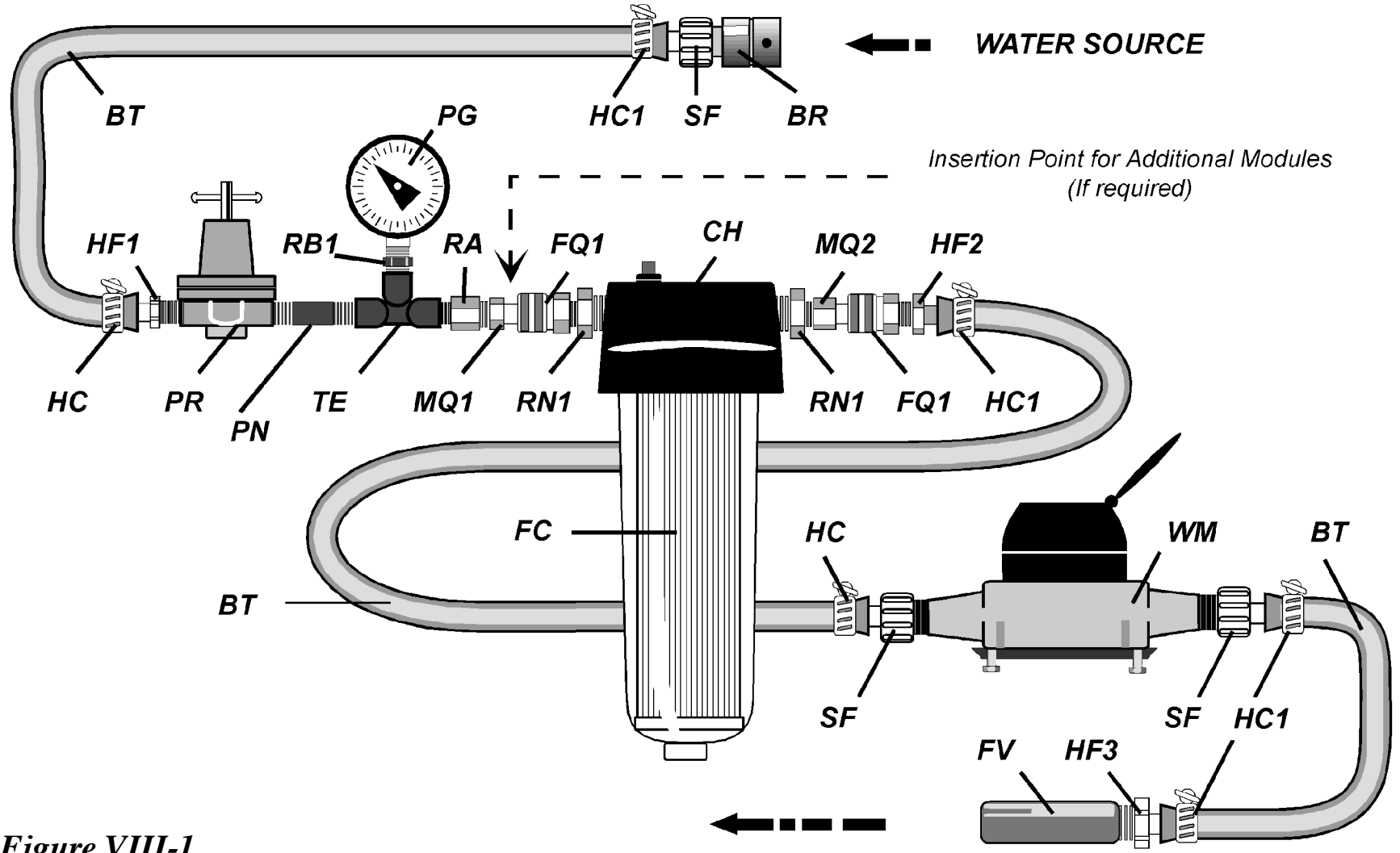


Figure VIII-1.
Standard Filter Apparatus

x. One PG — **P**ressure **G**auge 0-30 pound per square inch (PSI; Cole-Parmer Product No. G-68004-03; place in ¼" gauge port if using the 263A regulator).

