

## FIELD SAMPLING

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Sampling for microorganisms requires that sampling equipment be clean and sterile and that attention to sterile technique be followed. For more details on sampling for microorganisms, the reader is referred to U.S. Environmental Protection Agency (1978), Myers and Sylvester (1997), and Francy and others (2000), parts of which are included below. Details and references for the recommended analytical methods can be obtained at [Analytical and Field Methods](#).

### **Streamwater sample collection**

**Streamwater sampling technique.** The spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles.

- When collecting a sample for microorganisms, follow equal-width increment (EWI) or equal-discharge increment (EDI) methods described in Edwards and Glysson (1988), whenever possible, to ensure that a sample is representative of the flow in the cross section.

Because churn and cone splitters cannot be autoclaved, use a sterile 3-L bottle to composite subsamples when using EDI and EWI methods. If possible, composite by collecting subsamples at vertical locations in the cross section without overfilling the bottle.

- Collect a sample by the hand-dip method if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, the stream is well mixed, and (or) project objectives do not require an EWI or EDI sample.
  - Open a sterile bottle; grasp the bottle near the base, with hand and arm on downstream side of bottle.
  - Without rinsing, plunge the bottle opening downward, below the water surface. Allow the bottle to fill with the opening pointed slightly upward into the current.

- Remove the bottle with the opening pointed upward toward the water and tightly cap it, allowing about 2.5 to 5 cm of headspace.
- This procedure minimizes collection of surface film and avoids contact with the streambed.

Streamwater—Bacterial indicators and coliphage. Samplers that may be used to collect streamwater for bacterial indicators and coliphage include the US DH-95, US D-95, US D-96, US DH-81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

- Clean the equipment coming in contact with the water with dilute nonphosphate, laboratory-grade detergent, rinse it three times with hot tap and three times with deionized or distilled water, and sterilize it (preferably by autoclaving).
- Prepare a separate set of sterile equipment (bottles, nozzles, and caps) for sampling at each site.
- Collect approximately 800 mL of streamwater for bacterial indicators and coliphage.
- Leave at least an inch of headspace in the bottle to allow adequate mixing and aeration.
- Process the samples for *E. coli* and (or) enterococci within 6 hours. Store sample on ice in cooler or refrigerator before processing.
- For coliphage and *Clostridium perfringens*, send samples (at least 500 mL) on ice to the qualified laboratory to arrive within 48 hours after collection.

For *E. coli* in streamwater, use the mTEC, modified mTEC, or Colilert Quantitray methods. For enterococci, the mEI method is recommended, although the mE method also may be used. For *Clostridium perfringens*, use the mCP method. For coliphage, the single-agar layer method (USEPA Method 1602) and 100-mL sample volumes are recommended. This method quantifies somatic and F-specific coliphage in water.

Streamwater—*Cryptosporidium* and *Giardia*. The standard samplers used in the USGS can be used to collect streamwater samples for *Cryptosporidium* and *Giardia*. Special sterilization procedures, however, are needed for equipment used in the collection of samples for *Cryptosporidium* and *Giardia*. Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection. The following procedure is best done in the office with dedicated sampling equipment for each site; it may be done in the field as long as the bleach is stored and disposed of properly.

- Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water.
- Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes.
- Wash the equipment free of residual sodium hypochlorite with three rinses of sterilize deionized or distilled water; do not dechlorinate the equipment using sodium thiosulfate.

Because the 12 percent hypochlorite solution is toxic and corrosive, alternative (but less effective) cleaning and sterilization procedures may be used. Bottles, caps and nozzles are soaked in dilute liquinox for 30 minutes, scrubbed well, rinsed 3 times with tap water to remove detergent, rinsed 3 times with deionized water, wrapped in aluminum foil, and autoclaved for 20 minutes.

For analysis of water samples for *Cryptosporidium* and *Giardia*, USEPA Method 1623 is recommended.

- Collect 10 L of streamwater using standard sampling techniques. Composite the sample in a 10-L cubitainer (Hedwin Corporation, Baltimore, MD) that is presterilized by the manufacturer.
- Send 10-L samples in a cubitainer to a qualified laboratory to arrive within 48 hours after collection
- The sample does not have to be kept on ice during transport, but it is recommended.
- Analyses for both *Cryptosporidium* and *Giardia* are done from the same 10-L sample.

Streamwater—Enteric viruses. A different procedure is used to collect samples for enteric viruses than for bacterial indicators, coliphage, or protozoan pathogens.

- Because large volumes of water are needed, water samples are filtered in the field and EDI or EWI techniques are not possible.
- Collect a sample from several sections of the cross section, if possible, by placing the pump tubing in each vertical section.

The analyzing laboratory should instruct you on how to collect the sample, provide you with a specially designed sampling apparatus containing a Virosorb 1MDS filter (Cuno, Meriden, Conn.), and advise on the quantity of water needed (usually about 100 L of streamwater).

- Use a specially designed sampling apparatus to filter the sample on site.
- Clean the sampler with dilute nonphosphate, laboratory-grade detergent, rinse it with tap water and then deionized or distilled water, sterilize by circulating 10% household bleach through the sampler for 30 minutes, neutralize by circulating a sterile sodium thiosulfate solution for 5 minutes, and then rinse with sterile deionized or distilled water to remove any residuals.
- Place a Virosorb1MDS filter (Cuno, Meriden, Conn.), which removes viruses present in the water by charge interactions, in the sterile cartridge housing.
- After sampling, keep the 1MDS filter within the cartridge housing on ice. Send the cartridge housing and 1MDS filter to a qualified laboratory, to arrive within 48 hours after collection, for virus elution, concentration, and detection.

