Standard Operating Procedure for Dead-End Ultrafiltration Water Sampling in the Field for Bacterial Pathogens

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Prepared

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1. SCOPE AND APPLICABILITY

The procedure will be used to filter up to 100 L of water in the field.

2. SUMMARY OF METHOD

Using a field-portable peristaltic pump, volumes up to 100 L of water will be passed continuously through a REXEED 25S dialysis filter (ultrafilter); the filter having a pore size small enough to capture viruses, bacteria and parasites in the water sample. Parameters such as time, date, and Sample ID will be recorded on the filter using permanent marker. The ultrafilter will be kept at 4ºC (refrigerated or on ice packs) after sample collection.

3. HEALTH AND SAFETY WARNINGS

Sterile disposable gloves are recommended to be worn during all steps of this procedure to prevent possible exposure to waterborne pathogens and contamination of the ultrafilter or filtration components. Gloves are mandatory when removing caps from the ultrafilter, attaching tubing to the filter ports or otherwise touching exposed parts of the ultrafilter.

4. EQUIPMENT AND SUPPLIES

4.1 Supplies

1) Ultrafilter-related supplies
    a. Filtrate port tubing (Note this tubing is not sold pre-sterilized, although the likelihood of pathogen presence is infinitesimal: if concern over sterility exists, contact manufacturer for autoclaving instructions or consider using an approximately 3-foot piece of tubing from same lot as a control sample that could be flushed with sterile water that is them processed as a sample)
        1. Silicone Tubing L/S 36 (sold in 25-foot rolls; Masterflex Cole-Parmer Item # 96410-36);
        2. Alternative tubing (less expensive but doesn't work as well) – Silicone Tubing, Size 36 (sold in 25-foot rolls; Geotech Environmental Equipment, Item# 77050011, http://www.geotechenv.com/)
        3. Alternative tubing (needs to be used with the smaller DIN adapter and clamp) L/S 24 tubing (Masterflex, Cole-Parmer Item # 96410-24); Small Plastic hose clamp - (Grainger # 1ENJ3, Max 0.406")
    b. Ultrafilter [REXEED 25 S filters (Dial Medical Supply)] http://dialmedsupply.com/order/index.html **IMPORTANT**: the filters are very sensitive to freezing temperatures so take care if they have been shipped outside their normal packaging that the plastic filter housing is inspected carefully prior to use to ensure no cracks are visible or that otherwise the integrity of the plastic has been compromised. Upon receipt of filters from manufacturer, be sure to check the included TransTracker®C indicator to ensure the filters are ok to use.
    c. Plastic hose clamp (Cole-Parmer # 6832-04; Min ID: 0.402", Max ID: 0.449"); See photo in Appendix A 2. Also, Grainger Item# 1ENP7; Max 0.452 can substitute
    d. DIN Adapter (Molded Products #MPC-855, sold by the set, sterile, 2 pieces per set; https://moldedproducts.com/); See photo in Appendix A
        1. Alternative DIN Adapter – larger version, not pre-sterilized, **only** used with the L/S 36 tubing (Molded Products Item #: MPC-855NS.375)
            1. Advantage – can use Zip ties instead of clamps and thus, no pliers needed – (Cole Parmer 40 Pound Solid Nylon Cable, Zip Ties, 5.5" L, White; 1000/PK; Fisher Scientific Cat. NC9269761)
    e. Blood Port Caps (Molded Products # MA-40); See photo in Appendix A. *Only used if original caps from filter are not available for reuse.*
    f. Dialysate Port Storage Cap (Molded Products #MPC-60D); Described in this document as "rubber storage cap"; See photo in Appendix A. *Only used if original caps from filter are not available for reuse*

