**Cryptosporidium**

**Matrix Spike Sample Collection**

Send an extra 10-L bulk sample for spiking at the Cryptosporidium laboratory to coincide with the 1st and 2nd routine monitoring sample collections and again with every 20 samples if more than 40 source water samples are submitted. Follow the matrix spike sample collection protocol. 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 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 min). Complete the sample collection form including utility and sample ID information, date, time, 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.

**Sample Collection Recommendations**

Prior to sample collection, thoroughly flush stagnant water and debris in the sample line until the temperature and turbidity stabilize (approx. 2–3 min). Complete the sample collection form including utility and sample ID information, date, time, pH, and temperature.

4. Aseptically fill the 10-L matrix spike sample 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 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.

**E. coli**

Sample Collection

Wear gloves wherever 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 min). Complete the sample collection form including utility and sample ID information, date, time, pH, and temperature.

1. Water taps used for sampling should be free of sponges, 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 min). DO NOT close 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 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 heating and drying while in transit.
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 location, and cooler-type used (follow laboratory recommendations). NEVER USE DRY ICE.
4. Sign and date sample collection form, place in a water-tight bag in cooler.
5. Seal and secure cooler with tape.
6. Completeillet and attach to cooler, retain shipper’s copy.
7. 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.

**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.

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

**Unpressurized Sample Filtration**

**Pressurized Sample Filtration**

3. Assemble sampling unit, immediately after filling the carboy.

2. Chill the full carboy to between 1° and 10°C if filtration is not performed using hose, pump, funnel, etc. from the location described to the State.

1. Fill 30+ L carboy from a pressurized sample tap or manually collect the sample from a non-pressurized source.

- **Pressurized source**
  - Determine sample line water pressure at the location described to the State.
  - Install filter capsule.
  - Record start time and initial meter reading.
  - Slowly open sample tap, bleed out air, and adjust up to the following maximum values: 100 psig and 2 L/min.
  - When at least 0.1 L has passed through the filter, turn off sample tap allowing pressure to decrease until water stops.
  - Record stop time and final meter reading.
  - Hold inlet pointing up, remove the outlet tubing and allow water to drain.
  - Seal filter inside filter housing with rubber stoppers and place into a gallon-size zippered plastic bag.
  - Chill the sample as soon as possible to between 1° and 10°C until ready for shipment.

- **Unpressurized source**
  - Connect assembled sampling unit, without capsule/foam filter, to sample tap.
  - Flush the sampling unit for 2-3 minutes and test for leaks.
  - Turn on pump to flush the sampling unit for 2-3 minutes and test for leaks.
  - Slowly open sample tap, bleed out air, and adjust up to a maximum of 3-4 L/min.
  - When at least 10 L has passed through the filter, turn off pump.
  - Record stop time and final meter reading.
  - Hold inlet pointing up, remove outlet tubing and allow water to drain.
  - Seal filter inside filter housing with rubber stoppers and place into a gallon-size zippered plastic bag.
  - Chill the sample as soon as possible to between 1° and 10°C until ready for shipment.

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