

# Attachment C – Sample Collection Procedures

## Standard Operating Procedure Sample Collection Procedure for *Naegleria fowleri*

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**PURPOSE:** The following procedure is to be used for the collection of *Naegleria fowleri* samples.

### I. EQUIPMENT AND SUPPLIES

- Ultrafiltration protocol
- Chain of custody (COC) form
- Watch or stopwatch
- 1-L Graduated cylinder
- Ultrafilters (UF) [ELISIO-25H]
- UF Inlet Connection Tubing:
  - Various sample tap connectors
  - Tubing adapter - connect tap connector to UF Port 1
- UF Effluent Tubing with a Flow rate/totalizer connect to Port 3
- Tubing clamps
- Port caps/plugs (included with ultrafilter)
- 125-mL sample bottles (for measuring water quality)
- 60-mL syringes
- 50-mL tubes containing sodium thiosulfate
- Zip closure plastic storage bags
- Pliers
- Permanent markers
- Scissors
- Shipping container and bubble wrap
- Isopropyl alcohol and spray bottle

### II. GRAB SAMPLE COLLECTION:

1. Fill out all information fields on the Chain of Custody (COC) provided. NOTE: Do not use same COC for more than one Public Water System (PWS).
2. Using the spray bottle of isopropyl alcohol (or 5% chlorine bleach), disinfect the hydrant, hose bib or sample station fixed connection by thoroughly spraying the alcohol/bleach solution on the threads. Let sit for two minutes, then wipe clean/dry using a clean paper towel.
3. Open faucet wide-open and let water run out for **five minutes** to flush out stagnant water, and then close.
4. Using available sample containers, collect grab samples and measure water quality parameters (*i.e.*, free and total chlorine using a verified colorimeter kit). Dispose of this water when finished, rinse with deionized water and re-use bottle.
5. Fill two 50-mL tubes containing de-chlorinating agent (sodium thiosulfate) with the test water. Shake to dissolve completely and set aside for Step 13 of ultrafiltration procedure (III.B).
6. Label storage bag with System Name and PWS ID# and Sample Site ID. Place in a zip closure bag and place in tote for transport.
7. Enter sample information onto field form and transfer info to chain of custody after sampling.
8. Keep water samples for amoeba testing at ambient temperature. **Do not chill.**

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### III. WATER SAMPLE COLLECTION USING ULTRAFILTRATION

#### A. Ultrafilter (see page 4 for set up details):

ELISIO-25H ultrafilters are used to filter water from a pressurized source. See Figure 1 for ultrafilter setup. The ultrafilters have four ports. These ports are:

- Port 1: Influent port
- Port 2: Plugged port; no water should flow through this port
- Port 3: Effluent port
- Port 4: Plugged port; no water should flow through this port

Figure 1. Ultrafilter Setup



#### B. Ultrafiltration Procedure:

1. Label ultrafilter and all associated supplies (storage bag, etc.) with sample site ID and enter sample information onto chain of custody form.