2) 20-L cubitainers or other container with demarcations for measuring total volume passed through filter if not using a flow meter (having two containers will allow for water measurements to continue while one container is being emptied)
3) Black Permanent Marker
4) Ziploc bags
5) Spray bottle of 70% ethanol (to field-disinfect scissors, pliers, and gloved hands)
6) Pliers (to close the hose clamps)
7) Cooler or other suitable container for keeping filters chilled after collection and during transport to laboratory
8) Heavy duty tape (Gorilla Tape or equivalent since will be used to seal cooler for shipment and will need to withstand rough treatment)
9) Ice / freezer packs
10) Bench protector paper (Fisher Scientific, Cat. 14-206-65, or similar)
11) UPS or FedEx Shipping label(s) for Next Day Delivery, if shipping
12) Trash bag
13) Gloves (Sterile, disposable)
14) Scissors (to cut tubing)
15) 50 mL conical tubes or other clean, sterile container for storing clamps and caps before and after use.
16) Flat head and #1Phillips head screwdrivers (if the easy-load II pump head on geopump needs to be moved)
17) Umbrella (golf-type or other large one, if bench protector paper not available) if sampling in sunny conditions to protect ultrafilter during collection and prevent pump from overheating

NOTE: Ultrafilter sampling kits can be made ahead of time to facilitate field activities. One (1) kit is prepared per ultrafilter. See Appendix B for details.

4.2 Reagents

1) Sodium thiosulfate, Fisher Cat. No S446 (Only needed for chlorinated water)

4.3 Equipment

1) Peristaltic Pump (Geotech; http://www.geotechenv.com/peristaltic_geopump.html) with one battery (recommend purchasing extra batteries)
   a. Extra batteries for field sampling pump (Geotech – Item # 77250000)
   b. DC to AC converter for pump operation (Geotech – Item # 81400127) – this piece of equipment has alligator clips (to attach to a car battery) and a place to plug in the pump. It provides a way to power the pump in the field if the battery is dead.
2) Turbidity Meter (optional) with a resolution of 0.01 NTU, a range up to at least 200 NTU, and compliant with EPA 180.1 method or ISO 7027 standard.
3) Flow meter (optional and can be used instead of containers for measuring pump output). Recommend - Item# 113900-9511 Rust Automation & Controls Inc. Phone: 800-772-0878 rustco.com (or equivalent)

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by the US Food and Drug Administration or the Department of Health and Human Services. Alternative products may be used if they provide equivalent performance to the items cited in this SOP.

5. FILTRATION and SHIPPING PROCEDURES

5.1 Put on gloves.

5.2 In preparation for collecting the ultrafilter sample, place clean bench protector(s) on the area where sampling will be performed.

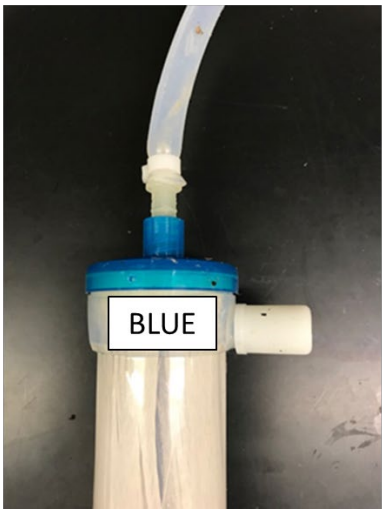
5.3 Remove ultrafilter from packaging. On the ultrafilter, write the date, time of collection, your initials, and sample number.

5.4 Place ultrafilter setup on the bench protector. The tubing is connected to the ultrafilter as shown in Figure 1 and the entire set-up with pump is shown in Figure 2 below.

