United States
Environmental
Protection Agency

Office of Ground Water
and Drinking Water
Washington, DC 20460

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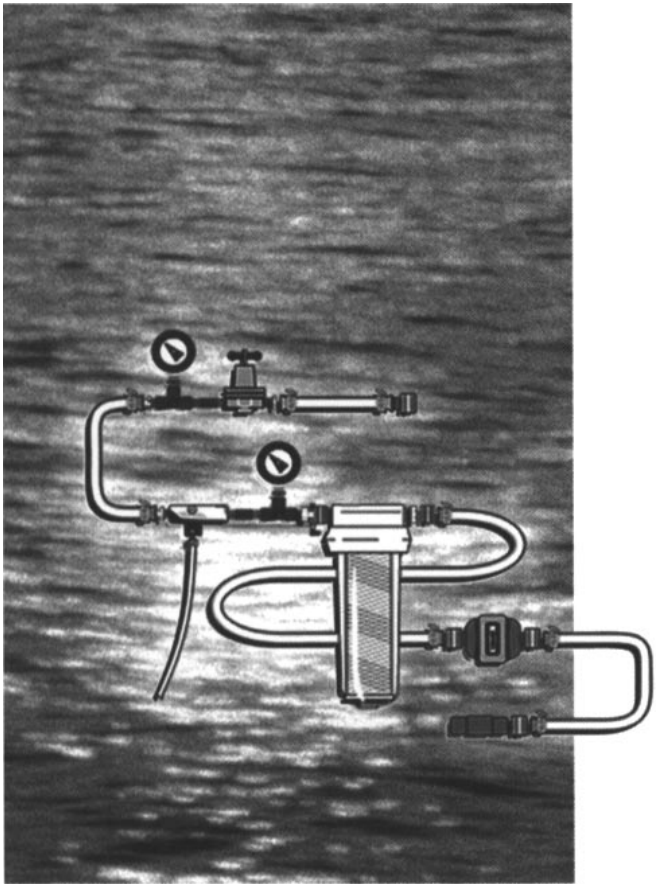
Information Collection Requirements Rule— Protozoa and Enteric Virus Sample Collection Procedures



About This Manual

This manual is designed to be brought into the field by drinking water utility personnel when collecting source and finished water samples for protozoa and viruses. The sample collection steps in this manual are consistent with those demonstrated in the accompanying video. To further associate the steps in this manual with the sampling demonstration on the video, the photos for each step are taken directly from the video.

Several graphic conventions are used throughout the manual to differentiate steps or denote special actions:

A step icon is used at the beginning of each step. These steps are parallel to those in the accompanying video.

Actions denoted by this icon are critical to ensuring that the sample will be valid and uncontaminated, such as putting on fresh latex gloves before handling the filter.

Text denoted by this icon provides additional information to the samplers, but may not be part of the actual collection procedure.

Collecting protozoan and virus samples correctly under the Information Collection Requirements Rule can be challenging. Please watch the demonstration video before collecting the samples, and be sure to follow each step in this manual when in the field.

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Protozoan and Enteric Virus Sample Collection Procedures as Defined by the Information Collection Requirements Rule

This manual describes the procedures for collecting source water and finished water samples for protozoan and enteric virus monitoring under the Information Collection Requirements (ICR) rule. This manual and the accompanying video comprise a two-part set of instructional materials that provide public water supply systems with the information needed to properly collect samples for protozoan and virus monitoring. All water utility personnel involved with ICR monitoring should watch the video and review this manual before collecting any samples.

The protozoan collection procedures described in this manual and in the video are based on the procedures in the ICR Protozoan Method for Detecting *Giardia* cysts and *Cryptosporidium* Oocysts in Water by a Fluorescent Antibody Procedure. The total culturable virus collection procedures described in this manual and in the video are based on the procedures in the Virus Monitoring Protocol for the Information Collection Rule. Both of these methods can be requested by calling the Safe Drinking Water Hotline, at (800) 426-4791.

Questions Commonly Asked by Drinking Water Utilities

What is the purpose of the ICR rule?

The ICR rule was developed by EPA to collect occurrence, exposure, and treatment data on drinking water pathogens and disinfectant by-products. The pathogen data are needed to determine whether current Surface Water Treatment Regulations should be revised to include new or more stringent treatment levels for some microbes. The disinfectant by-product data are needed to determine whether to regulate the chemical by-products that form when disinfectants react with organic chemicals in source water.

Although drinking water utilities will be involved in collecting both disinfectant by-product and waterborne pathogen data under the ICR rule, this manual describes the utility's role in collecting data on drinking water pathogen occurrence.

What pathogens are monitored under the ICR rule?

The ICR rule requires public water supply systems to monitor source water (and finished water in some cases) for the following pathogens:

- *Giardia* cysts
- *Cryptosporidium* oocysts
- Total culturable viruses
- Fecal coliform or *Escherichia coli* bacteria
- Total coliform bacteria

EPA is considering revising the current Surface Water Treatment Regulations because existing treatment levels for *Giardia* and viruses

may not be adequate to protect public health for systems supplied by poor-quality source water and because of the new threat posed by *Cryptosporidium*.

Giardia cysts in drinking water cause more reported waterborne disease outbreaks than any other single known pathogen. They also are more resistant to environmental stresses and disinfection than almost all other known waterborne pathogens.

Cryptosporidium oocysts in drinking water have caused major waterborne disease outbreaks in the U.S. and other countries and are even more resistant to disinfection than *Giardia*.

Several enteric viruses have caused waterborne disease and may be responsible for many, if not most, of the outbreaks where a causative agent was not identified (about half of all reported outbreaks). Adequate analytical methodology is not yet available for routine analysis for many enteric viruses, so EPA has required monitoring of total culturable viruses. Total culturable viruses are a group of enteric viruses commonly found in poor-quality waters and which EPA believes are at least somewhat representative of other pathogenic viruses. Monitoring for total culturable viruses is useful because this group contains pathogens and is a potential indicator of other viral pathogens.

Fecal coliforms, *E. coli*, and total coliforms have been used for decades to assess source water quality. Coliform bacteria are much more sus-

ceptible to environmental stress and disinfection than protozoa and viruses, and would be eliminated by any system that eliminated more resistant pathogens. However, the ICR rule requires drinking water utilities to submit coliform monitoring data as general indicators of water quality. Monitoring procedures for fecal coliform, *E. coli*, and total coliform densities have been established and are not addressed by this manual.

Which drinking water utilities have to collect protozoan and virus samples?

Public water supply systems that serve between 10,000 and 100,000 people and use surface water (or groundwater under the influence of surface water) are required to monitor their source water for *Giardia* cysts and *Cryptosporidium* oocysts.

Public water supply systems that serve more than 100,000 people and use surface water (or groundwater under the influence of surface water) are required to monitor their source water for *Giardia* cysts, *Cryptosporidium* oocysts, and total culturable viruses. If pathogen densities in the source water exceed 1 pathogen per liter during the first 12 months of monitoring, then public water supply systems also must sample finished water for the remaining months.

How often must samples be taken?

Public water supply systems that serve between 10,000 and 100,000 people must collect samples every two months for 12 months.

Systems that serve more than 100,000 people must take samples every month for 18 months.

However, these systems may discontinue monitoring if:

- Viruses are not detected in the source water during the first 12 months of monitoring, or
- Source water has been tested for either total coliforms or fecal coliforms at least five times per week for four months before and two months after the effective date of the ICR and the total coliform density is less than 100 colonies/100 mL or the fecal coliform density in 90 percent of all samples is less than 20 colonies/100 mL.

Where should samples be collected?

Samples must be taken at the intake of each treatment plant. If a plant has several sources of water, the system must sample the blended water from all sources. If this is not possible, the source with the highest expected pathogen concentration should be sampled.

Who will analyze the samples?