xi. One RA — **R**educing **A**daptor, ½" female NPT × ⅜" male NPT (Cincinnati Valve and Fitting Product No. SS-8-RA-6).

xii. One MQ1 — **M**ale **Q**uick Connect, ½" male NPT (Cincinnati Valve and Fitting Product No. SS-QF8-S-8PM; appropriate hose fittings and braided tubing can be substituted for quick connects).

xiii. Two FQ1 — **F**emale **Q**uick Connects, ½" female NPT (Cincinnati Valve and Fitting Product No. SS-QF8-B-8PF).

xiv. Two RN1 — **R**educing **N**ipples, ¾" male NPT × ½" male NPT (Cole-Parmer Product No. G-06349-35).

xv. One CH — **C**artridge **H**ousing with wench (Cuno Product No. AP11T).

xvi. One FC — **F**ilter **C**artridge, positively charged 1MDS, ZetaPor Virosorb (Cuno Product No. 45144-01-1MDS).

xvii. One MQ2 — **M**ale **Q**uick Connect, ½" female NPT (Cincinnati Valve and Fitting Product No. SS-QF8-S-8PF).

xviii. One HF2 — **H**ose **F**itting, ½" male NPT × ½"tubing ID (United States Plastic Product No. 62142).

xix. One WM — **W**ater **M**eter (Neptune Equipment Product No. ⅝" Trident 10). The water meter should be used in a horizontal position and protected from freezing. The order should specify that the meters be rated in gallons (1 gal = 0.1337 ft^3 or 3.7854 L). If not specified, meters may be rated in cubic feet (1 ft^3 = 7.481 gal or 28.316 L).

xx. One HF3 — **H**ose **F**itting, nylon, ¾" male NPT × ½" tubing ID (United States Plastic Product No. 61143).

xxi. One FV — **F**low Control **V**alve (Plast-O-Matic Valves Product No. FC075B-3-PVC).

b. Apparatus assembly — the standard filter apparatus consists of three modules: the regulator module, the cartridge housing module and the discharge module.

Teflon tape (Cole-Parmer Product No. G-08782-27) must be used on all threaded, non-compression fittings. It is recommended that apparatus assembly be performed by the analytical laboratory contracted by the utility to analyze ICR samples for viruses).

i. Regulator module — in order, as shown in **Figure VIII-1**, connect the backflow regulator (BR) to a swivel female insert (SF). Clamp a piece of braided tubing (BT) onto the tubing connector of the swivel female insert using a hose clamp (HC1). Clamp the other end of the tubing to a ⅜ × ½" hose fitting (HF1). Screw the fitting into the inlet of the pressure regulator (PR). Connect the outlet of the pressure regulator to the PVC TEE (TE) via a PVC nipple (PN). Connect the pressure gauge (PG) to the top of the PVC TEE using the reducing bushing (RB). Attach a reducing adaptor (RA) to the remaining connection on the PVC TEE. Add a male quick connect (MQ1) to the reducing adaptor.

ii. Cartridge housing module — Attach a female quick connect (FQ1) to a reducing nipple (RN1). Connect the reducing nipple to the inlet of the cartridge housing (CH). Attach another reducing nipple to the outlet of the housing. Attach a male quick connect (MQ2) to the reducing adaptor.

iii. Discharge module — attach a female quick connect (FQ1) to a hose fitting (HF2). Connect a piece of braided tubing to the hose fitting with a hose clamp (HC1). Clamp the other end of the braided tubing to a swivel female insert with another hose clamp. Attach a swivel female insert to the inlet of the water meter (WM). Attach another swivel female insert to the outlet of the meter and connect a piece of braided tubing with a hose clamp. Clamp the other end of the tubing to a hose fitting (HF3) with a hose clamp. Screw the fitting into the inlet of the flow control valve (FV). An additional hose fitting (not shown) may be added to the flow control valve for the attachment of a sufficient length of tubing to reach a drain. The discharge module does not have to be sterilized.

iv. Connect the cartridge housing module to the regulator module at the quick connect. The combined regulator and cartridge housing modules should be sterilized with chlorine as described in **Part 5**. Presterilize a 1MDS filter cartridge (FC) as described in **Part 5** and place it into the cartridge housing using aseptic technique. Replace the housing head of the cartridge housing and tighten with a cartridge housing wench. Check to ensure that the filter is adequately sealed by shaking the housing. Adequately sealed filters should not move. For convenience during shipping, the regulator and cartridge housing modules may be separated. Seal all openings into the modules with sterile aluminum foil.

