

Sampling Protocol for *Giardia*, *Cryptosporidium* and Microscopic Particulate Analysis

Courtesy of CH Diagnostic

This protocol satisfies sampling requirements for the following methods:

1. ICR protozoan method for detecting *Giardia* cysts and *Cryptosporidium* oocysts in water by a fluorescent antibody procedure- EPA ICR Microbial Laboratory Manual EPA/600/R-95/178
2. Microscopic Particulate Analysis (MPA) for Filtration Plant Optimization- EPA 910-R-96-001
3. Consensus Method for Determining Groundwaters Under the Direct Influence of Surface Water Using Microscopic Particulate Analysis (MPA)- EPA 910/9-92-029
4. Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA- EPA 821-R-99-006

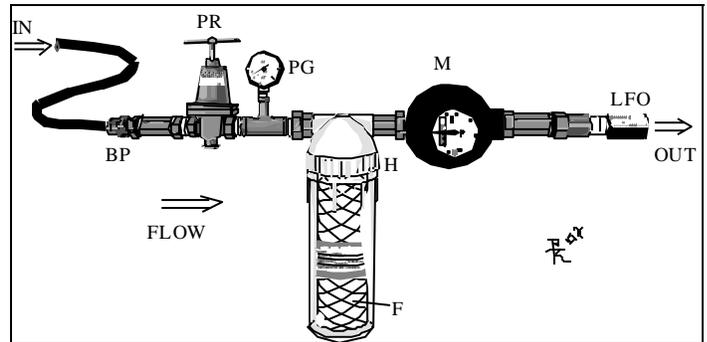
EQUIPMENT

1. Inlet hose (washing machine hose)
2. Backflow preventor (BP)
3. Pressure regulator (PR)
4. Pressure gauge (PG)
5. Filter housing (H) Fulflo LT10
- For 1623:** Substitute Gelman Envirochek™ Capsule, clear tubing (0.5 in I.D.), clamps, appropriate fittings.
6. 1 micron nominal porosity filter (F) Parker Hannifan M39R10A
- For 1623:** filter included in Gelman Envirochek™ Capsule
7. Meter (M)
8. Limiting flow orifice (LFO) 2L/min. (0.5 gal/min)

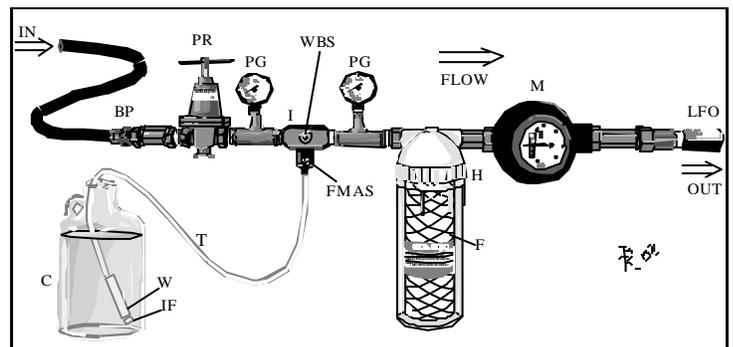
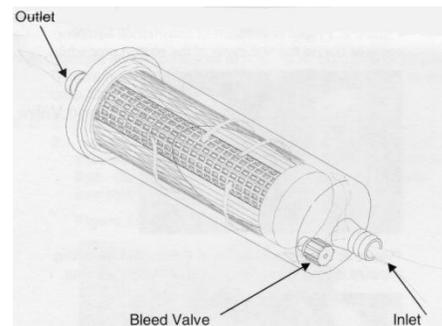
Additional Equipment for chlorinated samples:

9. Carboy (C) for sodium thiosulfate
10. Injector (I) with:
 - a. water bypass screw (WBS)
 - b. (Optional) fine metering adjustment (FMAS)
 - c. tubing (T)
 - d. injector filter (IF)
 - e. porcelain weight (W)
11. Second pressure gauge

Clean equipment, prior to use, by washing in hot water with detergent and bleach followed by hot water rinse and particle free water rinse, air dry and seal open ends of sampler for storage. Alternatively, borrow equipment from CH Diagnostic.