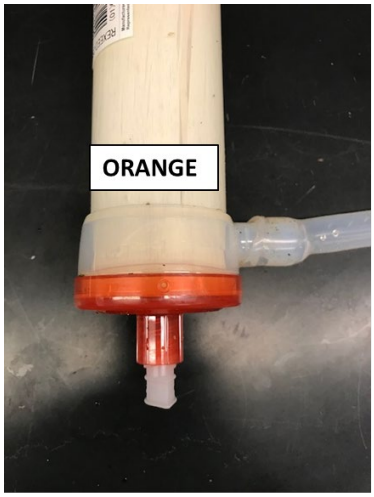
5.4.1 There is not necessarily an order to follow in terms of the set-up although it is preferred to allow the tubing to remain in the packaging for as long as possible since dirt and other debris will adhere easily to it. It may be easier to find one end of the tubing while the remainder stays in the bag during the attachment of the tubing to the filter. If using the smaller diameter DIN adapter, the clamp is placed on very end of the tubing, pinching it closed one click using fingers. The DIN adapter can be inserted next into the tubing and it should not fall out of the tubing since the clamp is closed enough to narrow the tubing opening (visually check to be sure the clamp placement is such that when closed, the clamp will cover the DIN adapter end sitting inside the tubing). Then using pliers, squeeze the clamp fully closed. If using the larger diameter DIN adapter, it will be stable enough within the tubing opening to accommodate the clamp being attached afterwards. After the DIN adapter is secured, it can be threaded into the blue end of the ultrafilter as shown in Figure 1 and the other end of the tubing cut to the desired length.

Figure 1. Ultrafilter Setup

End cap has been removed and set aside for later use. DIN adapter is screwed in and connected to new, unused L/S 36 tubing with hose clamp. Hose clamp is tightened using needle nose pliers.



Plastic cap is left on the side port (shown in photo). Ensure cap is hand-tightened so as to prevent leaking. A rubber storage cap can also be used.



Used or new L/S 36 tubing is attached to side port by pushing the end of the tubing onto the port. No hose clamp or tools are needed.

End cap is removed and set aside for later use. Blood port cap has been screwed on tightly by hand.

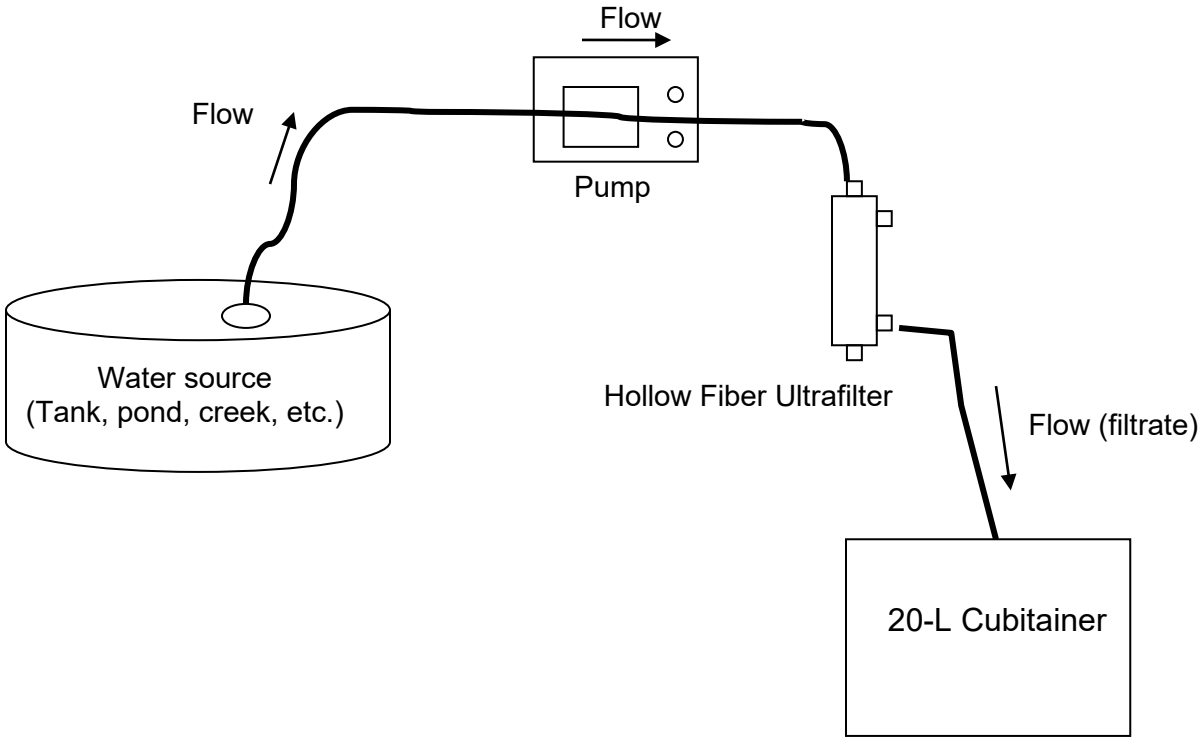
5.5 Place the tubing (L/S 36) into a 20-L cubitainer or other container for measuring total volume that has passed through the filter.