2. Prefilter module for waters exceeding 75 nephelometric turbidity units (NTU) and for any other conditions that prevent the minimum sampling volumes from being obtained (see **Figure VIII-2**).

a. Additional parts needed: One PC — 10 μm Polypropylene **P**refilter **C**artridge (Parker Hannifin Product No. M19R10-A); in addition, a female quick connect (FQ1), two reducing nipples (RN1), a cartridge housing (CH) and a male quick connect (MQ2) as described for the standard apparatus are needed.

b. Module assembly — in order, as shown for the prefilter module in **Figure VIII-2**, attach a female quick connect (FQ1) to a reducing nipple (RN1). Connect the reducing nipple to the inlet of the cartridge housing (CH). Attach another reducing nipple to the outlet of the housing. Attach a male quick connect (MQ2) to the reducing adaptor. Sterilize the unit with chlorine as described in **Part 5** and add a presterilized polypropylene prefilter cartridge using aseptic technique. Cover the ends with sterile aluminum foil. The prefilter module may be sent to the utility and stored in a clean location until needed.

3. Injector modules for source or finished water requiring pH reduction and for finished waters requiring dechlorination (see **Figure VIII-2**).

a. Additional parts needed:

i. Two FQ2 — **F**emale **Q**uick Connects, ½" male NPT (Cincinnati Valve and Fitting Product No. SS-QF8-B-8PM).

ii. Four ME — **M**ale **E**lbows, ⅜" male NPT (Cincinnati Valve and Fitting Product No. SS-6-ME).

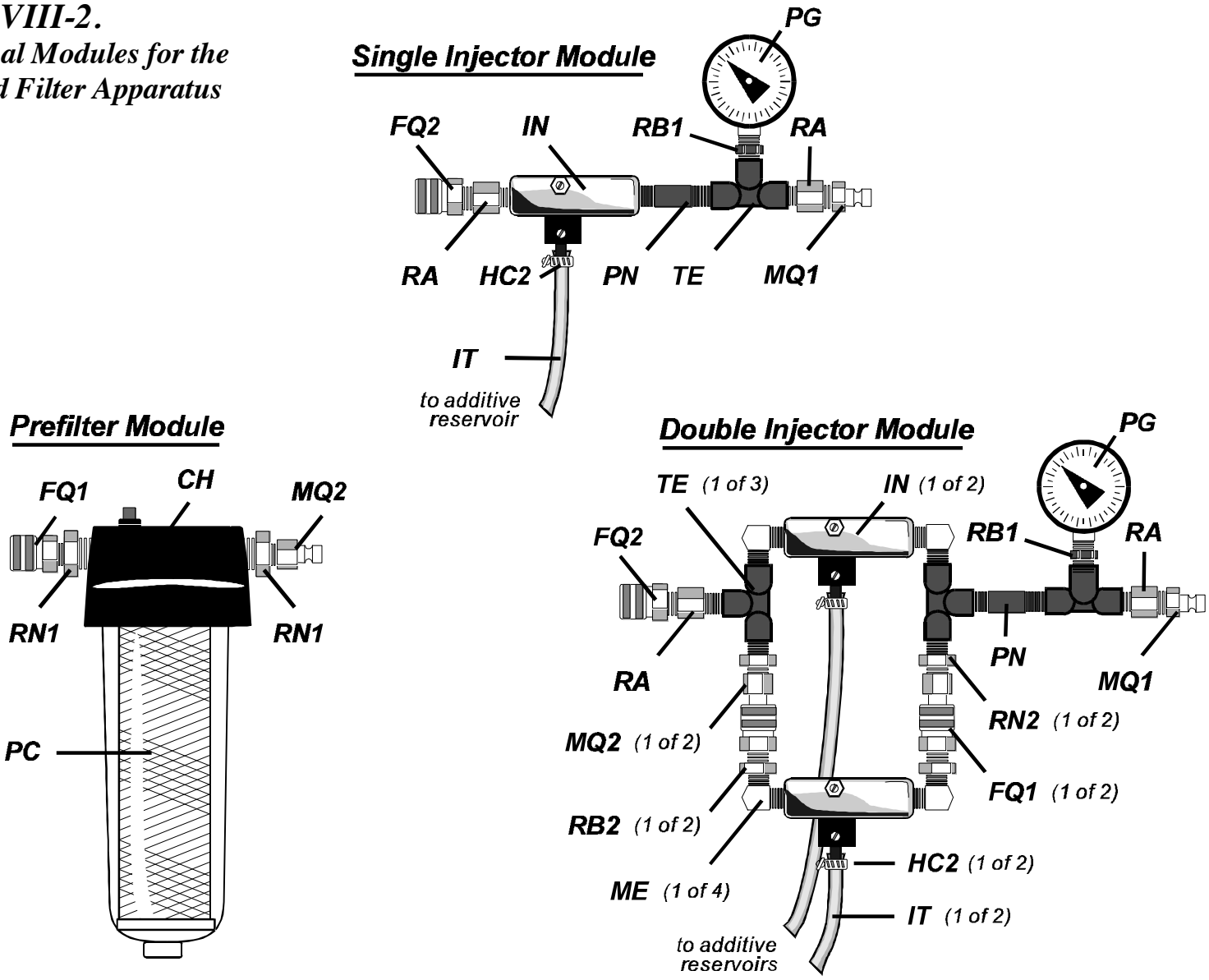
iii. Two RN2 — **R**educing **N**ipples, ⅜" male NPT × ½" male NPT (Cole-Parmer Product No. G-6349-85).

