LT2 Rule *Cryptosporidium* & *E. coli* Sample Collection Recommendations: A Pocket Guide

Cryptosporidium
10-L Bulk Water Collection

Thoroughly clean* all reused collection equipment and containers (new hoses, cubitainers, etc. should not need cleaning), and wear gloves whenever needed to prevent sample contamination throughout the collection procedure. Prior to sample collection, thoroughly flush stagnant water and debris in the sample line until the temperature and turbidity stabilize (approx. 2-3 mins).

Complete the sample collection form including utility and sample ID information, date, time, turbidity, pH, and temperature.

1. Fill the 10-L cubitainer from a pressurized sample tap or manually collect sample using hose, pump, funnel, etc. from the location described to the State.
2. Once the sample is collected, immediately place the cap on the cubitainer and tighten
3. Chill the sample as soon as possible to between 1° and 10°C before shipment

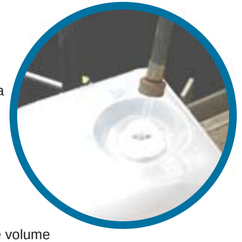
* Refer to the cleaning procedure located on the Packing and Shipping panel of this pocket guide

Cryptosporidium
Matrix Spike Sample Collection

Send an extra 10 L bulk sample for spiking at the *Cryptosporidium* laboratory concurrent with the 1st and 21st routine monitoring sample collections and again with every 20 samples if more than 40 source water samples are collected. Coordinate the collection of matrix spike samples with your laboratory. Matrix spike samples should be collected from the same location and at the same time as the associated routine sample. The matrix spike sample and routine sample should be the same volume. Label the cubitainer with the following information using a waterproof pen: PWSID, facility name, and sample collection date. Chill the sample as soon as possible to between 1° and 10°C before shipment.

10-L Matrix Spike Sample

Follow instructions for collecting a 10-L bulk water sample given on previous panel.



Matrix Spike Samples Greater than 10-L

Either ship the entire matrix spike volume as a bulk sample to the laboratory to be spiked and analyzed, **OR** filter all but 10 L of the matrix spike sample at your utility and collect the remaining 10 L as a bulk sample. Chill both the filter and bulk sample as soon as possible to between 1° and 10°C before shipment. Clearly label both the filter and bulk sample as comprising 2 parts of a single sample.

E. coli
Sample Collection

Wear gloves whenever needed to prevent sample contamination throughout the collection procedure.

Complete the sample collection form including utility and sample ID information, date and time, etc.

1. Water taps used for sampling should be free of aerators, hose attachments, etc.
2. Prior to sample collection, thoroughly flush stagnant water and debris in the sample line until the temperature and turbidity stabilize (approx. 2-3 mins): **DO NOT** rinse the sample bottle
3. Record the turbidity, pH, and temperature
4. Aseptically fill the **sterile** *E. coli* sample bottle from a pressurized sample tap or manually collect sample using, hose, pump, funnel, etc. from the location described to the State
5. Leave at least one inch of head space in the *E. coli* sample bottle, if possible. Collect at least 100 mL of sample for analysis
6. Immediately close the sample bottle
7. Chill sample as soon as possible to between 0° and 10°C until ready for immediate shipment

Packing and Shipping

1. Remove sample (filter, cubitainer, or sample bottle) from refrigerator or cooler just prior to shipping. Wrap sample in bubble wrap to prevent freezing
2. Place sample in cooler lined with two large plastic trash bags
3. Pack cooler with sealed ice bags or the equivalent number of gel ice packs sufficient to maintain the sample between 1° and 10°C during shipment. Use the amount of coolant appropriate for the season, geographic location, and cooler-type used (follow laboratory recommendations). **NEVER USE DRY ICE.**
4. Knot and seal the two large plastic trash bags
5. Sign and date sample collection form, place in a water-tight bag in cooler
6. Seal and secure cooler with tape
7. Complete airbill and attach to cooler, retain shipper's copy
8. Ship sample(s) to the laboratory via overnight delivery

***Recommended Sampling Equipment Cleaning Procedure**

(or alternatively follow the manufacturer's cleaning instructions)

Not for sterilization of *E. coli* sample bottles

- Thoroughly clean all reused influent equipment prior to the capsule/filter housing/bulk water container with a warm detergent solution and scrubbing with a brush (new equipment should not need cleaning)
- Expose equipment to hypochlorite solution **(Add 25 mL of 5% household bleach for every 1 gallon of pH 7 water)** for at least 30 minutes at room temperature
- Thoroughly rinse equipment with reagent grade water that is free of analytes and interfering substances. Dry equipment in an area free of potential *Cryptosporidium* contamination

LT2 Rule *Cryptosporidium* & *E. coli* Sample Collection Recommendations

Pocket Guide

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Cryptosporidium
Preparation for Sample Filtration

Thoroughly clean* all reused collection equipment and containers (new tubing, fittings, etc. should not need cleaning), and wear gloves whenever needed to prevent sample contamination throughout the collection and filtration procedure. Before connecting the sampling unit to the tap, thoroughly flush stagnant water and debris in the sample line until the temperature and turbidity stabilize (approx. 2-3 mins).

Complete the sample collection form including utility and sample ID information, date, turbidity, pH and temperature.