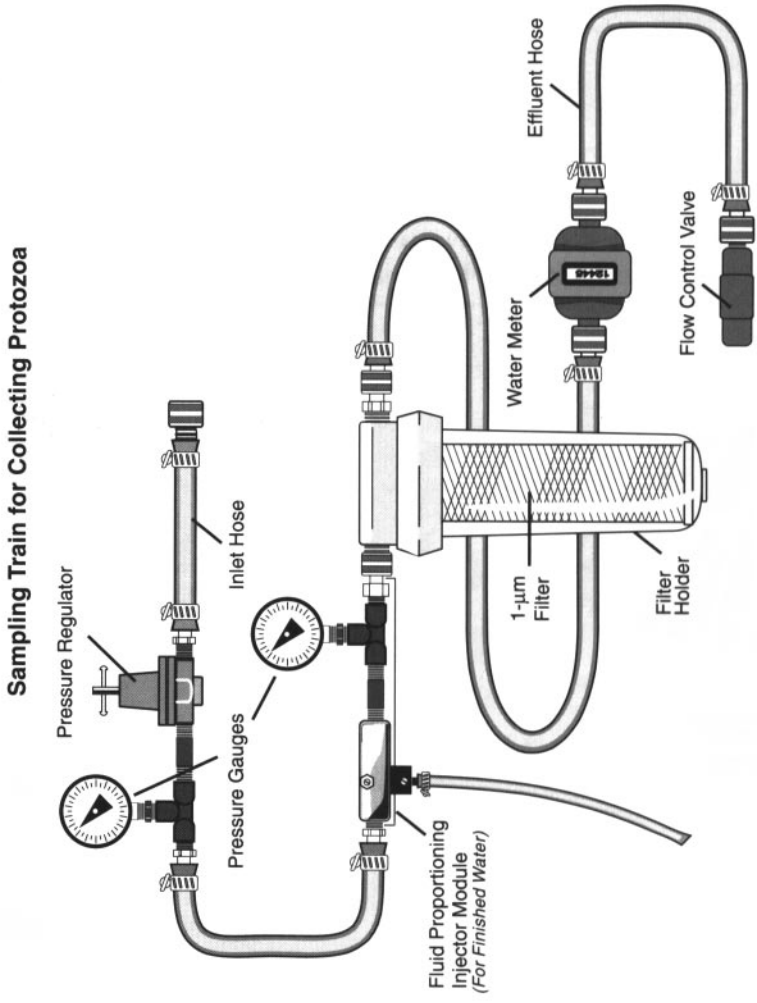
EPA has approved several laboratories to analyze the protozoan and virus samples. Before collecting samples, you must arrange to have them analyzed by an EPA-approved laboratory.

If you have not already located an approved laboratory, notify:

ICR Laboratory Coordinator
EPA Office of Ground Water & Drinking Water
26 West Martin Luther King Drive
Cincinnati, Ohio 45268.

EPA will provide you with a list of approved laboratories or other appropriate guidance.

Sample Collection Procedures for Detecting Protozoa in Water


Sampling Train for Collecting Protozoa
Pressure Regulator
Inlet Hose
Pressure Gauges
Fluid Proportioning Injector Module
(For Finished Water)
1-µm Filter
Filter Holder
Water Meter
Effluent Hose
Flow Control Valve

STEP 1 Each month, your laboratory will send you all of the equipment needed to collect samples for *Giardia* cyst and *Cryptosporidium* oocyst analyses. When you receive the sampling kit, check the contents of the carton. The sampling kit should contain the following items:

- ❑ Sampling train for collecting protozoa (left):
  - Inlet hose
  - Pressure regulator with pressure gauge
  - Fluid proportioning injector module, including an injector and pressure gauge*
  - 1-µm nominal porosity filter and holder made by Parker Hannifan or Filterite
  - Water meter
  - Effluent hose and flow control valve

*Needed for finished water sample collection only

- ❑ Plastic sample bags
- ❑ Ice packs for shipping the collected samples
- ❑ Sample labels

If you are missing any items, contact your laboratory immediately. Do not attempt to collect the samples without a complete sampling kit.

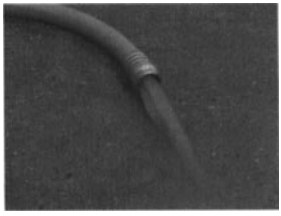
STEP 2 Once you have verified the contents of the sampling kit, place the ice packs in the freezer and repack the box for later use.

COLLECTING SOURCE WATER SAMPLES

When you are ready to collect your protozoa sample, bring the following items with you to the sampling location:

- ❑ Shipping container sent by the laboratory
- ❑ Sampling apparatus
- ❑ Plastic sample bags
- ❑ Sample labels
- ❑ Frozen ice packs
- ❑ Several pairs of new latex gloves
- ❑ pH meter
- ❑ Thermometer
- ❑ Turbidimeter

! If you will be collecting samples from both source water and finished water on the same day, perform the finished water sampling first. Using the sampling apparatus on source water first may cause false positives for finished water sample analyses.

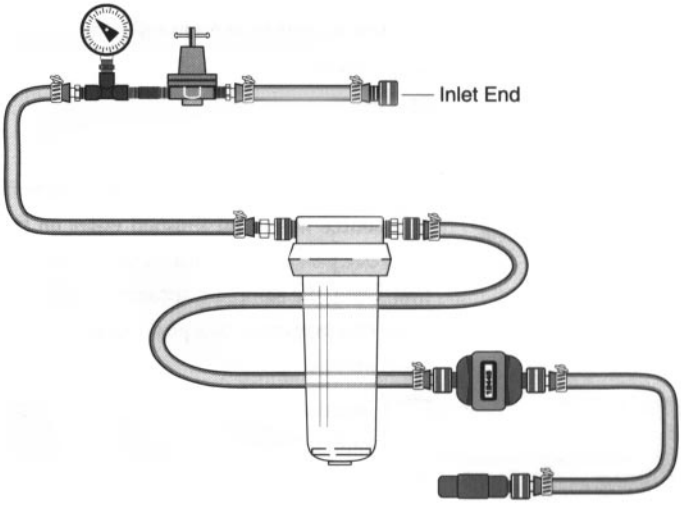


3 Turn on the water at the tap and allow the water to flow for 2 to 3 minutes or until any debris that has accumulated in the sampling line has cleared or the turbidity in the water becomes uniform.

Turn off the water at the tap.

4 Put on new latex gloves to prevent contamination from outside sources. Sterile technique must be used when sampling for *Giardia* and *Cryptosporidium*. Any contamination of the sampling apparatus may bias the final results.

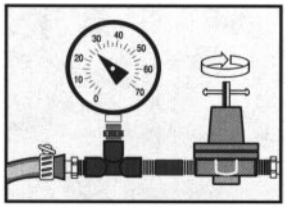
Assemble the sampling apparatus as shown below and connect the inlet end of the sampling apparatus to the sampling tap or to an extension hose connected to the tap.



! Be sure that the filter housing does *not* contain the filter.

Note the water meter reading, then slowly turn on the water.

5 Using the pressure regulator, adjust the water pressure to no more than 30 psi. Flush the sampling apparatus with 20 gallons/ 76 liters of water by allowing the water to flow through the system and out the effluent hose.



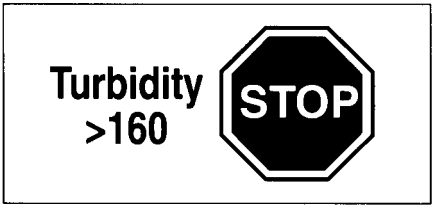
Sampling Step	Volume In GALLONS	Volume In LITERS	Volume In FT^3
System Flush	20	76	2.7

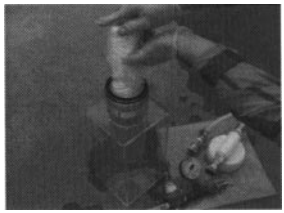
While the water is flushing the sampling apparatus, begin completing your sample label. Record the following information:

- Sampler's name
- Date
- Sample location

Stop Time:_____ Meter Reading:_____ Turbidity:_____
Start Time:_____ Meter Reading:_____ Turbidity:_____
Operator Name:_____ Total Volume Filtered:_____
Date:_____ Sampling Location:_____

6 Measure the turbidity of the source water flowing from the effluent hose. Record the readings on the sample label. If the turbidity is greater than 160 Nephelometric Turbidity Units (NTU), sampling should be rescheduled for a day when the turbidity is lower.





STEP 7 After the system has been flushed with 20 gallons / 76 liters of water, turn off the tap and disconnect the inlet and outlet hoses from the filter housing.

Using the filter wrench, open and drain the filter housing.

Open the filter packaging as aseptically as possible and carefully drop the filter into the filter housing.

! Be sure to hold the loose gasket in place using aseptic technique.

Reassemble the filter housing, and reconnect the inlet and outlet hoses. Place the filter housing in an upright position.

Slowly, turn on the tap and start the water flowing through the sampling apparatus.

Using the pressure regulator, adjust the pressure to no more than 30 psi.

Record the following information on the sample label:

- Time sampling started
- Initial water meter reading (including units)
- Turbidity

Stop Time: _____	Meter Reading: _____	Turbidity: _____
Start Time: _____	Meter Reading: _____	Turbidity: _____
Operator Name: _____	Total Volume Filtered: _____	
Date: _____	Sampling Location: _____	

Monitor the water meter to ensure that the flow rate does not exceed 1 gallon/min (approximately 4 liters/min).

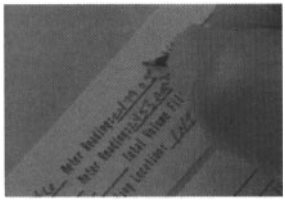
8 Allow at least 26 gallons/100 liters of water to pass through the filter. At a flow rate of approximately 1 gallon/minute, this will require about 30 minutes.