iv. Two RB2 — **R**educing **B**ushings, ⅜" female NPT × ½" male NPT (Cole-Parmer Product No. G-06349-34).

v. Three IN — In-line **IN**jectors (DEMA Engineering Product No. 203B ⅜" female NPT; a metering pump and appropriate connectors may be substituted for an injector).

vi. Three HC2 — **H**ose **C**lamps (Cole-Parmer Product No. G-06403-10).

Figure VIII-2.
Additional Modules for the Standard Filter Apparatus



vii. In addition, four reducing adaptors (RA), four PVC TEEs (TE), two PVC nipples (PN), two reducing bushings (RB1), two pressure gauges (PG), two female quick connects (FQ1), two male quick connects (MQ1) and two male quick connects (MQ2) as described for the standard apparatus are needed. Two union ball joints, ⅜" female NPT (not shown; Cincinnati Valve and Fitting Product No. SS-6-UBJ) and two PVC nipples may be used in place of the two reducing nipples (RN2), male quick connects (MQ2), female quick connects (FQ1) and reducing bushings (RB2) used with the double injector module.

b. Module assembly:

i. Single Injector Module — assemble the parts in order as shown for the single injector module in **Figure VIII-2**. Attach a female quick connect (FQ2) to a reducing adaptor (RA). Connect the adaptor to the inlet of the injector (IN). Connect the outlet of the injector to a PVC TEE (TE) via a PVC nipple (PN). Connect a pressure gauge (PG) to the top of the PVC TEE using a reducing bushing (RB1). Attach a reducing adaptor (RA) to the remaining connection on the PVC TEE. Add a male quick connect (MQ1) to the reducing adaptor.