Samples can be analyzed by a molecular technique such as reverse-transcriptase-polymerase chain reaction (RT-PCR) and (or) a cell-culture method (U.S. Environmental Protection Agency, 1996). A method that incorporates both techniques is integrated cell culture-polymerase chain reaction (ICC-PCR) (Reynolds and others 1996). Molecular

techniques target specific viruses, but cannot be used to detect the infectious state of a virus; it is usually a presence or absence method. The cell culture method detects infectious viruses, but does not identify the type of virus. Cell culture methods require about 2 weeks to complete, whereas RT-PCR results are available in a few days.

### **Ground-water sample collection**

Ground water sampling technique. Collecting ground-water samples by use of sterile technique requires knowledge of the type of well, its use, its construction, and its condition. Samples are collected once purging criteria have been met as described in Koterba and others (1995). Collect samples for analysis of microorganisms after all other water-quality sampling has been completed.

- In sampling wells with a dedicated pump, collect the sample directly from the tap into a sterile container.
- Swab the inside and outside rim of the tap with ethanol. Flame sterilize the tap and allow to dry and cool. Rinse the tap with sterile deionized or distilled water.

A tap that yields water directly from the well and before entering any kind of holding tank is preferred. Water collected after treatment is unsuitable for microbiological analysis.

For wells without in-place pumps, samples should be obtained by use of the following methods (in descending order from most to least desirable): (1) a peristaltic or vacuum pump with autoclavable silicon tubing, (2) a sterile bailer, (3) a chlorine-disinfected pump and tubing, or (4) a detergent-cleaned pump and tubing. Refer to Myers and Sylvester (1997) for a detailed discussion of ground-water sampling for microbiological analysis.

- If using a detergent-cleaned pump and tubing, collect additional field blanks to evaluate the effectiveness of the cleaning procedure.

Ground water—Bacterial indicators and coliphage. In sampling ground water for bacterial indicators and coliphage, simply fill a clean and sterile sample bottle directly from the tap or line.

- Use any autoclavable Teflon, glass, or polypropylene sample bottle.
- Clean the sample bottle and equipment coming in contact with the water with dilute nonphosphate, laboratory-grade detergent, rinse it three times with hot tap water and then three times with deionized or distilled water, and sterilize it by autoclaving.
- Prepare a separate set of sterile equipment for sampling at each site.
- Collect approximately 800 mL for bacterial indicators. Two 100-mL aliquots are recommended plating volumes for ground water.
- Leave at least an inch of headspace in the bottle to allow adequate mixing and aeration.
- Process the samples for total coliforms, *E. coli*, and (or) enterococci within 6 hours.

- For *Clostridium perfringens*, send samples on ice to the qualified laboratory to arrive within 48 hours after collection. A 500-mL to 1-L plating volume is recommended.
- For coliphage, collect a 1-L sample volume. Send the sample on ice to the qualified laboratory to arrive within 48 hours after collection.

For total coliforms and *E. coli* in ground water, use the mENDO/NA-MUG, the MI, or the Colilert Quantitray or Colilert presence/absence methods. For enterococci, the mEI method is recommended, although the mE method may also be used. For *Clostridium perfringens*, use the mCP method. For coliphage, the two-step enrichment procedure (USEPA Method 1601) and 1-L sample volumes are recommended. This method determines the presence or absence of somatic and F-specific coliphage in water.

Ground water—*Cryptosporidium* and *Giardia*. In most circumstances, sampling ground water for *Cryptosporidium* and *Giardia* is not recommended. If ground water is under the influence of surface water or suspected to be contaminated from protozoan pathogens, follow streamwater sampling instructions with some obvious modifications.

Ground water—Enteric viruses. A different procedure is used to collect samples for enteric viruses than for bacterial indicators or coliphage.

- Because large volumes of water are needed, water samples are filtered in the field.
- Collect a sample by attaching the intake line from a specially designed sampling apparatus directly to the tap or by inserting the intake line down the wellhead.

The analyzing laboratory should instruct you on how to collect the sample, provide you with a specially designed sampling apparatus containing a Virosorb 1MDS filter (Cuno, Meriden, Conn.), and advise on the quantity of water needed (usually about 2,000 L of ground water).

- Use a specially designed sampling apparatus to filter the sample on site.
- Clean the sampler with dilute nonphosphate, laboratory-grade detergent, rinse it with tap water and then deionized or distilled water, sterilize by circulating 10% household bleach through the sampler for 30 minutes, neutralize by circulating a sterile sodium thiosulfate solution for 5 minutes, and then rinse with sterile deionized or distilled water to remove any residuals.
- Place a Virosorb1MDS filter (Cuno, Meriden, Conn.), that removes viruses present in the water by charge interactions, in the sterile cartridge housing.
- After sampling, keep the 1MDS filter within the cartridge housing on ice. Send the cartridge housing and 1MDS filter to a qualified laboratory, to arrive within 48 hours after collection, for virus elution, concentration, and detection.

Samples can be analyzed by a molecular technique such as reverse-transcriptase, polymerase chain reaction (RT-PCR) and (or) a cell-culture method (U.S. Environmental Protection Agency, 1996). A method that incorporates both techniques is integrated cell culture polymerase chain reaction (ICC-PCR) (Reynolds and others 1996). Molecular techniques target specific viruses but cannot be used to detect the infectious state of a

virus; it is usually a presence or absence method. The cell culture method detects infectious viruses, but does not identify the type of virus. Cell culture methods require about 2 weeks to complete, whereas RT-PCR results are available in a few days.

## **REFERENCES**

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## Virus sampling apparatus

Pump  
Intake line  
Prefilter  
Pressure regulator  
Pressure gage  
1 MDS filter  
Water meter  
Flow constrictor  
Discharge line



## Protozoan sampling

Collect 10 liters of water by compositing into a sterile cubitainer