Sampling Step	Volume In GALLONS	Volume In LITERS	Volume In FT^3
Protozoa Flow Rate	1 per minute	4 per minute	.13 per minute
Protozoa Source Water Sample	26	100	3.5

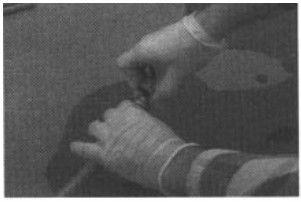
9 When the water meter indicates that 26 gallons/100 liters of water have passed through the filter, turn off the water at the tap.

Record the following information on the sample label:

- Time sampling stopped
- Final water meter reading (including units)
- Final turbidity
- Total volume filtered



Stop Time: _____ Meter Reading: _____ Turbidity: _____
Start Time: _____ Meter Reading: _____ Turbidity: _____
Operator Name: _____ Total Volume Filtered: _____
Date: _____ Sampling Location: _____

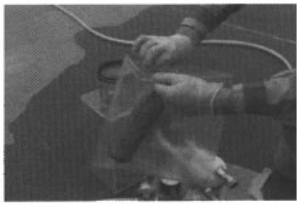


STEP 10 Disconnect the sampling apparatus from the water tap.

! Be sure to hold the inlet hose *above the level of the outlet hose opening* while the water drains from the housing. This will prevent backwash and loss of particulate matter from the filter.

Disconnect the inlet and outlet hoses from the filter housing.

! Put on fresh latex gloves.



STEP 11 As aseptically as possible, remove the filter from the housing and put it into a plastic sample bag.

STEP 12 Pour all of the water remaining in the filter housing into the same plastic bag.

STEP 13 Seal the plastic sample bag and place it inside the second plastic sample bag. Transfer the label or label information to the outside of the outer bag.

14 Put the bags containing the filter into the shipping container. Place the ice packs around, but not on, the sample bag to prevent freezing the sample. You may want to insert several inflated, empty sample bags between the sample and the ice packs.

15 Seal the container and follow the laboratory's instructions related to the cleaning, storage, and return of sampling equipment.

16 Ship the container by overnight courier to the laboratory. Call the laboratory and notify them of the sample shipment.

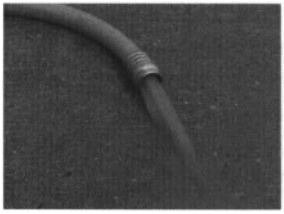
COLLECTING FINISHED WATER SAMPLES

If *Giardia* or *Cryptosporidium* concentrations in your source water samples exceed 1 per liter during the first 12 months of sampling, then you must monitor finished water as well as source water. If you are required to collect samples from both, collect the finished water sample first, then the source water sample.

Receiving and verifying the contents of your sampling kit are addressed in **STEPS 1** and **2** of the source water sampling section.

When you are ready to collect your finished water protozoa sample, bring the following items with you to the sampling location:

- ❑ Shipping container sent by the laboratory
- ❑ Sampling apparatus
- ❑ Fluid proportioning injector (for adding 2% thiosulfate solution to neutralize effects of chlorination or other disinfectant treatments)
- ❑ Plastic sample bags
- ❑ Sample labels
- ❑ Frozen ice packs
- ❑ Several pairs of new latex gloves
- ❑ Approximately 2 gal (4 L) of 2% sodium thiosulfate solution
- ❑ Sterile, 250- or 500-mL graduated cylinder
- ❑ Thermometer

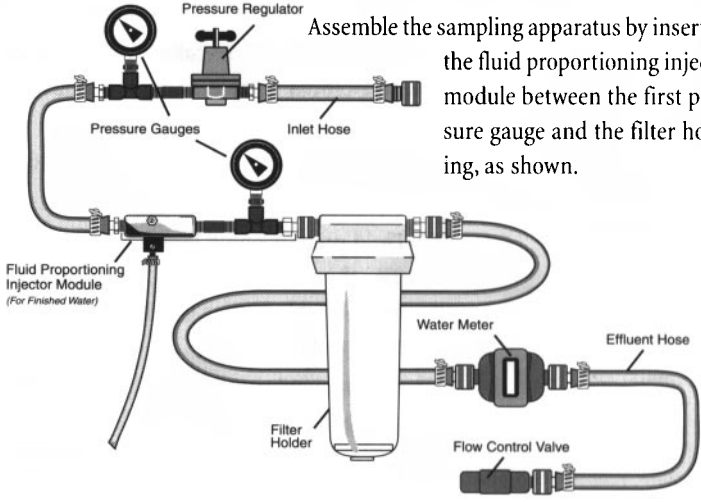


STEP 3 Turn on the water at the tap and allow the water to flow for 2 to 3 minutes or until any debris that has accumulated in the sampling line has cleared or the turbidity in the water becomes uniform.

Turn the water off at the tap

STEP 4 Put on new latex gloves to prevent contamination from outside sources. Sterile technique must be used when sampling for *Giardia* and *Cryptosporidium*. Any contamination of the sampling apparatus may bias the final results.

Assemble the sampling apparatus by inserting the fluid proportioning injector module between the first pressure gauge and the filter housing, as shown.

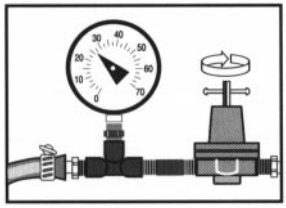


Connect the inlet end of the sampling apparatus to the sampling tap or to an extension hose connected to the tap.

Be sure that the filter housing does NOT contain the filter.

Note the water meter reading, then slowly turn on the water.

5 Using the pressure regulator, adjust the water pressure on the first pressure gauge to no more than 30 psi.



Flush the sampling apparatus with 20 gallons/ 76 liters of water by allowing the water to flow through the system and out the effluent hose.

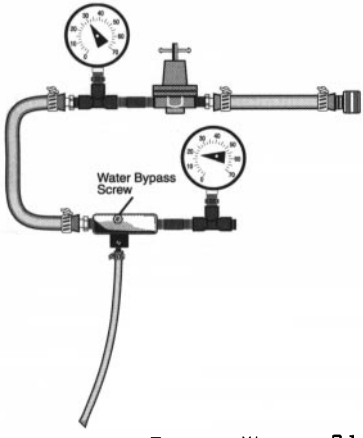
While the water is flushing the sampling apparatus, begin completing your sample label. Record the following information:

- Sampler's name
- Date
- Sample location

Stop Time: _____	Meter Reading: _____	Turbidity: _____
Start Time: _____	Meter Reading: _____	Turbidity: _____
Operator Name: _____	Total Volume Filtered: _____	
Date: _____	Sampling Location: _____	

6 Now, you must adjust the thiosulf injector.

First, using the water bypass screw, the larger top screw in the injector, adjust the pressure on the downstream pressure gauge to be at least 35% less than the pressure shown on the upstream gauge. For ample, if the upstream gauge reads 30 then the second gauge should read no m than 19psi.



Pour the 2% sodium thiosulfate solution into a graduated cylinder. Place the injector tube in the thiosulfate solution, and adjust the smaller injector screw, located on the bottom of the injector, so that the flow rate of the 2% thiosulfate solution is approximately 10 milliliters per minute.

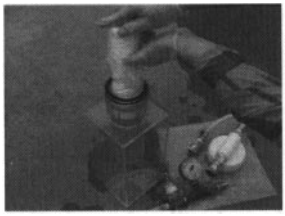
! If there is no suction visibly drawing down the thiosulfate solution, or if too much is flowing, adjust the water bypass screw further to increase or decrease the pressure differential between the two gauges. A greater differential between the upstream and downstream gauges increases the flow rate; a smaller differential decreases the flow rate.

After the thiosulfate flow rate is adjusted properly, transfer the injector tube to a carboy of thiosulfate. You will need to monitor this rate visually throughout sampling to ensure that an adequate amount of thiosulfate is being added to neutralize all of the disinfectants.

Turn off the water at the tap and empty the water in the filter housing.

7 Open the filter packaging as aseptically as possible and carefully drop the filter into the filter housing.

! Hold the loose gasket in place.



Reassemble the filter housing, and reconnect the inlet and outlet hoses.

Slowly, start the water flowing through the sampling apparatus.

Using the pressure regulator, adjust the pressure on the upstream pressure gauge to no more than 30 psi. Using the water bypass screw, readjust the downstream pressure gauge to read 35% less than the upstream gauge, if necessary.

Record the following information on the sample label:

- Time sampling started
- Initial water meter reading (including units)
- Turbidity

Stop Time: _____	Meter Reading: _____	Turbidity: _____
Start Time: _____	Meter Reading: _____	Turbidity: _____
Operator Name: _____	Total Volume Filtered: _____	
Date: _____	Sampling Location: _____	

Place the filter housing in an upright position.

Monitor the water meter to ensure that the flow rate does not exceed 1 gallon/min (approximately 4 liters/min).

STEP 8 Allow at least 264 gallons/1000 liters of water to pass through the filter. At a flow rate of approximately 1 gallon/minute, this will require about 4 hours and 45 minutes.