ii. Double Injector Module — assemble the parts as shown for the double injector module in **Figure VIII-2**. Assemble the main portion by attaching a female quick connect (FQ2) to a reducing adaptor (RA). Connect the adaptor to the top connector of a PVC TEE (TE). Add a male elbow (ME) to one of the connections on the PVC TEE. Attach a reducing nipple (RN2) to the other connection. If using a union ball joint in place of the quick connects, attach a PVC nipple (not shown) to the other connection. Add a male quick connect (MQ2) to the reducing nipple or add one portion of a union ball joint (not shown) to the PVC nipple. Connect the inlet side of an injector (IN) to the male elbow. Attach another male elbow to the outlet of the injector. Connect the male elbow to another PVC TEE. Connect a reducing nipple (RN2 or PVC nipple) to the other end of the second PVC TEE. Add a male quick connect (MQ2) to the reducing nipple as above (or add one portion of the second union ball joint to the PVC nipple). Connect the top connector of the second PVC TEE to a third PVC TEE via a PVC nipple (PN). Connect a pressure gauge (PG) to the top of the third PVC TEE using a reducing bushing (RB1). Attach a reducing adaptor (RA) to the remaining connection on the third PVC TEE. Add a male quick connect (MQ1) to the reducing adaptor. Attach two male elbows (ME) to the inlet and outlet of a second injector (IN). Connect two reducing bushings (RB2) or, if used, the bottom portion or the two union ball joints (not shown) to the male elbows. Connect a female quick connect (FQ1) to each reducing bushing. Orient the second injector so that the direction of flow is the same as the first injector (the arrows on the injectors should both point towards the pressure gauge side of the assembly). Connect the two female quick connects to the male

quick connects of the main portion to complete the assembly or, if used, connect the two portions of the union ball joints.

iii. Sterilize the single and double modules with chlorine as described in **Part 5**. Cover the ends, including the injector port, with sterile aluminum foil. Sterilize the inside and outside surfaces of the **I**njector **T**ubing (IT; injector tubing is supplied with each injector). Place the tubing in a sterile bag or wrapping in such a way that the ends may be removed without contaminating them. The injector modules may be shipped to the utility and stored in a clean location until needed.

4. Portable pH probe (Omega Product No. PHH-1X)

5. Portable temperature probe (Omega Product No. HH110).

6. Commercial ice packs (Cole-Parmer Product No. L-06346-85).

7. One liter polypropylene wide-mouth bottles (Nalge Product No. 2104-0032).

8. Insulated shipping box with carrying strap (17" × 17" × 13"; Cole-Parmer Product No. L-03748-00 and L-03742-30).

9. Miscellaneous — aluminum foil, data card (see **Part 9**), hosecock clamp, surgical gloves, screwdriver or pliers for clamps, waterproof marker.

10. Chemical resistant pump capable of supplying 30 PSI at 3 gal/min and appropriate connectors (for use where garden hose-type pressurized taps for the source or finished water to be monitored are unavailable and for QC samples). Follow the manufacturer's recommendations for pump priming.

MEDIA AND REAGENTS

1. 2% sodium thiosulfate ($Na_2S_2O_3$) — dissolve 100 g of $Na_2S_2O_3$ in a total of 5000 mL dH_2O to prepare a stock solution. Autoclave for 30 min at 121°C.

2. Hydrochloric acid (HCl) — Prepare 0.1, 1 and 5 M solutions by mixing 50, 100 or 50 mL of concentrated HCl with 4950, 900 or 50 mL of dH_2O, respectively. Prepare solutions to be used for adjusting the pH of water samples at least 24 h before use.

PROCEDURE

Operators must wear surgical gloves and avoid conditions that can contaminate a sample with virus. Gloves should be changed after touching human skin or handling components that may be contaminated (e.g., water taps, other environmental surfaces).

Step 1. Purge the water tap to be sampled before connecting the filter apparatus. Continue the purging for 3-3 min or until any debris that has settled in the tap line has cleared. Then turn off the water tap.

Source water sampling must be conducted at the plant intake, before impoundment, chlorination or any other treatment. Finished water sampling must be conducted at the point of entry into the distribution system. If it is necessary to use a pump for sampling, sterilize the pump with chlorine as described in ***Part 5*** *or flush with 20 gal of water to be sampled before each use.*

Step 2. Remove the foil from the backflow regulator (see **Figure VIII-1**) on a regulator module. Loosen the swivel female insert slightly to allow it to turn freely and connect the backflow regulator to the tap. Retighten the swivel female insert. Disconnect the cartridge housing module at the quick connect following the pressure gauge (the insertion point shown in **Figure VIII-1**), if connected, and cover the open ends leading into the modules with sterile foil.

