

**EXAMPLE PROCEDURE FOR FIELD-FILTERING SAMPLES FOR  
*CRYPTOSPORIDIUM* ANALYSIS USING PALL LIFE SCIENCES  
ENVIROCHEK™ OR ENVIROCHEK™ HV FILTERS**

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**DRAFT**

# Example Procedure for Field-Filtering Samples for *Cryptosporidium* Analysis Using the Pall Life Sciences Envirochek™ or Envirochek™ HV Filters

## 1.0 Required Materials

Check to make sure the following materials are available before collecting sample:

- Several pairs of new, powder-free latex gloves (Lab Safety Supply, cat. number 16285XL, or equivalent)
- Sample collection form
- Pall Life Sciences Envirochek™ or Envirochek™ HV sampling capsule (recommend that two filter capsules be kept on hand in case the first one clogs prior to filtering 10 L)
- Pump (if water is not from pressurized source) - Electric centrifugal(Simer, cat. number M40) or Electric peristaltic pump (Masterflex L/S variable-speed drive, Cole Parmer, cat. number LC-07554-90, or equivalent with Masterflex L/S Easy Load II pump head, Cole Parmer, cat. number LC-77200-60) or any equivalent pump that can create a flow of approximately 2 L/min
- Five lengths of 12.7-mm (0.5-in.) internal-diameter clear, vinyl, laboratory tubing (Cole Parmer, cat. number, 06405-19 or equivalent)
- Five pairs of clamps fit to tubing
- One coupling to fit 0.5-in. internal diameter tubing
- Pressure regulator (Watts Regulator, cat. number, 263A, or equivalent)
- Pressure gauge (filter's maximum operating pressure, original - 30 psi, HV - 60 psi) (Cole Parmer, cat. number, E-68004-04, or equivalent)
- Water meter (flow totalizer) (Omega Engineering, cat. number, FTB-4005, or equivalent)
- Flow rate meter (Blue-White, cat. number, F44375LEA 8, or equivalent)
- Flow control valve (Plast-o-matic, model number #FC050B-1/2-PV)
- Stopwatch
- Sample number label
- Cooler, approximately 16-quart (Coleman, model number, 6216-703, or equivalent)
- Two large plastic trash bags
- One 8-lb bag of ice or gel ice pack (VWR, cat. number, 15715-105, or equivalent)
- Three 13 × 18 inch ziplock bags (Associated Bag, cat. number, 270-42, or equivalent)
- Strapping tape
- Two self-adhesive plastic airbill sleeves
- Airbill for shipment

## 2.0 Collecting the Sample

If the sample will be collected from a pressurized source, use the sample collection procedures in Section 2.1. If the sample will be collected from an unpressurized source, use the sample collection procedures in Section 2.2.

### 2.1 Sample Filtration from a Pressurized Source

2.1.1 Before connecting the sampling system to the tap or source, turn on the tap and allow the water to flow for 2 to 3 minutes or until any debris that has accumulated in the source water lines has cleared or the turbidity in the water becomes visibly uniform. Turn off the tap.

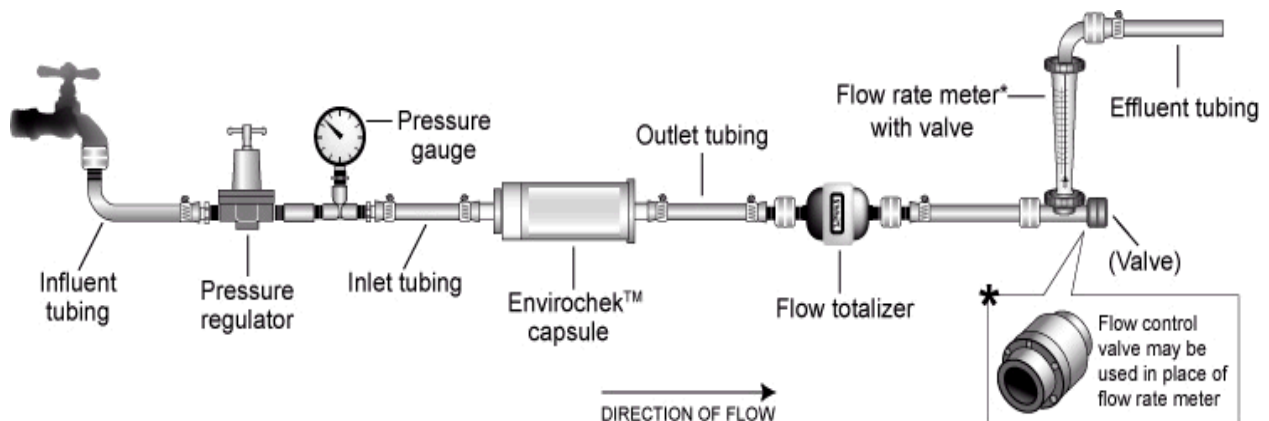
2.1.2 Put on a pair of powder-free latex gloves to prevent contamination from outside sources. Any contamination of the sampling apparatus may bias the final results.