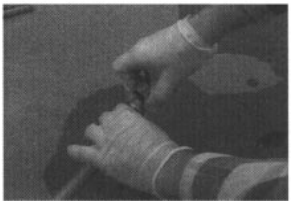
Sampling Step	Volume In GALLONS	Volume In LITERS	Volume In FT^3
Protozoa Flow Rate	1 per minute	4 per minute	.13 per minute
Protozoa Finished Water Sample	264	1000	36

STEP 9 When the water meter indicates that 264 gallons/1000 liters of water have passed through the filter, turn off the water at the tap.

Record the following information on the sample label:

- Time sampling stopped
- Final water meter reading (including units)
- Final turbidity
- Total volume filtered

Stop Time:_____ Meter Reading:_____ Turbidity:_____
Start Time:_____ Meter Reading:_____ Turbidity:_____
Operator Name:_____ Total Volume Filtered:_____
Date:_____ Sampling Location:_____



STEP 10 Disconnect the sampling apparatus from the water tap.

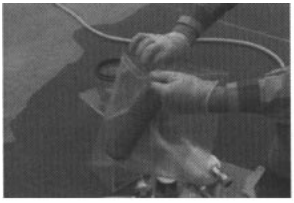
! Be sure to hold the inlet hose *above the level of the outlet hose opening* while the water drains from the housing. This will pre-

vent backwash and loss of particulate matter from the filter.

Disconnect the inlet and outlet hoses from the filter housing.

Put on fresh latex gloves.

11 As aseptically as possible, remove the filter from the housing and put it into a plastic sample bag.



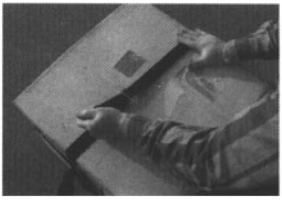
12 Pour all of the water remaining in the filter housing into the same plastic bag.

13 Seal the plastic sample bag and place it inside the second plastic sample bag.

Transfer the label or label information to the outside of the outer bag.

14 Put the bags containing the filter into the shipping container.

Place the ice packs around, but not on, the sample bag to prevent freezing the sample. You may want to insert several inflated, empty sample bags between the sample and the ice packs.



15 Seal the container and follow the laboratory's instructions related to the cleaning, storage, and return of sampling equipment.

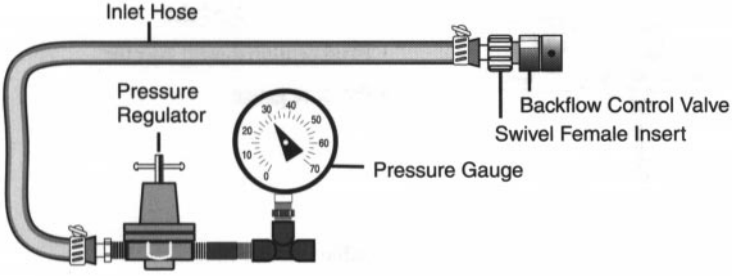
16 Ship the container by overnight courier to the laboratory.

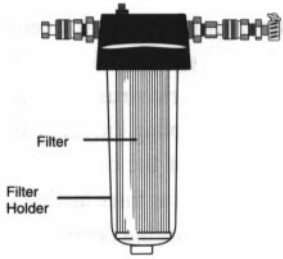
Call the laboratory and notify them of the sample shipment.

Sample Collection Procedures for Detecting Enteric Viruses in Water

STEP 1 Each month, your laboratory will send you all of the equipment needed to collect samples for enteric virus analyses. When you receive the sampling kit, immediately check the contents of the carton. The sampling kit will be shipped as three modules, and should contain the following items:

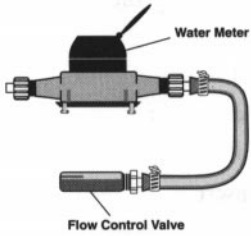
- ❑ Plastic sample bags
- ❑ Ice packs for shipping the collected samples
- ❑ Sample data sheet
- ❑ Regulator Module (below):
  - Backflow control valve
  - Swivel female insert
  - Inlet hose
  - Pressure regulator with pressure gauge





❑ Cartridge Housing Module:

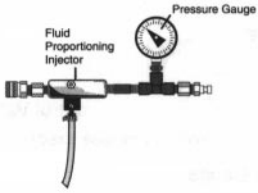
- 1-MDS Zetapor Virosorb filter inside a filter holder



❑ Discharge Module:

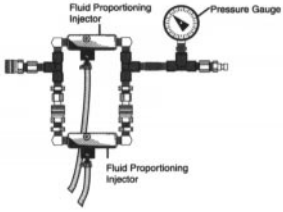
- Water meter
- Flow control valve

The laboratory will also ship three additional modular sections, as required by your facility. These may include:



❑ Single Injector Module:

- Fluid proportioning injector
- Pressure gauge

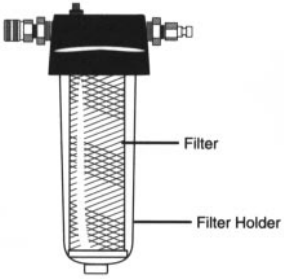


❑ Double Injector Module:

- Two fluid proportioning injectors, in parallel
- Pressure gauge

❑ Prefilter Module:

- 10 µm polypropylene filter inside a filter holder



! The ends of each module should be wrapped in foil to ensure that the equipment remains free of contamination. If your modules are unprotected or compromised, please contact your laboratory immediately for further instructions.

If you are missing any items, contact your laboratory immediately. Do not attempt to collect the samples without a complete sampling kit.

Once you have verified the contents of the sampling kit, place the ice packs in the freezer and repack the box.

COLLECTING SOURCE WATER SAMPLES

When you are ready to collect your virus sample, bring the following items with you to the sampling location:

- ❑ Shipping container sent by the laboratory
- ❑ Regulator Module
- ❑ Cartridge Housing Module
- ❑ Discharge Module
- ❑ Single Injector Module (for adding 0.1-molar hydrochloric acid to adjust pH, if necessary)
- ❑ Prefilter Module (for filtering sediment from highly turbid water, if necessary)
- ❑ Approximately 2 gal (4 L) of 0.1-molar hydrochloric acid solution (for adjusting pH, if necessary)
- ❑ Sterile, 250- or 500-mL graduated cylinder
- ❑ Plastic sample bags
- ❑ Sample data sheet
- ❑ Frozen ice packs
- ❑ Several pairs of new latex gloves
- ❑ pH meter
- ❑ Thermometer
- ❑ Turbidimeter

2 Turn on the water at the tap and allow the water to flow for 2 to 3 minutes or until any debris that has accumulated in the sampling line has cleared or the turbidity in the water becomes uniform.

3 Put on new latex gloves to prevent contamination from outside sources. Sterile technique must be used when sampling for enteric viruses. Any contamination of the sampling apparatus may bias the final results.

Turn off the water at the tap.

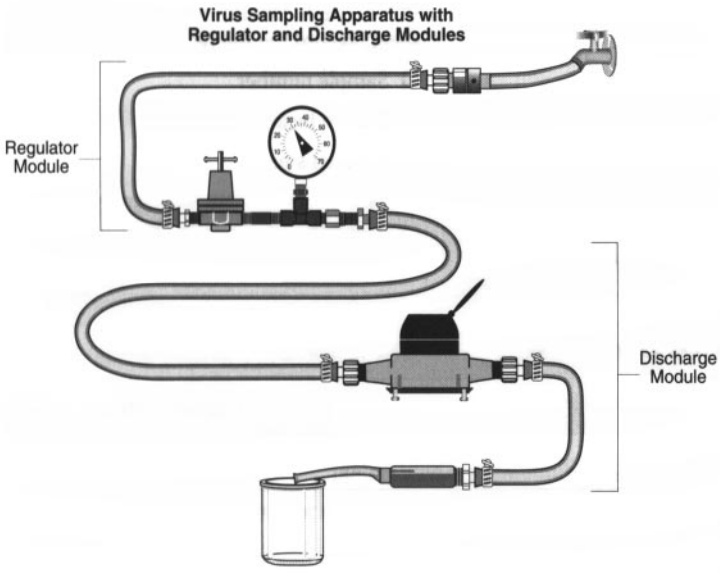
Remove the foil from the backflow regulator on the Regulator Module and connect it to the water tap or to an extension hose connected to the tap.

Remove the foil from the other end of the Regulator Module and from the Discharge Module. Connect the Discharge Module to the Regulator Module.

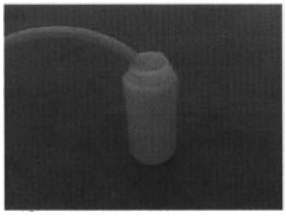
Place the end of the Discharge Module, or an extension hose connected to the Discharge Module, into a 1-liter plastic bottle.

Note the water meter reading, then slowly turn on the water.

Using the pressure regulator, adjust the water pressure to no more than 30 psi.



