Field Methods For

Giardia and *Cryptosporidium*

Sample Collection

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1. Introduction

Freshwater is an essential resource throughout the world. Humans require water for drinking, recreation, aesthetics, food processing, agriculture, and industry (Warrington 1994). Aquatic and terrestrial wildlife, both plant and animal, needs freshwater to survive.

Canada enjoys the highest per capita freshwater availability in the world (Homer-Dixon 1992). Population growth and development create monumental demands for adequate clean freshwater (Wetzel 1983). Freshwater availability heavily influences patterns of settlement. Limitations in quantity and quality of water affect potential land usage. Drinking water requirements often have the most stringent water quality criteria in British Columbia (Nagpal 1995).

Water may be unsuitable for drinking due to many factors. Unpleasant flavors, odors, colors, or turbidity may render water unpalatable. More importantly, chemicals or organisms that adversely affect health may be present. Pathogens such as viruses, bacteria, worms, and protozoa occur naturally in freshwater and can be a serious health hazard to humans.

Protozoans are a significant problem in drinking water, because they can cause major public health problems. *Giardia* and/or *Cryptosporidium* (G/C) have caused recent epidemics of gastrointestinal disease in British Columbia, Ontario, and the United States. In the 1993 *Cryptosporidium* outbreak in Milwaukee, Wisconsin, approximately 403,000 people became ill, 4,400 were hospitilized, and at least 69 were killed (Solo-Gabriele and Neumeister 1996).

Giardia lamblia and *Cryptosporidium parvum* are parasitic, intestinal protozoans responsible for disease outbreaks in humans (Warrington 1988). When ingested in contaminated water, they cause giardiasis ("beaver fever") and cryptosporidiosis. Symptoms include diarrhea, abdominal cramps, nausea, vomiting, chills, fever, dehydration, headaches, and malaise. Both parasites produce cysts that withstand harsh environmental conditions, lying dormant until ingestion. The levels of chlorine normally used to disinfect drinking water do not kill cysts. Both organisms reproduce in humans, domestic pets, livestock, and wildlife, then are shed in fecal matter and spread via contaminated water (Anon. 1996). Healthy individuals usually recover naturally from these parasites in less than a month. Children, seniors, and immunocompromised individuals (*i.e.* AIDS or chemotherapy patients) can suffer prolonged and life-threatening infections (Solo-Gabriele and Neumeister 1996).

Giardia and *Cryptosporidium* generally occur in very low numbers in aquatic environments (West 1991). Therefore, testing potential drinking water sources requires filtering large volumes of water through small-pore filter cartridges (1µ nominal). The cysts are then extracted from the filters for examination. Methods are complex, inefficient, and imprecise (Isaac-Renton 1995, Reofer *et al.* 1996). Recovery efficiencies averaged 2.9 percent (range 0.8 to 22.3 percent) in studies of commercial laboratories (Reofer *et al.* 1996). The maximum recovery expected with this study is 25 percent (Wallis 1997).

No standard methods for G/C sampling are currently available. This report details a practical field method for G/C determination in freshwater, based on methods used by other agencies (Isaac-Renton 1995) for field surveys.

2. General Considerations

2.1. Preparing to Go to the Field

These methods were developed for a project sampling ambient sites in relatively remote areas. This is in contrast to most *Giardia/Cryptosporidium* sampling, which is typically done near or at water treatment plants.

Preliminary site selection can be accomplished using detailed maps (e.g. TRIM, NTS topographical, and BC Forest Recreation). Choose sites that represent the variety of conditions found in the watersheds being studied. Include small and large streams and lakes, major rivers and lesser tributaries, headwaters and lower reaches. Pick sites upstream and downstream of potential sources of impact on water quality. Choose areas that experience the range of impact from forestry operations (*i.e.* recently clearcut to pristine) or other land disturbances such as agriculture or settlement. Access to the water is generally most expedient at bridges, campsites, and boat launches. When these are not available, access can be from roadsides near to streams, but considerable road dust tends to contaminate such sites. Samples should be collected upstream of bridges to avoid bridge and traffic contamination.

2.2. Locating Sites in the Field

The exact location of field sites should be carefully considered. Suitable sites should take only a few minutes to access on foot. Safety is the primary concern when choosing how to access the sampling site from the road. Look for road access points that are at or near the same elevation as the water body being sampled. Look for well-used paths, avoiding steep slopes with loose rock. Make detailed notes about the access used. Mark the access point with flagging tape to help relocate the site on subsequent visits. Record directions and roads travelled to get to the sites. It is useful to clock the mileage between sites and to easily-identified landmarks for future reference.

2.3. Field Notes/Observations

Detailed field notes aid immensely in the interpretation of the results of G/C sampling. Useful to note are: GPS position, elevation, date, time, weather, temperature, station depth, stream velocity, water color, estimated turbidity, algal or periphyton growth, aquatic organisms (*i.e.* fish, insects), and any other distinguishing features. Include all field measurements taken (*i.e.* dissolved oxygen, water temperature). Site photographs showing access point from the road, and views downstream, upstream, and across stream are helpful (Boyle 1996, Pehl 1997).