2.1.3 Determine the pressure of the water source with the pressure gauge.

2.1.4 Assemble the sampling system, minus the Envirochek™ capsule. In place of the Envirochek™ capsule, insert a 0.5-in. barbed connector between the outlet tubing from the sample valve or the pressure regulator or gauge and the inlet tubing of the flow totalizer, flow meter or control valve. For high pressure (> 20 psig) sites, the sampling system should be assembled in the following order, as shown in **Figure 1** below:

- Reinforced influent tubing
- Pressure regulator
- Pressure gauge
- Reinforced inlet tubing
- Envirochek™ capsule
- Reinforced outlet tubing
- Flow totalizer (mechanical or graduated collection device)
- Flow control valve or flow rate meter with valve
- Effluent tubing to drain

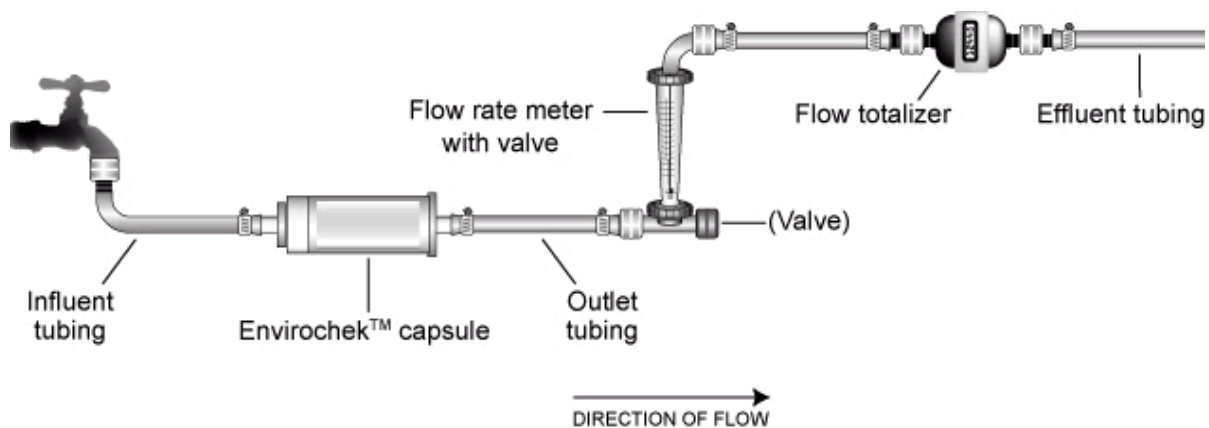
**Figure 1. Sample System Setup for Collecting *Cryptosporidium* Samples from a Pressurized Source (> 20 psig)**



For low pressure (1 to 20 psig) sites, the sampling system should be assembled at the sample port valve in the following order, as shown in **Figure 2** below :

- Influent tubing
- Envirochek™ capsule
- Outlet tubing
- Flow rate meter with valve
- Flow totalizer (mechanical or graduated collection device)
- Effluent tubing to drain

**Figure 2. Sample System Setup for Collecting *Cryptosporidium* Samples from a Pressurized Source (1 - 20 psig)**















2.1.5 Connect the sampling system, with the connector in place of the Envirochek™ capsule, to the pressurized water system, using appropriate fittings and clamps.