4 Flush the sampling apparatus with 20 gallons / 76 liters of water by allowing the water to flow through the system, out the effluent hose into the 1-liter plastic bottle.



Sampling Step	Volume In GALLONS	Volume In LITERS	Volume In FT^3
System Flush	20	76	2.7

While the water is flushing the sampling apparatus, begin completing your sample data sheet. Record the following information:

- ❑ Sample number
- ❑ System location
- ❑ Sampler's name

SAMPLE DATA SHEET			
SAMPLE NUMBER:			
SYSTEM LOCATION:			
SAMPLER'S NAME:			
WATER pH:	**WATER TEMPERATURE:**	**°C**	**TURBIDITY:** **NTU**
INIT. METER READING:	**(CHECK UNITS)**	**__ft³**	**__gallons**
date:	**time:**		
FINAL METER READING:	**(CHECK UNITS)**	**__ft³**	**__gallons**
date:	**time:**		
TOTAL SAMPLE VOLUME:		**liters**	
(Final-Initial meter readings x 28.316 (for readings in ft³) or x 3.7854 (for readings in gallons))			
CONDITION ON ARRIVAL:			
COMMENTS:			

5 Measure the pH, temperature, and turbidity of the source water flowing from the effluent hose. Record the readings on the sample data sheet.

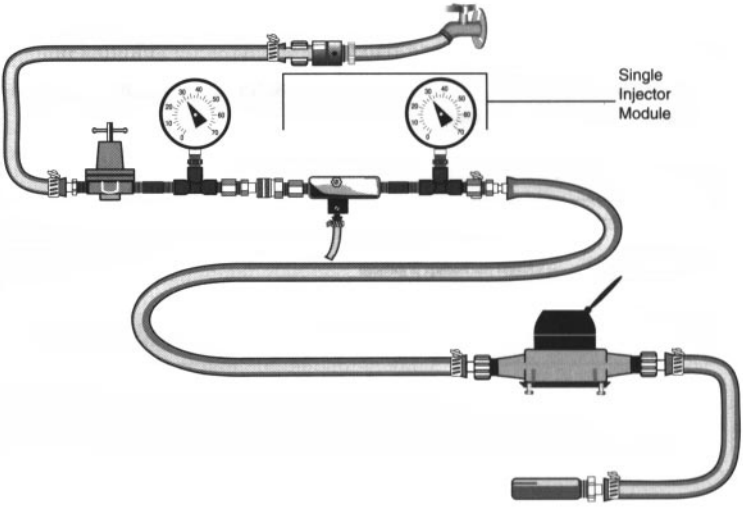
SAMPLE DATA SHEET			
SAMPLE NUMBER:			
SYSTEM LOCATION:			
SAMPLER'S NAME:			
WATER pH:	**WATER TEMPERATURE:** °C	**TURBIDITY:**	NTU
INIT. METER READING:	**(CHECK UNITS)**	__ft^3	__gallons
date:	**time:**		
FINAL METER READING:	**(CHECK UNITS)**	__ft^3	__gallons
date:	**time:**		
TOTAL SAMPLE VOLUME:		**liters**	
(Final-Initial meter readings x 28.316 (for readings in ft^3) or x 3.7854 (for readings in gallons))			
CONDITION ON ARRIVAL:			
COMMENTS:			

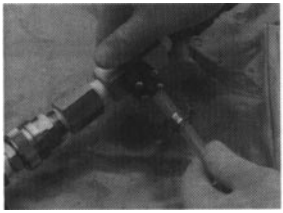
6 Turn off the water at the tap and decide whether you need to insert additional modules into the sampling train.

For source water sampling, you may need to use the Single Injector Module and/or the Pre-filter Module.

First, determine if you need to use the Single Injector Module.

If your pH value is greater than 8.0, you need to insert the Single Injector Module between the Regulator and Discharge Modules before proceeding.

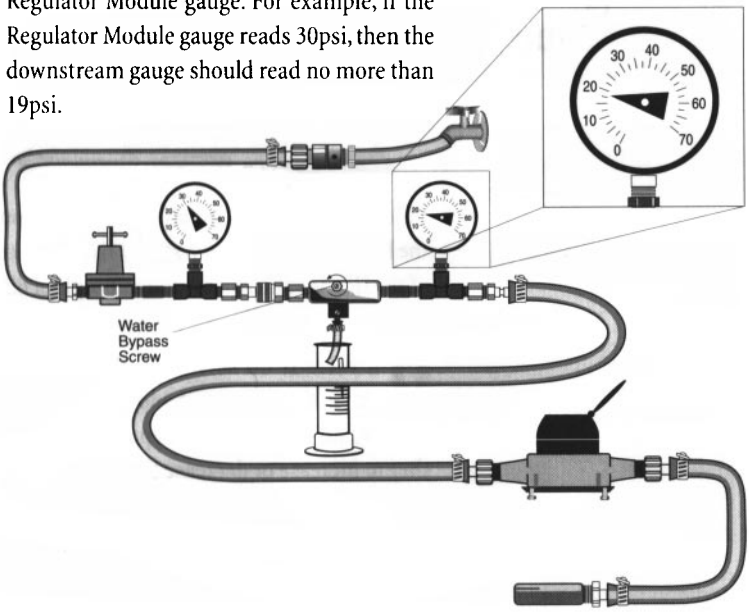


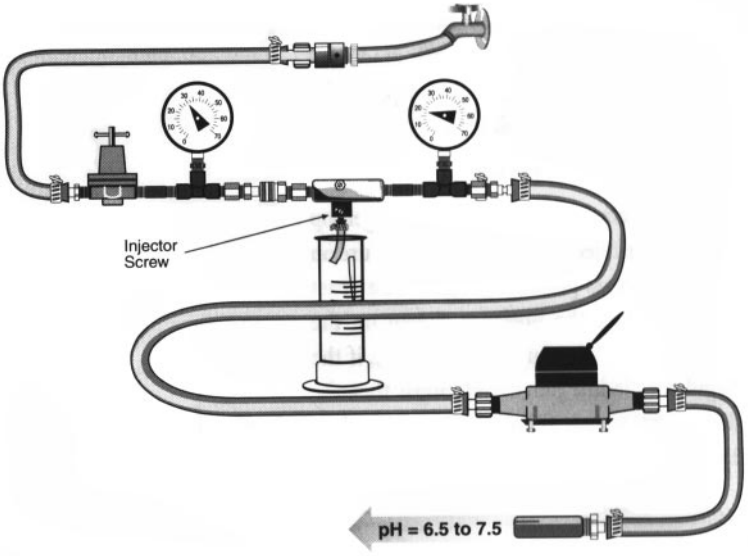


Using aseptic technique, connect the sterile tubing to the injector. Fill the sterile graduated cylinder with 0.1-molar HCl and place the tube in the graduated cylinder.

Turn on the water at the tap.

Using the water bypass screw—the larger top screw in the injector—adjust the pressure on the downstream pressure gauge to be at least 35% less than the pressure shown on the Regulator Module gauge. For example, if the Regulator Module gauge reads 30psi, then the downstream gauge should read no more than 19psi.





Adjust the smaller injector screw, located on the bottom of the injector, so that the flow rate of the HCl is sufficient to maintain a pH of 6.5 to 7.5.

! If there is no suction visibly drawing down the HCl, or if too much HCl is flowing, adjust the water bypass screw further to increase or decrease the pressure differential between the two gauges. A greater differential between the upstream gauges increases the flow rate; a smaller differential decreases the flow rate.

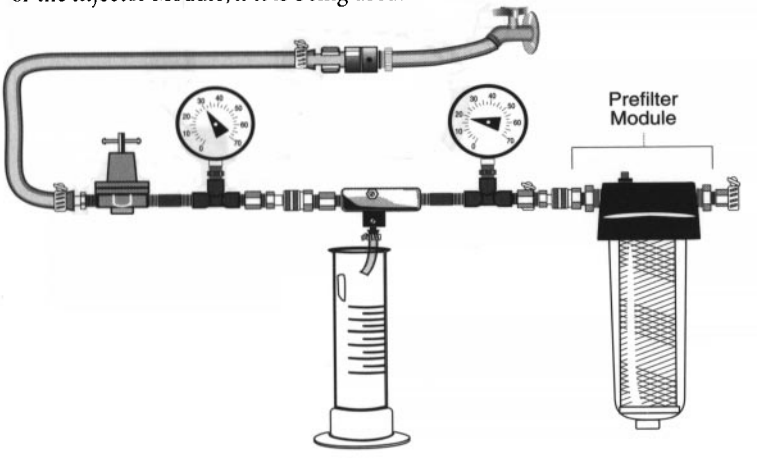
After the HCl flow rate is adjusted properly, transfer the injector tube to a carboy of HCl. Periodically check the pH to ensure that sufficient HCl is being added to maintain a pH of 6.5 to 7.5.

Record the adjusted pH on the Sample Data Sheet.