Step 3. Remove the foil from the ends of the discharge module and from the free end of the regulator module. Connect the discharge module to the regulator module. Place the control flow valve or tubing connected to the outlet of the flow control valve into a one liter plastic bottle. Note that the injector module, the prefilter module and the cartridge housing module must not be attached to the apparatus at this stage of the procedure!

Step 4. Slowly turn on the tap and adjust the pressure regulator until the pressure gauge on the regulator module reads 30 PSI. If the tap is incapable of 30 PSI, adjust the regulator to achieve the maximum pressure. Pressures less than 30 PSI will result in a reduced flow rate and thus longer sampling times. Flush the apparatus assembly with at least 20 gal of the water to be sampled. While the system is being flushed, measure the pH, the temperature and the turbidity on the water collecting in and overflowing from the one liter plastic bottle. Record the values onto the **Sample Data Sheet** (see **Part 9**).

The pH meter should be calibrated before each use for the pH range of the water to be sampled.

The turbidity reading may be taken from an in-line turbidimeter connected to the tap being used.

Step 5. If the sample has a pH above 8.0 **or** contains a disinfectant, turn off the water at the tap and disconnect the discharge module from the regulator module. Remove the foil from the

ends of a single injector module (see **Figure VIII-2**) and connect the module to the male quick connect of the regulator module. Reattach the discharge module.

Step 6. If the sample has a pH above 8.0 **and** contains a disinfectant, turn off the water at the tap and disconnect the discharge module from the regulator module. Remove the foil from the ends of a double injector module (see **Figure VIII-2**) and connect the module to the male quick connect of the regulator module. Reattach the discharge module.

Step 7. If an injector module has been added, remove the foil from the injector port(s) and attach the injector tubing to each port. Add a hosecock clamp to each injector tubing and tighten completely to prevent flow into the injector(s). Turn the fine metering adjustment screw on each injector (the smaller screw) clockwise as far as it will go to minimize the flow rate until the injectors are adjusted (note that the injectors were designed to have a minimum flow rate of 20-30 mL/min; thus completely closing the fine metering adjustment screw does not stop the flow). Place the other end of each tubing into the appropriate sterile graduated container containing 0.1 M HCl or 2% thiosulfate. Take care not to touch or contaminate the surfaces of the injector tubing that will be placed in the graduated containers. Slowly turn on the tap again and readjust the pressure regulator, if necessary.

Step 8. If a single injector module has been added, continue to flush the apparatus and adjust the water bypass screw on the injector (the larger adjustment screw) until the pressure gauge on the injector module is about 35% less than the pressure gauge on the regulator module (e.g., 19 PSI when the gauge on the regulator module reads 30 PSI; a minimum of a 35% pressure drop is required to achieve suction). Loosen the hosecock clamp and observe whether suction is occurring. If not, slowly increase the pressure drop until suction starts.

a. If the pH value of the water sample is greater than 8.0, ensure that the injector tubing is placed into a graduated container containing 0.1 M HCl. While continuing to measure the pH in the one liter plastic bottle, adjust the fine metering adjustment screw on the injector to add sufficient HCl to give a pH of 6.5 to 7.5. It may be necessary to use the hosecock clamp to reduce the flow rate to less than 20-30 mL/min or to use a more dilute or concentrated HCl solution with some water samples. When the pH stabilizes at a pH of 6.5 to 7.5, continue with Step 10. Record the adjusted pH onto the **Sample Data Sheet**.

b. If the water to be sampled contains a disinfectant, ensure that the injector tubing is placed into a graduated container containing 2% thiosulfate. Adjust the fine metering adjustment screw on the injector to add thiosulfate at a rate of 10 mL/gal (2.6 mL/L or 30 mL/min at a flow rate of 3 gal/min; note that at this rate, approximately 3-4 L of thiosulfate solution will be required per sample). When the proper rate is achieved, record the addition of thiosulfate on the **Sample Data Sheet** and continue with Step 10.