2.1.6 Slowly turn the tap to fully open. Allow a minimum of 20 L to flush the system. During this period, perform the following steps:

2.1.6.1 Measuring flow rate with the flow rate meter or the flow totalizer and a stopwatch, adjust the flow rate to approximately 2 L/min (approximately 0.5 gpm). Using the pressure regulator, adjust the pressure to a maximum of 30 psi if using the Envirochek™ or 60 psi if using the Envirochek™ HV. Observe the system for leaks and take the necessary corrective action if any are present.

2.1.6.2 Record the following information on the sample collection form:




-  Public water system (PWS) name
-  PWS address
-  Sampler name
-  Sample ID (optional)
-  Public Water System Identification (PWS ID) number
-  Public Water System facility ID number

-  Facility name
-  Sample collection point ID
-  Sample collection point name
-  Sample collection date
-  Source water type (optional [but required for *E. coli* sample forms])
-  Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both “*Cryptosporidium* field sample” and “*Cryptosporidium* matrix spike” sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.1.6.3 After the system has been flushed, any optional water quality parameters such as temperature, pH, and/or turbidity should be measured now.


2.1.7 Turn off the water at the sample port valve when the flow rate has been adjusted and the system has been flushed.

2.1.8 Record the following information on the capsule filter label with a waterproof pen:

-  PWS ID
-  Facility name
-  Date of sample collection

Cover the label with clear packing tape to keep the label dry.

2.1.9 Record the following information on the sample collection form:

-  Current meter reading

2.1.10 Remove the connector and in its place, install the Envirochek™ capsule in line, securing the inlet and outlet ends with the appropriate fittings/clamps.

**Note!** Retain the vinyl caps provided with the Envirochek™ capsule. These caps will be needed to seal the capsule for shipment.

2.1.11 Slowly turn on the pressurized water source. Record the following information on the capsule filter label or sample collection form:



-  Start time

2.1.12 Vent the residual air in the capsule using the bleed valve by turning it counter-clockwise. When the filter housing is full of water, close the bleed valve.

2.1.13 Monitor the water meter. When the targeted volume (actual sample volumes will be dependant on the utility, but volumes are typically 10 L [2.64 gal] to 50 L [13.2 gal]) has passed through the Envirochek™ capsule, shut off the water source. Allow the pressure to decrease until the water stops.

2.1.14 Record the following information on the capsule filter label and/or sample collection form:

-  Stop time (when the water was shut off)

-  Final meter reading
-  Comments to laboratory, if needed

- 2.1.15 With the capsule inlet pointed up, loosen the outlet end of the Envirochek™ capsule and allow water to drain as much as possible. Water drainage from the capsule through the outlet is acceptable, as the sample has passed through the membrane. Opening the vent valve during the draining will speed the process. Be sure to close the valve when finished.
- 2.1.16 Disconnect the inlet end of the Envirochek™ capsule, making sure not to spill any of the water remaining in the capsule through the inlet port. This water is part of your sample.
- 2.1.17 Seal the inlet of the capsule with the vinyl end cap that was previously saved.
- 2.1.18 Seal the outlet of the capsule with the vinyl end cap that was previously saved. Place the Envirochek™ capsule in a plastic ziplock bag for shipment.
- 2.1.19 Immediately following sample collection, place the bag containing the filter housing in a refrigerator to chill prior to packing the shipping cooler for shipment. If no refrigerator is available, and the sample will not be shipped for several hours, place the bag in the shipping cooler with ice to chill. Replace the ice before shipping.

**Note!** Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be <10°C (but not frozen), and the laboratory must have the filter processed within 96 hours of sample collection. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.