5.6 If a flow totalizer meter will be used to measure the volume of water filtered, screw a 3/4" GHT adapter onto each end, cut tubing attached to red side port and push tubing onto both sides of the meter (ensure directional flow of the meter is correct), no clamps are needed.

5.7 Place the influent tubing into the body of water and ensure the end of the tubing will stay below the surface of the water and away from plant material or other large debris which may be present. Be cautious not to disturb the water significantly near the filtration site.

5.8 Verify the set up is correct. See Figure 2 below.

Figure 2. Dead End Ultrafiltration Field Setup



5.9 Plug the pump into the battery.

5.10 Start the pump by flipping the switch to ON and turning the knob. Ensure that the pump is in the appropriate “Forward/Reverse” setting. Also, take note that with the geopump geotech peristaltic pump, the easy-load II pump head can be in one of

two positions (Series #1 Drive or #2 Drive) and either will work fine for the purposes of this method. However, the position to the right (Series #2 Drive; see picture in Appendix C) will result in faster pumping speeds and thus, shorter sampling times. A #1Phillips head screwdriver will be needed to move the easy-load II pump head from one position to the next. The pump will begin pumping the water through the influent port tubing into the filter and out the side port through the filtrate port tubing. Make sure no ports on ultrafilter are leaking.

5.10.1 ***IMPORTANT* DO NOT TURN OFF THE PUMP ONCE YOU START PROCESSING THE SAMPLE UNLESS ABSOLUTELY NECESSARY**.

5.11 If sampling outdoors and the ultrafilter and pump is in the sun, cover the ultrafilter and pump with a clean bench protector or large umbrella to shield it from the sun.

5.12 Empty the cubitainer immediately after it is filled. Do this 5 times for a 20 Liter sample. Adjust accordingly for containers of different volumes. Do not empty the cubitainer back into your water source.

5.13 When the water to be collected contains free chlorine or chlorination is suspected, treat ultrafilters with 1% sodium thiosulfate solution.

5.13.1 Fill a 500-ml bottle with non-chlorinated water and add 5 g sodium thiosulfate, shake to dissolve.

5.13.2 Place the influent tubing into the 1% sodium thiosulfate solution immediately after the filtration process and pump the entire volume through the filter without pumping air into the ultrafilter.

5.14 When pumping is completed and the pump is stopped, open the pump head and remove the filtration tubing from the pump head.

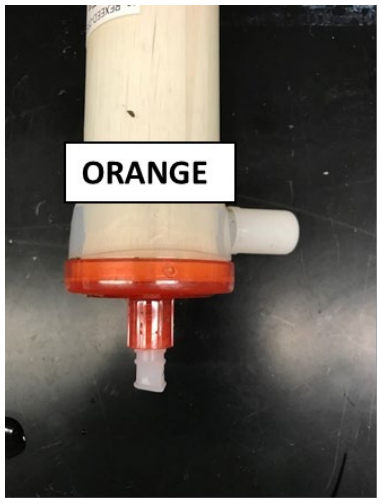
5.15 Unplug the pump from the battery.