3. Quality Assurance/Quality Control

3.1. Field Quality Assurance

To ensure the quality of research results, it is essential to protect samples from contamination and deterioration in the field. Proper field procedures, techniques, and sample handling must be followed (Cavanagh *et al.* 1996). Truly representative water samples must be free from

sediments and dust, as cysts accumulate in them (Hibler 1988). Protect filters from road dust by storing them in plastic bags. Avoid contacting filters with bare hands. Choose intake sites carefully to avoid contamination from disturbed sediments. If sediments are disturbed while placing intake hose, allow them to settle before beginning filtration. Maintain correct flow rates to capture maximum cysts (see protocol). Once sample is collected, cool immediately and keep cool until analysis. Deliver samples to laboratory promptly. Thoroughly clean and decontaminate equipment between samples.

3.2. Field Quality Control

3.2.1. Replicate Samples

Sequential replicate samples test the variation in cyst concentrations at a site at any given time (the heterogeneity of the sample source water). They can be collected using one filtration apparatus. Time limitations in the field may make this technique impractical.

3.2.2. Split Samples

A single sample can be split between filters in order to obtain identical samples. These samples are used to test the variability between laboratories or the consistency within a laboratory (Cavanagh *et al.* 1996). An effective device for splitting samples is shown in Figure 1. This configuration requires two additional hoses – one from the pump to the second filter housing, the other as an outlet. Only one intake hose and pump are required (flow rate is reduced). Using an in-line "Y" allows the source water to be randomly divided between two filters. If the filter housings are placed at the same height, the flow rate will be the same through both filters. Flow rates can be also be adjusted by valves in the "Y".

Figure 1. Configuration of apparatus for splitting G/C samples.

4. Filtration Apparatus

A basic, lightweight G/C filtering apparatus is shown in Figure 2. The essential components are a filter, filter housing, and flow meter. More complex units are available, but may be impractical for remote locations or extensive field studies.

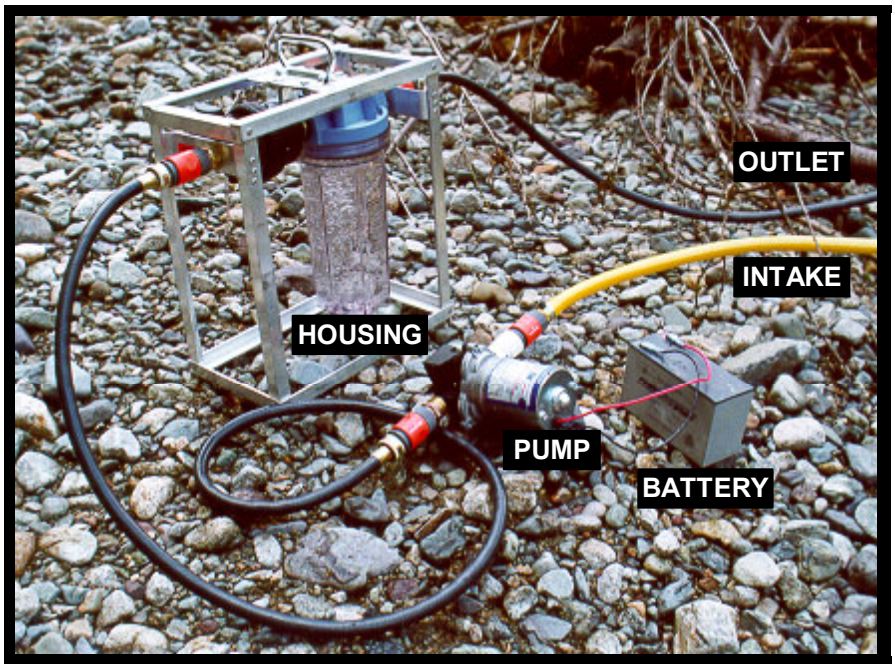

Figure 2. G/C field filtration apparatus.

The filters used in G/C sampling are 25-cm (10") polypropylene, string-wound cartridges. They must have a nominal pore size of 1 µ. A standard drinking water filter housing holds the filter. A mechanical water flow meter installed in line measures the volume of water filtered, so that cyst concentrations can be determined.

Water from a stream or lake must be pumped through the filter. A SHURflo® 12V diaphragm pump with a 9.5 litres/min (2.5 US gal) flow rate, or similar pump, is recommended (available from Van Isle Water Services, 600B Frances Ave., Victoria, BC). A small, gel-pack, 12V battery powers the pump and can be recharged overnight. The pump is connected to the filtration unit with a high-pressure (laundry) hose. Using standard, NPS threaded (¾"), garden fittings allows the use of readily available accessories such as quick-connectors, flow control valves, and splitters ("Y"s). The intake hose should have a coarse screen to prevent leaves and debris from clogging and damaging the pump. An outlet hose completes the equipment.