Pressurized source

1. Determine sample line water pressure at the location described to the State
2. Connect assembled sampling unit, **without capsule/foam filter,** to sample tap
3. Flush the sampling unit for 2-3 minutes and test for leaks

Unpressurized source

1. Fill 30+ L carboy from a pressurized sample tap or manually collect the sample using hose, pump, funnel, etc. from the location described to the State
2. Chill the full carboy to between 1° and 10°C if filtration is not performed immediately after filling the carboy
3. Assemble sampling unit, **without capsule/foam filter,** and insert influent tube into the center of the carboy
4. Turn on pump to flush the sampling unit for 2-3 minutes and test for leaks

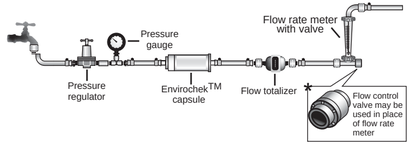
* Refer to the cleaning procedure located on the Packing and Shipping panel of this pocket guide

Cryptosporidium
Pressurized Sample Filtration

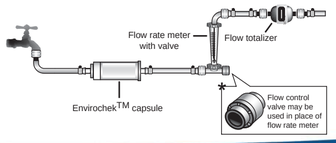
Envirochek™ or Envirochek™ HV Capsules

1. Install filter capsule
2. Record start time and initial meter reading
3. Slowly open sample tap, bleed out air, and adjust up to the following maximum values:
   - 100 psig and 2 L/min
4. When at least 10 L has passed through the filter, turn off sample tap allowing pressure to decrease until water stops
5. Record stop time and final meter reading
6. Hold inlet pointing up, remove outlet tubing and allow water to drain
7. Open bleed valve to speed the draining process and disconnect inlet tubing
8. Seal the capsule with vinyl end caps, close the bleed valve, and place in gallon-size zippered plastic bag
9. Chill the sample as soon as possible to between 1° and 10°C until ready for shipment

Tap pressure >100 psig



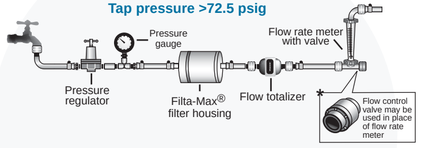
Tap pressure 1-100 psig



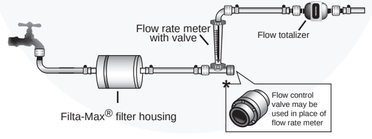
Filta-Max® Filter

1. Install foam filter inside filter housing
2. Record start time and initial meter reading
3. Slowly open sample tap and adjust up to the following recommended values:
   - 72.5 psig and 3-4 L/min
4. When at least 10 L has passed through the filter, turn off sample tap allowing pressure to decrease until water stops
5. Record stop time and final meter reading
6. Hold inlet pointing up, remove the outlet tubing and allow water to drain
7. Disconnect the inlet tubing
8. Seal filter inside filter housing with rubber stoppers and place into a gallon-size zippered plastic bag
9. Chill the sample as soon as possible to between 1° and 10°C until ready for shipment

Tap pressure >72.5 psig

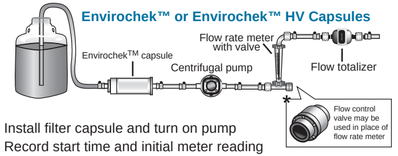


Tap pressure 7.5 - 72.5 psig



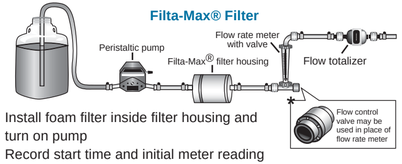
Cryptosporidium
Unpressurized Sample Filtration

Envirochek™ or Envirochek™ HV Capsules



1. Install filter capsule and turn on pump
2. Record start time and initial meter reading
3. Bleed out air and adjust flow rate up to a maximum of 2 L/min
4. When at least 10 L has passed through the filter, turn off pump
5. Record stop time and final meter reading
6. Hold inlet pointing up, remove outlet tubing and allow water to drain
7. Open bleed valve to speed the draining process and disconnect inlet tubing
8. Seal the capsule with vinyl end caps, close the bleed valve, and place in gallon-size zippered plastic bag
9. Chill the sample as soon as possible to between 1° and 10°C until ready for shipment

Filta-Max® Filter



1. Install foam filter inside filter housing and turn on pump
2. Record start time and initial meter reading
3. Adjust flow rate up to a maximum of 3-4 L/min
4. When at least 10 L has passed through the filter, turn off pump
5. Record stop time and final meter reading
6. Hold inlet pointing up, remove outlet tubing and allow water to drain
7. Disconnect inlet tubing
8. Seal filter inside filter housing with rubber stoppers and place into a gallon-size zippered plastic bag
9. Chill the sample as soon as possible to between 1° and 10°C until ready for shipment

This pocket guide is intended as an outline and supplement to the Source Water Monitoring Guidance Manual for Public Water Systems and the on-line Sample Collection Training Module. This guide is intended to provide useful information and recommendations, but does not substitute for the LT2 Rule.

The guidance manual is available for download and study at: http://www.epa.gov/safewater/disinfection/lt2/pdfs/guide_lt2_swmonitoringguidance.pdf

The on-line training module can be accessed at: http://www.epa.gov/safewater/lt2/training/modules.html

Copies of this pocket guide can be ordered by using the document number listed below and contacting the National Service Center for Environmental Publications at 1-800-490-9198

Office of Water (4601M)
EPA 817-K-06-002
April 2006