Gelman Envirochek™ Sampling Capsule



OTHER SUPPLIES

(1) Turbidimeter; (2) pH meter; (3) Thermometer; (4) Sanitary latex gloves; (5) Sample labels; (6) Heavy duty ziploc 2 gallon freezer bags; (7) Frozen "blue" ice packs and cooler for shipping; (8) For chlorinated water: sodium thiosulfate and reagent grade water (see page 4), sanitary 250 mL graduated cylinder; (9) For non-pressurized source: 2-4 Liter/minute gas or electric pump

SAMPLING LOCATIONS AND TIMING

Ground Water should be sampled as close to the source as possible to avoid inclusion of organisms growing in holding tanks, distribution pipes, or blended waters. Sampling taps and spring boxes should be cleaned and flushed prior to sampling. EPA Consensus Method recommends a minimum of two samples: one following a heavy rain event, snowmelt or irrigation season; and one in late summer or after an extended dry period. CH Diagnostic recommends 3 samples collected during periods when water quality data indicate greatest probability of surface water impact on ground water. These data include temperature, total dissolved solids, pH, conductivity, turbidity, dissolved oxygen, hardness, etc. that are monitored in the ground water and any adjacent surface water.

Raw Surface Water should be sampled prior to any chemical addition. It is preferable to collect the sample after any presedimentation basins or recycled water additions, but only if no chemicals were added prior to this point. Sampling after recycle input should be taken after adequate in-line mixing. The main objective of raw water collection is to get a representative sample of the organisms entering the treatment system.

Finished Surface Water should be sampled before chlorination. If this is not possible, sodium thiosulfate must be injected to neutralize the chlorine (see page 4). Collect samples prior to post-treatment storage.

Treatment Plant Evaluation: Raw water sampling should be initiated before finished water sampling. The amount of time between start of raw sampling and start of finished sampling should be equivalent to the detention time of the system. Finished water sampling should begin immediately after filters are put on line and encompass a full cycle run (or 24 hour period) including at least one backwash.

SAMPLING PROCEDURE

1. Source water measurements

- Run sample tap for 2-3 minutes to clear in-line debris and allow turbidity to become uniform. Measure and record turbidity, temperature and pH of sample source.

2. Flush equipment

- Assemble clean sampling apparatus as shown on page one; **however, do not install filter, limiting flow orifice, or Gelman Envirochek™ capsule yet.** Use the additional equipment if sampling chlorinated water.
- Ensure proper flow direction by checking arrows on meter and in/out indications on pressure regulator and filter housing.
- Flush sampling apparatus with 50 gallons (190 Liters, 6.7 ft³) of water being sampled. Allow water to flow through entire sampling apparatus (except for filter or capsule and limiting flow orifice).

3. Adjust pressure

- Attach the limiting flow orifice.
- Use pressure regulator to adjust water pressure to **10 psi** for unchlorinated samples and **25 psi** in the first gauge for samples requiring dechlorination.
- **If sampling a chlorinated source, follow injector adjustments on page 4.**

4. Install filter

- Using a filter wrench, open and drain filter housing.
- Put on new latex gloves. Open filter packaging and aseptically place filter into filter housing.
- Reassemble filter housing, ensuring o-ring seals properly.
- **For 1623:** Retain vinyl caps from capsule. Attach capsule with appropriate lengths of tubing. Orient the capsule with the large space at the inlet side of the unit. Secure with band clamps.

5. Begin Sampling

- Record date, time and initial meter reading. The one's unit on the meter is marked with an unchanging zero and is read from the rotating dial. The dial reads to tenths of gallons.
- Turn water on slowly with unit in upright position. Invert unit to expel all air from filter housing. When housing is full of water, return to upright position. **Increase water flow to no more than one gpm** and maintain this rate for entire sampling period (limiting flow orifice will prevent flow over 2L/min).

For 1623: Open purge valve of capsule by turning the plug about half a turn in the counter-clockwise direction and allow the incoming water to displace trapped air in capsule. When capsule is full of water, close the purge valve. **Increase water flow to no more than 2L/min.**

- **If sampling a chlorinated source, follow the dechlorination step on page 4.**
- Monitor pressure gauge: 10 psi for unchlorinated samples and 25 psi (read from the first gauge) for samples requiring dechlorination.

6. Sample the following amounts

ICR- raw water:	26.4 gal (100 liters, 3.5 ft ³)
ICR- finish water:	264.2 gal (1000 liters, 35 ft ³)
MPA- raw surface water:	minimum- 26.4 gal (100 liters, 3.5 ft ³); optimum- 1440 gal (5450 liters, 192.5 ft ³) or 24 hrs if flow <1 gpm
MPA- finished surface water:	minimum- 264 gal (1000 liters, 35 ft ³); optimum- 1440 gal (5450 liters, 192.5 ft ³) or 24 hrs if flow <1 gpm
MPA- groundwater:	minimum- 500 gal (1900 liters, 66.8 ft ³); maximum- 1000 gal (3785 liters, 133.7 ft ³)
1623	EPA recommends: 10 L per capsule with 2 capsules collected the first time at each source and every 20 th sample thereafter. CH Diagnostic recommends: 20 L minimum and 4,000 L max.