5.16 Remove all tubing from the ultrafilter and place end caps and storage caps on the ports, being careful not to allow water in the ultrafilter to escape. ***IMPORTANT* Be sure endcaps in particular are attached as snug as possible as they have a tendency to leak if not secure. Also ensure all caps are sterile.** See Figure 3 below. Also, be sure that water does not exit the filter as you are removing the tubing and securing the caps. Collect all contaminated blood port caps, DIN adapters, and rubber storage caps and place them in a conical tube or zip-loc bag for return to laboratory. These pieces may also be decontaminated and reused by soaking in a 10% bleach solution for 30 minutes.

Figure 3. Ultrafilter preparation for storage/shipment

Screw on the end cap.

Plastic cap is left on the side port. Ensure cap is hand-tightened so as to prevent leaking. A rubber storage cap can also be used (shown in photo).



Place plastic cap on side port and hand-tightened so as to prevent leaking (shown in photo). A rubber storage cap can also be used.

Leave the blood port cap in place.

5.17 Tubing that came in contact with the water source should be disposed of in a trash bag and **not reused** to collect another sample. The tubing used to pass water from the filter to the cubitainer may be reused since it did not come in contact with potentially contaminated water.

5.18 Dispose of trash (tubing, gloves, bench protectors, paper towels, etc.) in trash bag and dispose as regular trash.

5.19 Label the freezer bag and ultrafilter with the Sample ID, Date, and your initials.

5.20 Place the filter inside the bag and seal the bag and gently place in a chilled cooler for transport.

5.21 Keep L/S 36 tubing (filtrate tubing) in a zip-loc bag for use in collection of the next water sample collected for the study.

5.22 Keep ultrafilter chilled (wet ice / freezer packs or in a refrigerator) until shipment or processing.

5.23 Before shipping, confirm that each ultrafilter sample included in shipment is properly labeled directly on the ultrafilter with Date, Sample ID, and Sampler Initials.

5.23.1 Confirm that each ultrafilter has covers on all ports/openings and that it is secure in a sealed Zip-loc bag. Confirm that the sample bag is also labeled with Date, Sample ID, and Sampler Initials.

5.23.2 Place a layer of ice or freezer packs on the bottom of the cooler.

5.23.3 Place ultrafilters in the cooler on top of the ice or freezer packs.

5.23.4 Place a generous layer of ice or freezer packs over the ultrafilters and seal cooler with heavy duty tape for shipment.

6. TROUBLESHOOTING

Most Common Issues Observed

6.1 Pulling air instead of water through tubing

• Symptom: Pump is struggling to pull water from source

• Remedy: Stop pump, **slowly** release pressure in tubing by lifting the pump head clamp and resume pumping after checking to be sure intake tubing end is submerged under the water

6.2 Clogging of tubing or filter

• Symptom: Debris is observed in tubing near connection with filter inlet or flow of water out of filter visibly decreasing

• Remedy: Stop pump, consider if area where debris can be simply cut out and remainder of tubing reattached; if filter is clogging, it may be a hard stop and end to sampling with current ultrafilter

6.3 Split tubing

• Symptom: Water is leaking where tubing is threaded thru pump head due to crack in tubing

• Remedy: Stop pump, remove tubing from pump head and replace influent tubing piece, clear out any visible sand or debris that might be causing friction in pump head and resume pumping

7. Appendix A

Photos of Hose Clamp, Blood Port Cap, DIN Adapter, and Rubber Storage Cap

Photo 1. Hose clamp
(Cole-Parmer # 6832-04; Min ID: 0.402”, Max ID: 0.449”)

Photo 2. Blood Port Cap
(Molded Products # MA-40)

Photo 3. DIN Adapter
(Molded Products #MPC-855)

Photo 4. Rubber Storage Cap
Dialysate Port Storage Cap (Molded Products #MPC-60D)

8. Appendix B

List of Supplies in Ultrafilter Kit

Each 1-gallon Zip-loc bag contains the following:

2 bench protectors
1 clean/unused 50 mL conical tube
2 hose clamps
2 rubber storage caps
2 blood port caps
1 – 2.5-gallon Zip-loc bag (for storage of the ultrafilter after sample collection)

9. Appendix C

Picture of Peristaltic Pump in Use