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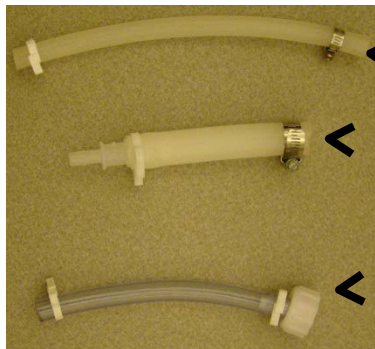
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2. Use new inlet hardware (e.g., hydrant adapter, tap connector, etc.) and tubing for each sample location/site.
3. Remove the cap from Port 1 and return to ultrafilter storage bag.
4. Attach tap connector to the sample tap (several are provided to fit different taps; hose bibb vs. small smooth tap). Using the screw-on adaptor and clamp, attach UF inlet tubing to Port 1. Use the pliers to ensure the clamp is snug around the tubing.
5. Remove the cap from Port 3 and return to ultrafilter zip closure plastic storage bag.
6. Attach the tubing with the flow totalizer to Port 3; it does not need to be clamped to the ultrafilter. Ensure directional flow of the meter is correct as indicated by the arrow on the flow meter. This tubing is not part of the sample stream, and can be re-used with multiple filters.
7. Keep the remaining caps in place. If an end port starts leaking, switch cap to a blue cap.
8. Record flow meter start reading on the chain of custody. If using the digital flow meter, be sure to zero the meter. Carefully, open faucet partially, record the filtration start time and check to make sure water is flowing into Port 1 and slowly out of flow meter (via Port 3), and that there are no leaks.
9. Hold ultrafilter with inlet (Port 1) end up until entire space is filled with water. It can then be placed in any orientation. If placed on ground, use the tote lid and keep out of pooling water.
10. If using the analog flow meter, measure flow rate out of outlet tubing using a watch/timer and a graduated cylinder. Record the flow rate on the back of the COC. Ensure flow is less than 3 Liters per minute. Reduce flow as necessary.
11. Continue ultrafiltration to filter at least 10 L (2.64 gal) for raw water and 100 L (26.4 gal) for treated/finished water.
12. Close sample station or hose bib valve to stop filtration and record the filtration end time and the flow meter end reading on the chain of custody.
13. Unscrew the tubing from the ultrafilter Port 1 and connect a 2" length of 1/8" ID tubing to the inlet. Attach the 60-mL syringe (with the plunger removed) to the other end of the small diameter tubing. Fill the syringe with the 50-mL sodium thiosulfate solution and gently push through the ultrafilter with the syringe plunger. Some water will flow out of outlet port as you do this. Repeat with the second 50-mL tube of sodium thiosulfate solution.
14. Detach the syringe and re-cap Port 1 using original cap. Detach tubing from Port 3 and recap.
15. Put the ultrafilter in the labeled zip closure plastic storage bag and store ultrafilters in a tote and maintain at ambient temperature. (DO NOT USE FREEZER PACKS or ICE).
16. Package ultrafilters in bubble wrap and ship (overnight priority) the ultrafilters with the signed COCs to the designated laboratory; use the lab address on the COC.

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## Sample Collection Procedure for *Naegleria fowleri*