Step 9. If a double injector module is being used, continue to flush the apparatus and turn the water bypass screws on each injector clockwise as far as possible. Then turn the water

bypass screws on each regulator one half turn counter clockwise. Continue turning the screws evenly one half turn counter clockwise until the pressure gauge on the double injector module is 35% less than the pressure gauge on the regulator module. Ensure that the tubing from one injector is placed into a graduated container containing 0.1 M HCl and the other into a graduated container containing 2% sodium thiosulfate. Loosen the hosecock clamps. Since there may be slight differences between the injectors and since the pressure reading after the injectors reflects an average pressure drop from both injectors, some additional adjustment of the water bypass screws may be required to obtain suction on each injector. After confirming that each injector is drawing fluid, adjust the flow of HCl and thiosulfate as in Step 8a-8b above. Record the final pH and the addition of thiosulfate on the **Sample Data Sheet** and continue with Step 10.

Step 10. After adjusting the injectors, if required, and flushing the system with at least 20 gal, turn off the flow of water at the sample tap and remove the discharge module. If the water sample has a turbidity greater than 75 NTU, remove the foil from each end of the prefilter module and connect the prefilter module (see **Figure VIII-2**) to the end of the regulator module or to the end of one of the injector modules, if used. Remove the foil from the cartridge housing module and connect it to the end of the regulator module, or to the end of the injector module or the prefilter module, if used. Connect the discharge module to the cartridge housing module.

Step 11. Record the sample number, location, date, time of day and initial gallon (or cubic feet) reading from the water meter onto the **Sample Data Sheet**.

Use the unique utility-specific sample numbers assigned by the ICR Joint Application Design database.

Step 12. Slowly turn on the water with the filter housing placed in an upright position, while pushing the red vent button on top of the filter housing to expel air. When the air is totally expelled from the housing, release the button, and open the sample tap completely. Readjust to 30 PSI, if necessary. Check the thiosulfate usage rate or the pH of the discharged water if an injector(s) is being used and readjust, if necessary.

Step 13. Sample a minimum volume for source water of 200 L (7.1 ft^3, 52.8 gal) and for finished water of 1500 L (53.0 ft^3, 396.3 gal). Samples for source and finished waters must not exceed 300 L (10.6 ft^3, 79.3 gal) and 1800 L (63.6 ft^3, 475.5 gal), respectively. For source water the total amount of sample that can be passed through a filter will depend upon water quality, however, it should be possible to obtain the minimum volume using the procedures described above.

Samples should be monitored periodically during the sampling. If the filter clogs, contact the approved analyst for further instructions. Since the flow rate may change during sampling due to filter clogging, thiosulfate addition and the adjusted pH of the sample must be checked regularly.

Step 14. Turn off the flow of water at the sample tap at the end of the sampling period and record the date, time of day, and final gallon (or cubic feet) reading from the water meter onto the **Sample Data Sheet**. Although the final water meter reading may be affected by the addition of HCl and/or thiosulfate, the effect is considered insignificant and may be ignored.

Step 15. Loosen the swivel female insert on the regulator module and disconnect the backflow regulator from the tap. Disconnect the cartridge housing module and the prefilter housing module, if used from the other modules. Turn the filter housing(s) upside down and allow excess water to flow out as waste water. Turn the housing(s) upright and cover the quick connects on each end of the modules with sterile aluminum foil.

Step 16. Pack the cartridge housing module(s) into an insulated shipping box. Add 6-8 small ice packs (prefrozen at -20°C) around the cartridge housings to keep the sample cool in transit (the number of ice packs may have to be adjusted based upon experience to ensure that the samples remain cold, but not frozen). Drain and add the regulator and injector modules used. Place the **Sample Data Sheet** (protected with a closable plastic bag) in with the sample and ship by overnight courier to the contracted, approved laboratory for virus analysis. Notify the laboratory by phone upon the shipment of sample.

*The approved laboratory will elute virus from the 1MDS filter (and prefilter, if appropriate) and analyze the eluates as described in **Parts 2-3**. After removing the filter, the laboratory will clean, sterilize the apparatus components with chlorine and dechlorinate with sodium thiosulfate as described in **Part 5**. After flushing with sterile dH_2O, a new 1MDS cartridge (and prefilter, if appropriate) will be added, the openings sealed with sterile aluminum foil, and the apparatus returned to the utility for the next sample. The discharge module can be stored at the utility between samplings. Openings should be covered with aluminum foil during storage.*