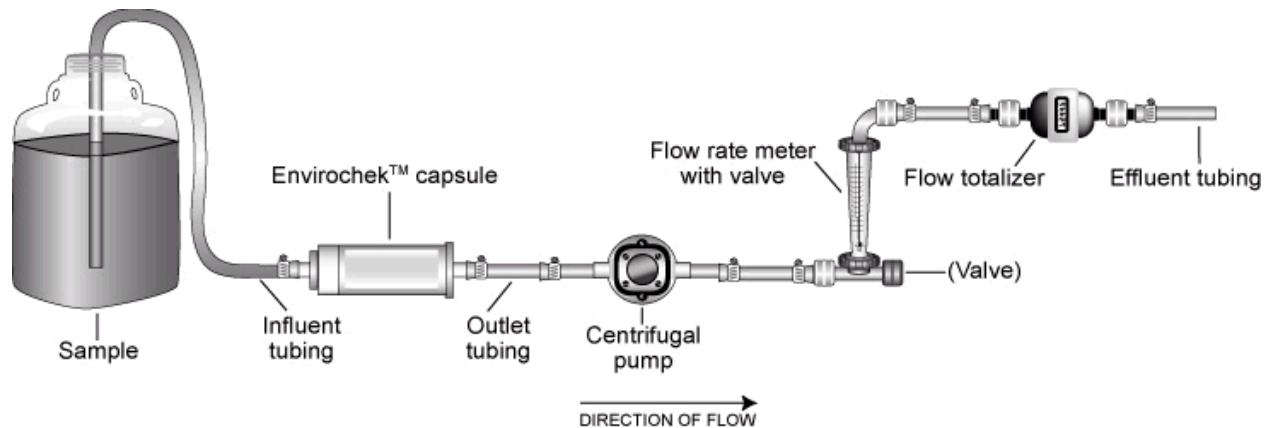
As a recommendation, it is best to have the filter processed by the laboratory within 24 hours of sampling.

## 2.2 Sample Filtration Using an Unpressurized Source

- 2.2.1 Put on a pair of powder-free latex gloves to prevent contamination from outside sources. Any contamination of the sampling apparatus may bias the final results.
- 2.2.2 If sampling from a source of unlimited volume, it may be desirable to pre-flush the sampling system. Assemble the sampling system, minus the Envirochek™ capsule. In place of the Envirochek™ capsule, insert a 0.5-in. coupling to connect the influent tubing to the inlet tubing of the pump. The sampling system should be assembled in the following order, as shown in **Figure 3** below:



- Influent tubing
- Envirochek™ capsule
- Outlet tubing
- Centrifugal or peristaltic pump
- Tubing
- Flow rate meter with valve
- Flow totalizer (mechanical or graduated collection device)
- Effluent tubing to drain

**Figure 3. Sample System Setup for Collecting *Cryptosporidium* Samples from an Unpressurized Source**



When assembling sample chain, make sure that it is as airtight as possible in order to create a vacuum. To accomplish this, make sure that clamps are used at each connection and that rubber washers are inserted into the hose connections on the inlet and outlet ends of the centrifugal pump.




- 2.2.3 Place the inlet end of the inlet tubing in sample source, away from any walls, bottom, or other environmental surfaces.
- 2.2.4 Turn on the pump and allow a minimum of 20 L to flush the system. If sampling source water from a carboy, continuously refill the carboy as necessary to flush the system. As a recommendation, the carboy should not be removed from the sampling chain and should be refilled using a separate container. Observe the system for leaks and take the necessary corrective action if any are present. During this period, perform the following steps:
  - 2.2.4.1 Measuring flow rate with the flow rate meter or the flow totalizer and a stopwatch, adjust the flow rate to approximately 2 L/min (approximately 0.5 gpm) by varying the pump speed or adjusting the valve (if pump is not variable speed).
  - 2.2.4.2 Record the following information on the sample collection form:
    - ✎ Public water system (PWS) name
    - ✎ PWS address
    - ✎ Sampler name
    - ✎ Sample ID (optional)
    - ✎ Public Water System Identification (PWS ID) number
    - ✎ Public Water System facility ID number
    - ✎ Facility name
    - ✎ Sample collection point ID
    - ✎ Sample collection point name
    - ✎ Sample collection date

-  Source water type (optional [but required for *E. coli* sample forms])
-  Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both “*Cryptosporidium* field sample” and “*Cryptosporidium* matrix spike” sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.2.4.3 After the system has been flushed, any optional water quality parameters such as temperature, pH, and/or turbidity should be measured now.


2.2.5 Turn off the pump when the flow rate has been adjusted and the system has been flushed.

2.2.6 Record the following information on the capsule filter label:

-  PWS ID
-  Facility name
-  Date of sample collection

Cover the label with clear packing tape to keep the label dry.

2.2.7 Record the following information on the sample collection form:

-  Current meter reading

2.2.8 Install the Envirochek™ capsule in line, securing the inlet and outlet ends with the appropriate fittings/clamps.

**Note!** Retain the vinyl caps provided with the Envirochek™ capsule. These caps will be needed to seal the capsule for shipment.