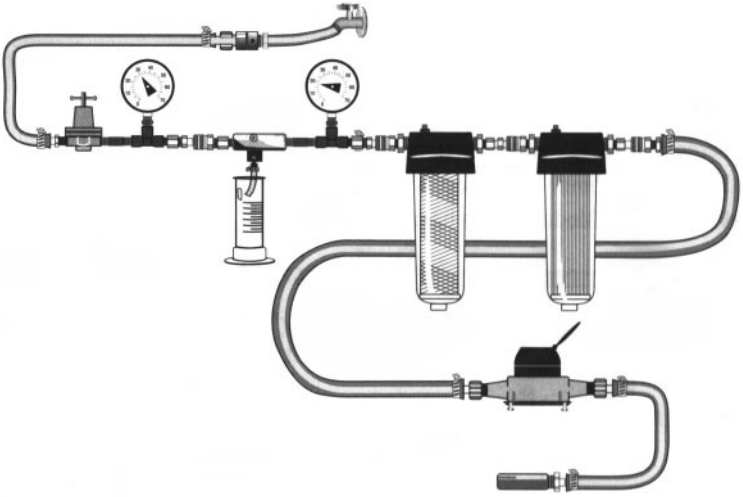
Next, determine if you need to use the Prefilter Module.

Turn off the water at the tap, and note the turbidity. If the turbidity is greater than 75 NTU, or for conditions where the 1-MDS filter is expected to clog before sampling is completed, you will need to use the Prefilter Module.

Disconnect the Discharge Module and connect the Prefilter Module to the Regulator Module or the Injector Module, if it is being used.



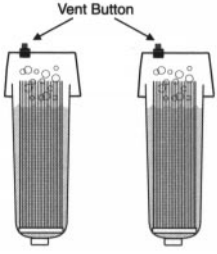
7 Connect the Cartridge Housing Module containing the 1-MDS filter to the Prefilter Module. Then, reconnect the Discharge Module to the outlet end of the Cartridge Housing Module.



Record the following information on the Sample Data Sheet:

- ❑ Date sampling started
- ❑ Time sampling started
- ❑ Initial water meter reading (including units)

SAMPLE DATA SHEET			
SAMPLE NUMBER:			
SYSTEM LOCATION:			
SAMPLER'S NAME:			
WATER pH:	**WATER TEMPERATURE:** °C	**TURBIDITY:**	NTU
INIT. METER READING:	**(CHECK UNITS)**	__ft^3	__gallons
date:	**time:**		
FINAL METER READING:	**(CHECK UNITS)**	__ft^3	__gallons
date:	**time:**		
TOTAL SAMPLE VOLUME:		**liters**	
(Final-Initial meter readings x 28.316 (for readings in ft^3) or x 3.7854 (for readings in gallons))			
CONDITION ON ARRIVAL:			
COMMENTS:			



STEP 8 Slowly, start the water flowing through the sampling apparatus.

Push the red vent buttons on top of the filter housings to expel air in the filters. When the air is totally expelled from the filters, release the button and open the water tap completely.

Using the pressure regulator on the Regulator Module, adjust the pressure regulator to no more than 30 psi.

Using the water bypass screw on the injector, adjust the pressure regulator on the Single Injector Module to be at least 35% less than the pressure shown on the Regulator Module gauge.

Allow 53 - 80 gallons / 200 - 300 liters of water to pass through the filter.

Sampling Step	Volume In GALLONS	Volume In LITERS	Volume In FT^3
Sampling Source Water	53 - 80	200 - 300	7 - 11

! If the virus filter clogs before 53 gallons/ 100 liters are collected, contact the approved analyst at your laboratory for further instructions.

STEP 9 When the water meter indicates that 53 - 80 gallons / 200 - 300 liters of water have passed through the filter, turn off the water at the tap.

Record the following information on the Sample Data Sheet:

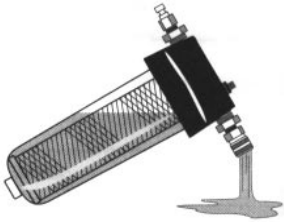
- ❑ Date sampling ended
- ❑ Time sampling ended
- ❑ Final water meter reading (including units)

SAMPLE DATA SHEET			
SAMPLE NUMBER:			
SYSTEM LOCATION:			
SAMPLER'S NAME:			
WATER pH:	**WATER TEMPERATURE:** °C	**TURBIDITY:**	NTU
INIT. METER READING:	**(CHECK UNITS)**	__ft^3	__gallons
date:	**time:**		
FINAL METER READING:	**(CHECK UNITS)**	__ft^3	__gallons
date:	**time:**		
TOTAL SAMPLE VOLUME:		**liters**	
(Final-Initial meter readings x 28.316 (for readings in ft^3) or x 3.7854 (for readings in gallons))			
CONDITION ON ARRIVAL:			
COMMENTS:			

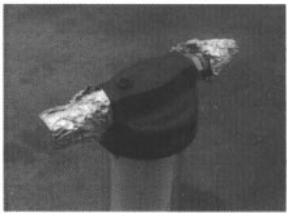
Put on fresh latex gloves.

Carefully, disconnect the sampling apparatus from the water tap.

10 Disconnect the Cartridge Housing Module from the sampling train.



Turn the filter housing upside down and allow excess water to flow out as waste water.



Turn the housing upright, and cover the module ends with sterile foil.

Do not attempt to open the filter housing.

If you are using the Prefilter Module, disconnect it from the sampling train, repeat the draining procedure, and cover the module ends with sterile foil.

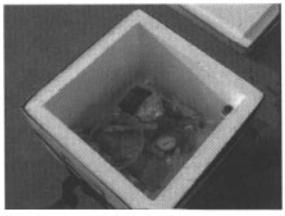
The filters and filter housings are shipped to the laboratory intact. The Discharge Module may be retained at the utility and reused.

Place the filter housings into an insulated shipping box.

Set the ice packs around the housings.

Return the Regulator Module and the Injector Module to the laboratory for cleaning and sterilization.

Place the Sample Data Sheet in a plastic bag and pack it on top of the sampling apparatus.



You may need to use additional packing material to ensure that the contents of the box will not shift during transport.

11 Seal the container and ship it by overnight courier to the laboratory. Call the laboratory and notify them of the sample shipment.

COLLECTING FINISHED WATER SAMPLES

1 If the concentration of any pathogen in your source water samples exceeds 1 per liter during the first 12 months of sampling, then you must monitor finished water as well as source water.

Sampling of finished water begins in the same manner as sampling of source water described previously, as follows:

When you are ready to collect your finished water virus sample, bring the following items with you to the sampling location:

- ❑ Shipping container sent by the laboratory
- ❑ Regulator Module
- ❑ Cartridge Housing Module
- ❑ Discharge Module
- ❑ Single Injector Module (for adding 2% thiosulfate solution to neutralize effects of chlorination or other disinfectant treatments)
- ❑ Double Injector Module (for adding 2% thiosulfate solution to neutralize effects of chlorination or other disinfectant treatments while adding 0.1-molar hydrochloric acid to adjust pH, if necessary)

- ❑ Approximately 2 gal (4 L) of 2% sodium thiosulfate solution
- ❑ Approximately 2 gal (4 L) of 0.1-molar hydrochloric acid solution (for adjusting pH, if necessary)
- ❑ 2 sterile, 250- or 500-mL graduated cylinders
- ❑ Plastic sample bags
- ❑ Sample data sheet
- ❑ Frozen ice packs
- ❑ Several pairs of new latex gloves
- ❑ pH meter
- ❑ Thermometer

2 Turn on the water at the tap and allow the water to flow for 2 to 3 minutes or until any debris that has accumulated in the sampling line has cleared or the turbidity in the water becomes uniform.

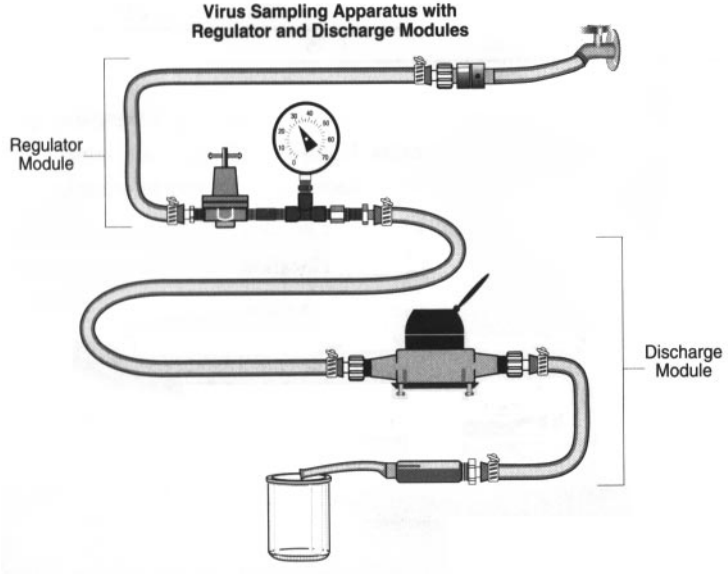
Turn off the water at the tap.

