FIELD CONCENTRATION (SAMPLING) PROTOCOL FOR *CRYPTOSPORIDIUM* AND *GIARDIA* FROM PRESSURIZED SOURCES

Note: This protocol does not consider any *E. coli* sampling that may be required under LT2ESWTR.

EQUIPMENT

Field Concentrating Apparatus.

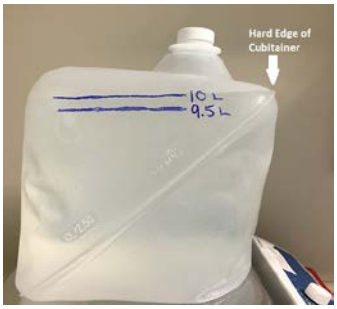
- Influent Hose with Pressure Regulator (<60 psi).
- "Hose Mender" device (only used if influent and effluent hose need to be flushed prior to sampling).
- Capsule Filter (Gelman HV Envirochek®). Back-up Capsule Filter (in case of clogging).
- Effluent Hose with Flow Restrictor (1/2 gpm).

10 Liter Cubitainer (10 liter, to capture and measure effluent for 10 Liter samples). For higher volume sample, an alternate effluent measuring device must be provided by sampler.

Sterile Gloves.

Re-sealable Bags (4, 2 per filter).

Bubble Wrap.



SAMPLING

(Using Envirochek® HV Capsule Filter).

Set-up:

Put on pair of gloves (powder free to prevent contamination). Flush stagnant water from tap until temperature and turbidity stabilizes (2-3 minutes). Record Temperature and Turbidity on LT2 Chain of Custody Record.

Connect *Influent* hose to pressurized source (garden hose thread tap).

Turn on tap and flush for 2-3 minutes.

Complete appropriate areas of LT2 Chain of Custody Record.

Turn off flow using ball valve (or main tap if more convenient).

Remove Capsule Filter from bag. Remove and KEEP end-caps. End-caps will be re-installed after completion of sampling.

Install capsule filter (note Flow Direction) to Influent and Effluent hoses using Quick Clamps.

Prepare cubitainer to catch filter effluent (take care not to tear cubitainer). Pull to extend recessed cap and neck from cubitainer body. Cubitainer will be kept for future use.

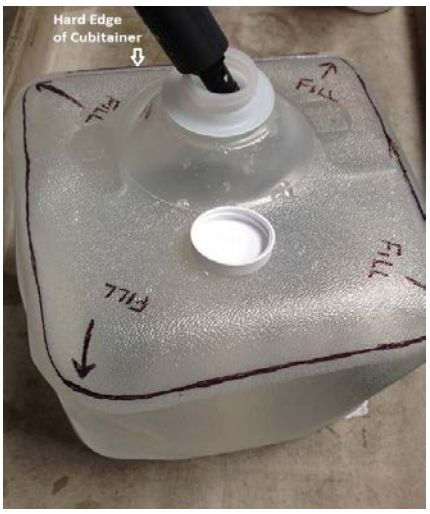
Sampling (Regular Sample)

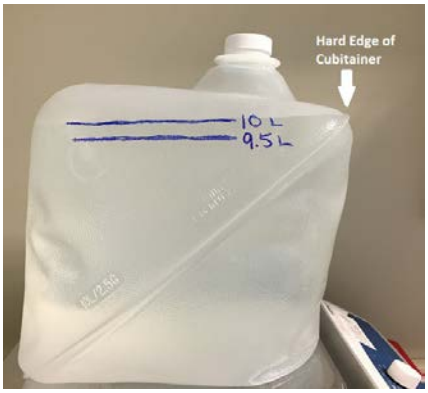
Direct effluent hose into 10 liter cubitainer.

Slowly turn on flow allowing capsule filter to completely fill. Use air bleed valve on capsule filter if necessary.

Fill cubitainer with 10 liters of filtrate (at least to top of shoulder of cubitainer). Target sample volume is 10 Liters (or more if taken consistently over entire LT2 sampling program). MINIMUM sample volume is 9.5 liters or, if filter clogging occurs, volume filtered through 2 capsule filters.

Turn off flow and allow pressure to subside.





Keeping Filter inlet above the outlet, allow water to drain from capsule filter. TAKE CARE NOT TO ALLOW BACKFLUSHING AT INLET PORT AS THIS MAY CAUSE LOSS OF ORGANISM. Open bleed valve to speed draining process.

Disconnect tubing from capsule filter.

Seal the capsule filter with end-caps, close bleed valve and place capsule filter in re-sealable plastic bag. Double bag.

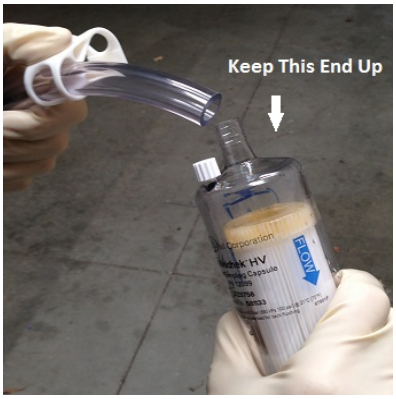
Complete Chain of Custody. Make sure to note Facility ID, Facility name, date of sample collection, filtration start & stop time, and volume filtered.

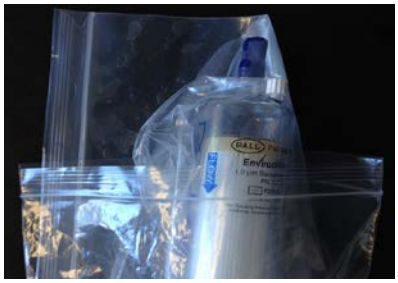
Filtrate in cubitainer can be discarded. Retain cubitainer for future use.

Sampling (Matrix Spike Sample)

Initially, and once every 20 regular samples per-site, 10 liter matrix spike samples are to be taken. Take as grab sample only and in conjunction (sample pair) with a regular field sample.

If sampling a matrix spike volume larger than 10 liters (match regular sample volume), a 10 liter grab is taken and the balance of the sample volume is concentrated through a capsule filter.





PACKING AND SHIPPING

Chill immediately. Pre-ice prior to shipment.

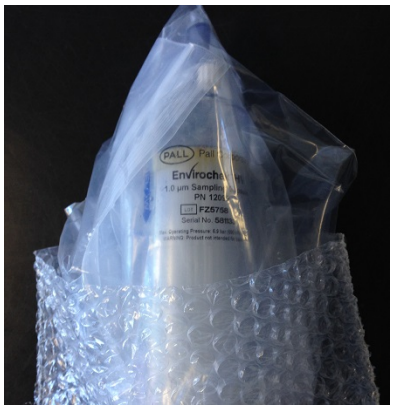
Bubble-wrap filter prior to packing.

Sample arrival at Eurofins: 1° - <20°C

Any evidence of freezing will invalidate sample.

Ship to laboratory same day and/or via overnight delivery.

Holding time: 96 hours from sample collection.



APPARATUS CLEANING PROCEDURE (adapted from the EPA LT2 Rule *Cryptosporidium* & *E. coli* Sample Collection Recommendations: A Pocket Guide.)

Thoroughly clean all reused *influent* equipment prior to the capsule filter. Use a warm detergent solution and scrubbing brush. Expose equipment to hypochlorite solution (add 25mL 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.