2.2.9 Refill the carboy with the sample to be filtered. As a recommendation, the carboy should not be removed from the sampling chain and should be refilled using a separate container.




2.2.10 Turn on the pump. Record the following information on the capsule filter label and/or sample collection form:

-  Start time

2.2.11 Monitor the water meter continuously refilling the carboy as needed. When the targeted volume (actual sample volumes will be dependant on the utility, but volumes are typically 10 L [2.64 gal] to 50 L [13.2 gal]) is reached, remove tubing from the carboy. Allow pump to evacuate water from capsule and then shut off the pump.



2.2.12 Record the following information on the capsule filter label and/or sample collection form:

-  Stop time (when the pump was shut off)
-  Final meter reading
-  Comments to laboratory, if needed

2.2.13 With the capsule inlet pointed up, loosen the outlet end of the Envirochek™ capsule and allow water to drain as much as possible. Water drainage from the capsule through the outlet is acceptable, as the sample has passed through the membrane. Opening the vent valve during the draining will speed the process. Be sure to close the valve when finished.

2.2.14 Disconnect the inlet end of the Envirochek™ capsule, making sure not to spill any of the water remaining in the capsule through the inlet port. This water is part of your sample.

2.2.15 Seal the inlet of the capsule with the vinyl end cap that was saved previously.

2.2.16 Seal the outlet of the capsule with the vinyl end cap that was saved previously. Place the Envirochek™ capsule in a plastic ziplock bag for shipment.

2.2.17 Immediately following sample collection, place the bag containing the filter housing in a refrigerator to chill prior to packing the shipping cooler for shipment. If no refrigerator is available, and the sample will not be shipped for several hours, place the bag in the shipping cooler with ice to chill. Replace the ice before shipping.

**Note!** Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be <10°C (but not frozen), and the laboratory must have the filter processed within 96 hours of sample collection. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.


As a recommendation, it is best to have the filter processed by the laboratory within 24 hours of sampling.

### **3.0 Packing the Sample**

3.1 Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, create two 4-pound ice bags by putting half an 8-pound bag of ice into two separate ziplock bags. To prevent leaks place each ice pack into an additional ziplock bag. Gel packs or blue ice may be used instead of wet ice, as long as the sample is maintained in the appropriate temperature range. Seal the ziplock bags, expelling as much air as possible, and secure top with tape.

**Note!** Shipping companies may delay sample shipments if leakage occurs. Double liners and ziplock bags around ice will prevent leakage and delays.

3.2 Place the capsule in a plastic ziplock bag (if this step has not already been performed) and place the bag in the cooler. Place the ice packs around, but not on, the bag containing the capsule to prevent freezing.

- 3.3 If you will be monitoring sample temperature during shipment, place in the cooler the temperature monitoring device (e.g., extra sample bottle for measuring sample temperature upon receipt at the laboratory, thermometer vial, or ThermoChron™ iButton). Seal each liner bag by twisting top of bag and tying in a knot.
- 3.4 Peel the backing off one of the plastic airbill sleeves and attach the sleeve to the inside of the cooler lid.
-  Sign and date the sample collection form.
- Fold the completed sample collection form, and place it inside the plastic sleeve.
- 3.5 Close the cooler lid, seal the horizontal joints with duct tape, and secure the lid with tape by taping the cooler at each end, perpendicular to the seal.
- Note!* Shipping companies may delay sample shipments if leakage occurs. Be sure to seal the cooler joints.
- 3.6 Peel the backing off of the second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

#### **4.0 Shipping and Tracking**

- 4.1 Ship samples on the day of collection and use a reliable shipping service for next-day delivery. If samples are not shipped the day of collection, the sample must be maintained at <math><10^{\circ}\text{C}</math> (but not frozen) by chilling in a refrigerator or cooler filled with ice.
- 4.2 Contact the laboratory to notify them of the sample shipment. Request that the laboratory contact you the next day if the sample is not received.
- 4.3 Using the airbill number on the shipper's copy of the airbill, track the sample shipment using the shipping company's web page or by contacting the shipping company over the phone.
- 4.4 If problems are encountered with the shipment, communicate with the shipping company to resolve, and update the laboratory regarding the status of the shipment.