3 Put on new latex gloves to prevent contamination from outside sources. Sterile technique must be used when sampling for enteric viruses. Any contamination of the sampling apparatus may bias the final results.

Remove the foil from the backflow regulator on the Regulator Module and connect the module to the water tap or to an extension hose connected to the tap.

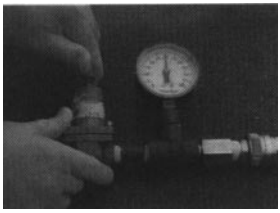
Remove the foil from the other end of the Regulator Module and from the Discharge Module and connect the Discharge Module to the Regulator Module.

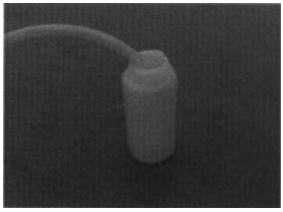
Place the end of the Discharge Module, or an extension hose connected to the Discharge Module, into a 1-liter plastic bottle.



Note the water meter reading, then slowly turn on the water.

Using the pressure regulator, adjust the water pressure to no more than 30 psi.





STEP 4 Flush the sampling apparatus with 20 gallons / 76 liters of water by allowing the water to flow through the system, out the effluent hose into the 1-liter plastic bottle.

Sampling Step	Volume In GALLONS	Volume In LITERS	Volume In FT^3
System Flush	20	76	2.7

While the water is flushing the sampling apparatus, begin completing your sample data sheet. Record the following information:

- ❑ Sample number
- ❑ System location
- ❑ Sampler's name

SAMPLE DATA SHEET

SAMPLE NUMBER:

SYSTEM LOCATION:

SAMPLER'S NAME:

WATER pH: **WATER TEMPERATURE:** °C **TURBIDITY:** NTU

INIT. METER READING: **(CHECK UNITS)** __ft^3 __gallons

date: **time:**

FINAL METER READING: **(CHECK UNITS)** __ft^3 __gallons

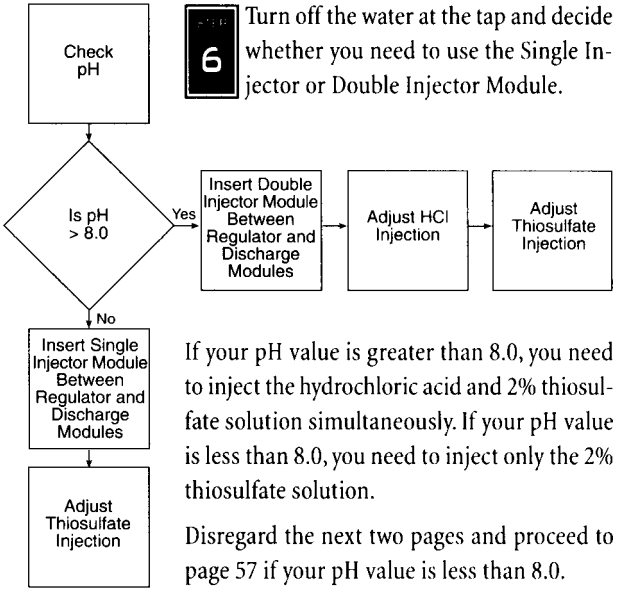
date: **time:**

TOTAL SAMPLE VOLUME: **liters**

(Final-Initial meter readings x 28.316 (for readings in ft^3) or x 3.7854 (for readings in gallons))

5 Measure the pH, temperature, and turbidity of the source water flowing from the effluent hose. Record the readings on the sample data sheet.

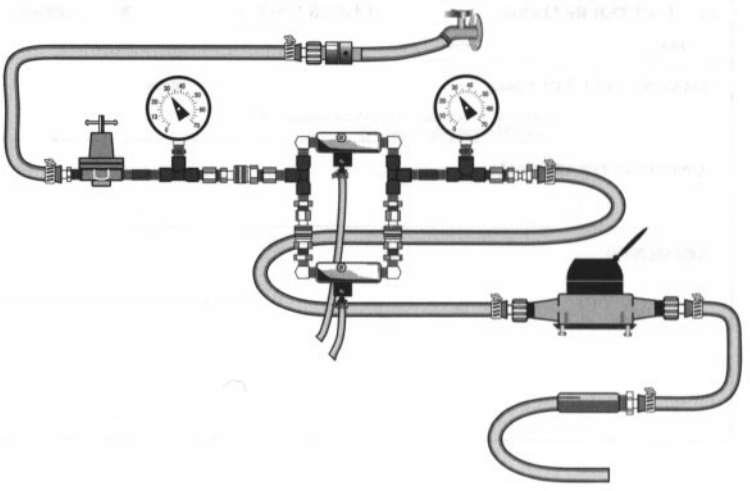
SAMPLE DATA SHEET			
SAMPLE NUMBER:			
SYSTEM LOCATION:			
SAMPLER'S NAME:			
WATER pH:	**WATER TEMPERATURE:**	**°C**	**TURBIDITY:** **NTU**
INIT. METER READING:	**(CHECK UNITS)**	**__ft³**	**__gallons**
date:	**time:**		
FINAL METER READING:	**(CHECK UNITS)**	**__ft³**	**__gallons**
date:	**time:**		
TOTAL SAMPLE VOLUME:		**liters**	
(Final-Initial meter readings x 28.316 (for readings in ft³) or x 3.7854 (for readings in gallons))			
CONDITION ON ARRIVAL:			
COMMENTS:			



6 Turn off the water at the tap and decide whether you need to use the Single Injector or Double Injector Module.

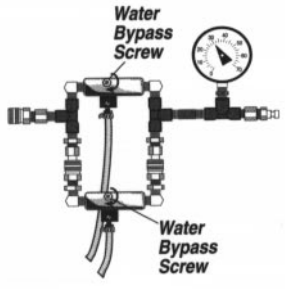
If your pH value is greater than 8.0, you need to inject the hydrochloric acid and 2% thiosulfate solution simultaneously. If your pH value is less than 8.0, you need to inject only the 2% thiosulfate solution.

Disregard the next two pages and proceed to page 57 if your pH value is less than 8.0.



pH > 8.0 Insert the Double Injector Module between the Regulator and Discharge Modules before proceeding.

! Ensure that both injectors are completely closed before proceeding.

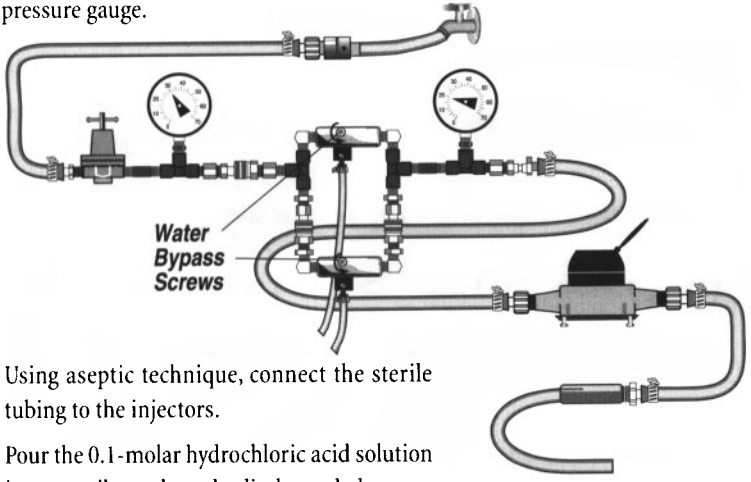


Adjust the water bypass screws on each injector clockwise as far as possible.

Turn on the water.

Next, turn each of the screws one half turn counterclockwise.

Continue opening the water bypass screws in half-turn increments until the reading on the second pressure gauge is approximately 35% less than that shown on the Regulator Module pressure gauge.



Using aseptic technique, connect the sterile tubing to the injectors.

Pour the 0.1-molar hydrochloric acid solution into a sterile graduated cylinder and place one of the injector tubes into it.

Pour the 2% thiosulfate solution into a second, sterile graduated container. Place the tube

from the second injector into the thiosulfate solution.

If there is no suction visibly drawing down the 2% thiosulfate or the HCl, or if too much is flowing, adjust the water bypass screws further to increase or decrease the pressure differential between the two gauges, until the flow is regulated properly.

Adjust the smaller injector screw on the hydrochloric acid injector to add sufficient hydrochloric acid to maintain a pH of 6.5 to 7.5.

After adjusting the injector, transfer the injector tube to the carboy of 0.1-molar hydrochloric acid. As sampling proceeds, periodically check the pH to ensure that it remains between 6.5 and 7.5.

Record the adjusted pH on the Sample Data Sheet.