If filter plugs before prescribed amount is attained, collection of less volume is permissible. If plugging is anticipated, sample may be collected at a reduced flow rate to encompass a longer sampling period. Make a note on analysis request form if filter plugged.

7. End sampling

- Shut off water. Record stop date and time, final meter reading, turbidity and total volume sampled.
- Disconnect lower section of filter housing while maintaining housing in upright position.
- With new latex gloves, aseptically remove filter from housing and place in plastic zip-loc bag.
- Pour all of the remaining water from filter housing into the same bag. Seal bag.
- **Record sample name on bag with a permanent marker.**
- Do not allow bag to touch any environmental surface before placing inside a second bag.
- Sample may be refrigerated at 2-5°C for up to 15 hours before shipping. **DO NOT FREEZE**

For 1623: Remove inlet hose from the water source while holding capsule in an upright position. Seal the inlet with vinyl cap. Never pour any water out of the upper chamber. While continuing to hold capsule in an upright position, disconnect the outlet hose. Leave remaining water in the capsule. Seal the outlet with vinyl cap. Samples must be received at CH Diagnostic within 24 hours of sample collection.

8. Ship sample

WRAP SAMPLE IN SOME FORM OF INSULATION (e.g. bubble wrap). Wrap ice pack sheets around filters (inside bubble wrap) so that ice is **NOT IN DIRECT CONTACT** with filters (frozen filters may compromise test results). Place into insulated shipping container. **DO NOT USE DRY ICE.** Avoid using wet ice whenever possible. Initial type of analysis to be performed on CH Diagnostic Analysis Request form. Place completed form in zip-loc bag and in box with filters. Ship by **priority over-night** courier to CH Diagnostic.

9. Clean up

Discard inlet hose. Clean equipment by washing in hot water with detergent and bleach followed by hot water rinse and particle-free water rinse, air dry, and seal open ends of sampler for storage. Alternatively, return equipment borrowed from CH Diagnostic at your earliest convenience. Equipment may be shipped separately from the sample by regular ground courier.

ADDITIONAL PROCEDURES FOR CHLORINATED WATER SAMPLES

2% Sodium Thiosulfate Solution (make prior to sampling):

3.14 grams of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) per 100 mL reagent grade water makes a 2% solution. 10 mL of 2% sodium thio. solution is needed for each gallon of chlorinated water that is sampled. Plan sample volume to determine appropriate amount of sodium thiosulfate solution. For example:

A 264 gal (1000 L) sample, requires 94.2 grams sodium thio. dissolved in 3 L reagent grade water.

A 500 gal (1900 L) sample, requires 157 grams sodium thio. dissolved in 5 L reagent grade water.

A 1,000 gal (3785 L) sample, requires 314 grams sodium thio. dissolved in 10 L of reagent grade water.

A 1,440 gal (5450 L) sample, requires 455.3 grams sodium thio. dissolved in 14.5 L of reagent water.

Make 0.5 liter marks with permanent marker on carboy that will hold sodium thiosulfate solution.

Injector Adjustments (make while adjusting pressure in step 3, page 2):

Sample tap must supply water with at least 25 psi. If not, install 1-5 gal/min gas or electric pump after sample filter housing. Pump must be capable of producing at least 25 psi.

Adjust injector during 50 gallon flush period by placing injector tubing with injector filter and weight into 250 mL graduated cylinder filled with reagent grade water.

Use water bypass screw to adjust pressure on downstream pressure gauge to 16 psi, while upstream pressure is at least 25 psi.

Check for 10 mL/min injection rate. Coarse adjustments may be made with water bypass screw. If injector has fine metering adjustment screw, use it to fine tune injection rate.

If there is no suction visibly drawing down the water in the graduated cylinder, or if too much is flowing, make sure the first gauge has at least 25 psi and adjust the water bypass screw further to increase or decrease the pressure differential between the two gauges. Greater differential between the upstream and downstream gauges increases the flow rate; a smaller differential decreases the flow rate.

Dechlorination Step (performed during sampling in step 5, page 3):

Pour 100 mL sodium thio. solution into graduated cylinder.

Adjust injector screws as necessary to 10 mL/min (see injector adjustment section above).

Move injector tube to large carboy filled with sodium thio. Monitor level of sodium thiosulfate solution in the carboy which should go down approximately 0.5 L every 1.5 hrs.