Sealable bags ("Ziplock") and labels are needed to store the filters after the sample is collected. The labels should indicate the site name, site number, and date sample was collected.

5. Collecting Samples

PROTOCOL

Place the intake hose end in a location where sediments are undisturbed. The end should be positioned at the appropriate sample depth (usually 0.1m in streams, 0.5m in lakes), elevated above the substrate surface, and away from any edge influences. Ensure that the outlet hose discharges downstream of the intake location.

Connect all the components as shown in Figure 1, without a filter. Make sure the filter housing and all hose fittings are tight. A small amount of Vaseline on the housing O-ring improves the seal. Check that the flow will be in the correct direction through the apparatus.

Connect the pump to the battery and record the time and flow meter reading. Flush the apparatus with at least 50 litres of source water. Disconnect the battery.

Unscrew the filter housing. Without touching the filter (hold with plastic bag or gloves), place it in the housing. Replace housing (containing filter), ensuring black O-ring is in place. Connect the battery and record time and meter reading. Filter at least 100 litres of raw source water. If water is clear and time permits, up to 1000 litres can be filtered. Filter smaller volumes in turbid waters, because turbidity interferes with recovery of cysts. The optimum flow rate is 6 litres/min, but 4 to 10 litres/min is acceptable. Adjust with a flow control valve, if necessary. When the required volume has been filtered, disconnect the battery and record the time and meter reading.

The sample filter can now be removed and placed into a sealable plastic sample bag. It is important not to touch the filter, as it may contain viable cysts. Wear gloves or invert the bag over your hand before grasping the filter. Include a small amount of sample water in the bag to keep the filter moist. Each filter must be placed in a separate clean, labelled bag. Seal the bag and quickly place in a cooler containing ice packs. Keep cool until analysis.

Drain water from housing, pump, and hoses before storing. Follow cleaning/decontamination procedures after collecting the last sample each day. Fill out a laboratory requisition for each site, including site name and number, date, volume filtered, and duration filtered.

6. Shipping

Samples must be kept cool until analysis, either refrigerated or in coolers containing ice packs. Coolers can be delivered to the analytical laboratory via courier or air cargo. For best results, the laboratory should receive samples within 24 hours. If shipping is delayed, it is crucial that samples be kept cool. Cysts may be detected in several-day-old samples, if stored properly (Wallis 1997).

7. Cleaning/Decontaminating Equipment

The filtration apparatus, pump and all hoses used must be cleaned between samples. When several samples are being collected in one day, clean by flushing thoroughly with at least 50 to 60 litres of source water at each new site before inserting the filter. This procedure is effective as long as the equipment does not dry out between sites. At the end of each sampling day, the filtration apparatus must be decontaminated. To decontaminate, set up equipment as for sampling and pump at least 50 litres of hot, soapy (dish detergent) water through apparatus. Drain water, and then pump 50 litres of hot, clear water through. Disassemble, drain, and air dry overnight.

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Appendix 1 G/C Field Checklist

GENERAL:

Field book___
Pencils___
Waterproof felt markers ___
Maps ___
Tape: packing ___ masking ___
GPS ___
Camera ___
Coolers: Lg ___ Sm ___
Ice packs ___
Requisition forms ___
Gloves ___
Flagging tape ___
Shipping labels ___
Waybills ___

G/C SAMPLING EQUIPMENT (clean/decontaminated):

Filtration apparatus ___
Filters ___
Hoses: Intake ___
Outlet ___
Connecting ___
Quick-connectors ___
Pump ___
Batteries: small ___ large____
Battery charger ___
Detergent ___
Sealable plastic bags ___
Labels for bags ___

SAFETY:

First aid kit ___
Latex gloves ___
Radio & manual ___
Rope ___

PERSONAL GEAR:

Rain gear ____
Patch kit ___
Gum boots ___
Waders (hip, chest) ___
Toilet paper ___
Flashlight ___
Food ___
Knife ___
Hat/shades ___
Sun screen ___
Mosquito spray ___

Appendix 2 G/C Sampling Protocol Shortlist

1. Position intake hose correctly
2. Place outlet hose downstream
3. Connect components tightly
4. Check flow direction
5. Connect battery
6. Record time and meter reading
7. Flush with ≥ 50 litres source water
8. Disconnect battery
9. Insert filter (do not touch)
10. Replace housing and filter
11. Connect battery
12. Record time and meter reading
13. Filter 100–1000 litres source water at 4-10 litres/min
14. Disconnect battery
15. Record time and meter reading
16. Remove filter (do not touch)
17. Seal filter in labelled bag with some water
18. Place filter in cooler
19. Drain apparatus
20. Clean/decontaminate equipment