Next, using the formula below, calculate the rate of thiosulfate injection and adjust the thiosulfate injector to deliver 10 mL of thiosulfate per gallon of flow.

$$\left(\begin{array}{c}\text{Water}\\ \text{Flow}\\ \text{Rate}\end{array}\right) \frac{\text{gallons}}{\text{minute}} \times \frac{\text{10 ml Thiosulfate}}{\text{1 gallon water}} = \left(\begin{array}{c}\text{Thiosulfate}\\ \text{Injection Rate}\end{array}\right) \frac{\text{ml}}{\text{minute}}$$

After the thiosulfate flow rate is adjusted, transfer the injector tube to the carboy of thiosulfate.

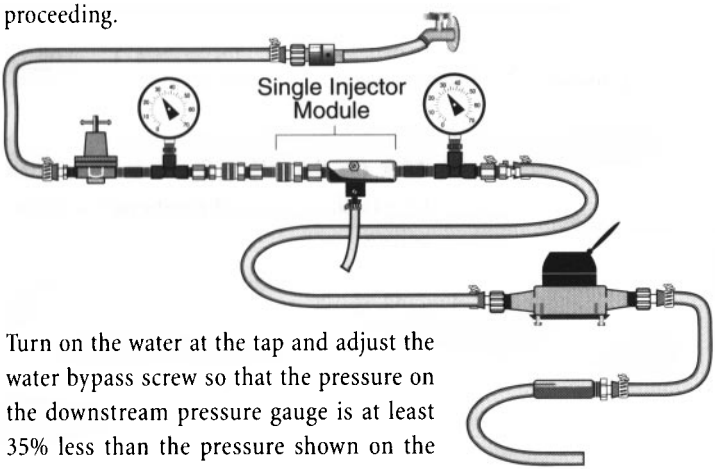
Monitor the thiosulfate flow rate visually throughout sampling.

Disregard the next section and proceed to step 7 (page 58).

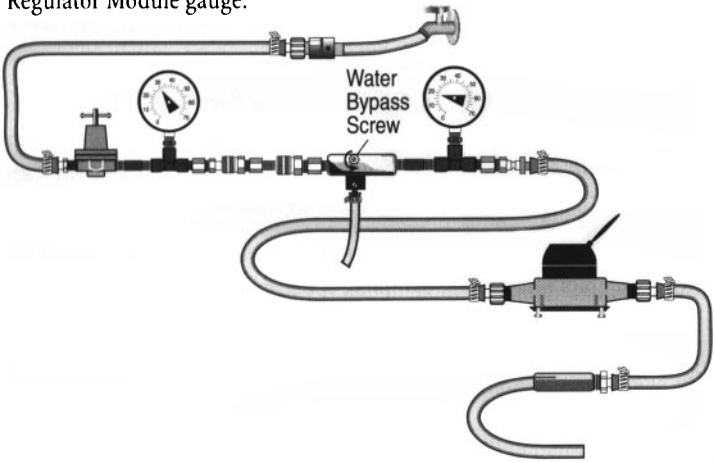
pH < 8.0

If your pH value is less than 8.0, it does not need to be adjusted, and you can use the Single Injector Module to inject the 2% sodium thiosulfate solution.

Insert the Single Injector Module between the Regulator and Discharge Modules before proceeding.



Turn on the water at the tap and adjust the water bypass screw so that the pressure on the downstream pressure gauge is at least 35% less than the pressure shown on the Regulator Module gauge.



Pour the 2% thiosulfate into a graduated cylinder.

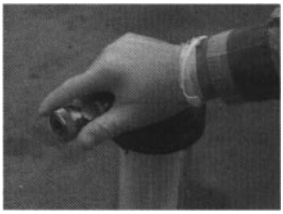
Next, using the formula below, calculate the rate of thiosulfate injection and adjust the thiosulfate injector to deliver 10 mL of thiosulfate per gallon of flow.

$$\left(\begin{array}{c}\text{Water}\\ \text{Flow}\\ \text{Rate}\end{array}\right) \frac{\text{gallons}}{\text{minute}} \times \frac{\text{10 ml Thiosulfate}}{\text{1 gallon water}} = \left(\begin{array}{c}\text{Thiosulfate}\\ \text{Injection Rate}\end{array}\right) \frac{\text{ml}}{\text{minute}}$$

After the thiosulfate flow rate is adjusted, transfer the injector tube to the carboy of thiosulfate.

Monitor the thiosulfate flow rate visually throughout sampling.

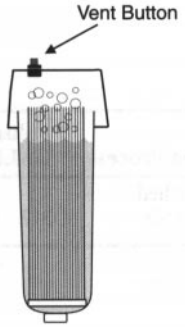
! If there is no suction visibly drawing down the thiosulfate, or if too much is flowing, adjust the water bypass screw further to increase or decrease the pressure differential between the two gauges, until the flow is regulated properly.



7 Connect the Cartridge Housing Module. Then reconnect the Discharge Module to the outlet end of the Cartridge Housing Module.

Slowly, start the water flowing through the sampling apparatus.

Push the red vent button on top of the filter housing to expel air in the filter. When the air is totally expelled from the filter, release the button and open the water tap completely.



Using the pressure regulator on the Regulator Module, adjust the pressure to no more than 30 psi.

Using the water bypass screw on the injector, adjust the pressure gauge on the Single Injector Module to be at least 35% less than the pressure shown on the Regulator Module gauge .

Record the following information on the Sample Data Sheet:

- ❑ Date sampling started
- ❑ Time sampling started
- ❑ Initial water meter reading (including units)

SAMPLE DATA SHEET			
SAMPLE NUMBER:			
SYSTEM LOCATION:			
SAMPLER'S NAME:			
WATER pH:	**WATER TEMPERATURE:**	**°C**	**TURBIDITY:** **NTU**
INIT. METER READING:	**(CHECK UNITS)**	**__ft³**	**__gallons**
date:	**time:**		
FINAL METER READING:	**(CHECK UNITS)**	**__ft³**	**__gallons**
date:	**time:**		
TOTAL SAMPLE VOLUME:		**liters**	
(Final-Initial meter readings x 28.316 (for readings in ft³) or x 3.7854 (for readings in gallons))			
CONDITION ON ARRIVAL:			

STEP 8 Collect 317 - 396 gallons or 1200 to 1500 liters of finished water.

Sampling Process	Volume In GALLONS	Volume In LITERS	Volume In FT^3
Virus Finished Water Sample	317 - 396	1200 - 1500	43 - 53

STEP 9 When the water meter indicates that 317 - 396 gallons / 1200 - 1500 liters of water have passed through the filter, turn off the water at the tap.

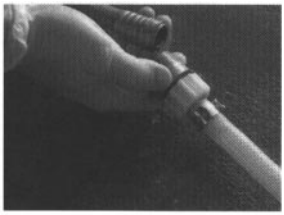
Record the following information on the Sample Data Sheet:

- ❑ Date sampling ended
- ❑ Time sampling ended
- ❑ Final water meter reading (including units)

SAMPLE DATA SHEET			
SAMPLE NUMBER:			
SYSTEM LOCATION:			
SAMPLER'S NAME:			
WATER pH:	**WATER TEMPERATURE:**	**˚C**	**TURBIDITY:** **NTU**
INIT. METER READING:	**(CHECK UNITS)**	**__ft³**	**__gallons**
date:	**time:**		
FINAL METER READING:	**(CHECK UNITS)**	**__ft³**	**__gallons**
date:	**time:**		
TOTAL SAMPLE VOLUME:		**liters**	
(Final-Initial meter readings x 28.316 (for readings in ft³) or x 3.7854 (for readings in gallons))			

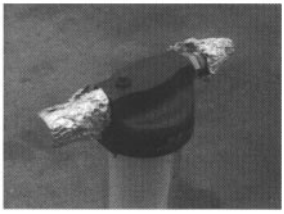
Put on fresh latex gloves.

Carefully, disconnect the sampling apparatus from the water tap.



Disconnect the Cartridge Housing Module from the sampling train. Turn the filter housing upside down and allow excess water to flow out as waste water.

Turn the housing upright, and cover the module ends with sterile foil.



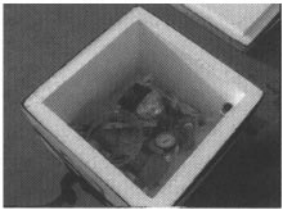
Do not attempt to open the filter housing.

The filter and filter housing are shipped to the laboratory intact. The Discharge Module may be retained at the utility and reused.

STEP 10

Place the filter housing into an insulated shipping box.

Set the ice packs around the housing.



Return the Regulator Module and the Injector Module to the laboratory for cleaning and sterilization.

Place the Sample Data Sheet in a plastic bag and pack it on top of the sampling apparatus. Seal the container.

You may need to use additional packing material to ensure that the contents of the box will not shift during transport.

STEP 11 Ship the container by overnight courier to the laboratory. Call the laboratory and notify them of the sample shipment.

Credits and Acknowledgments

The use of Manufacturer Trade Names in the production does not constitute endorsement by the U.S. Environmental Protection Agency.

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