### Sample Tap Connectors

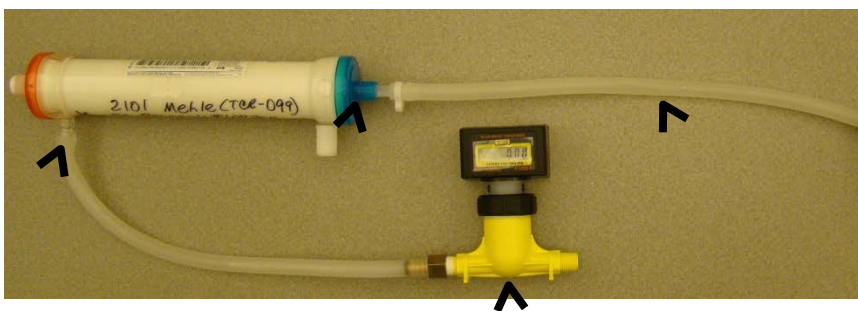


3/8" connector

3/8" to 5/8" connector

Hose bibb 3/8" connector

### Regular Sample Setup



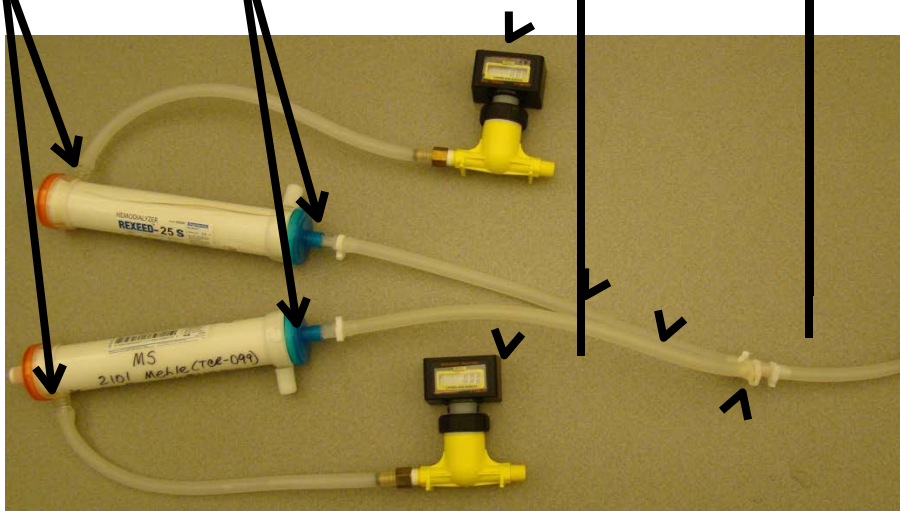
Port 3 – Outlet  
line to Flow

Port 1 – Filter  
Inlet

Water Flow Meter

Filter Inlet Line

### Matrix Spike Sample Setup



"Y" Connect Inlet split

# Standard Operating Procedure

## Sample Collection Procedure for *Perfluoroalkyl Substances (PFAS)*

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**PURPOSE:** The following procedure is to be used for the collection of Perfluoroalkyl Substances (PFAS) samples. PFAS contamination during sampling can occur from multiples sources including clothing, personal care products, etc. Continually washing hands and wearing nitrile gloves can reduce accidental contamination of the samples.

### I. General Preparation

1. Supplies: non-waterproof labels, COC, PFAS bottles and blanks with preservatives, sealable plastic bags (clean polypropylene or High Density Poly Ethylene (HDPE) material bags), ice bags (double bagged), deionized water, nitrile gloves and ice chests.
2. Wash hands thoroughly and use nitriles gloves when handling sample collection materials and collecting samples. Always put on a new pair of nitrile gloves prior to handling the sample bottles for each site.
3. Using gloves, prepare sample collection materials before leaving the office. Using gloves, affix the labels to the bottles and place in an individual sealed plastic bag. Minimize handling the sample bottles. Sample labels should have the water system name and ID number, Point of Collection ID, collector name, collection date and time the sample was taken. Do not write info on the bottles with a permanent marker.
4. Collect the PFAS sample first prior to collecting samples for any other parameters.

### II. Sampling Procedure

1. For ground water systems, all samples shall be collected directly from the raw water sample tap on the well discharge line while the well is operating. For surface water system, samples shall be collected from the point of entry to the distribution systems which is after all treatment. Remove any attachment from the tap such as hoses, filters, screens or aerators.
2. Flush the water for about 15 minutes or until the water temperature reaches a constant temperature, and then reduce water flow to a smooth (laminar) stream about the size of a pencil, if possible.
3. After steps 1 and 2, put on a new pair of nitrile gloves prior to handling the sample bottles for each site.
4. PFAS Sampling methods:
  - a. For PFAS (Method 537 or 537.1):
    - a. For each site collect 2 samples using 250 mL (8 oz) bottles with 1.25 Trizma (de-chlorinating agent in the bottle).
    - b. For each site, use 1-250 mL (8oz) with 1.25 Trizma as the field blank.
  - b. For PFAS (Method 533):
    - a. For each site collect two samples using 250 mL (8 oz) bottles with 250 mg ammonium acetate (de-chlorinating agent in the bottle).
    - b. For each site, use one - 250 mL (8oz) with 250 mg ammonium acetate as the field blank. Do not place bottle caps on any surface and avoid contact with the inside of the sample bottles or its cap.



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### Sample Collection Procedure for *Perfluoroalkyl Substances (PFAS)*

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- c. For the field blanks, fill with deionized water to the bottom of the neck and do not overfill. Agitate until preservative is dissolved. Use one field blank per method per site.
- d. For the sample, fill with sample point tap water to the bottom of the neck and do not overfill. Agitate until preservative is dissolved.
- e. Place the samples in an individual sealed plastic bag (ultra-clean polypropylene or HDPE material bags) and then place into ice chest with ice bags surrounding the sample bags. Do not have other sample parameter bottles in the ice chest.
- f. The samples must be stored and shipped (**overnight priority**) on wet ice (double bagged) to the designated laboratory. Pack the bottles upright in the ice chest and limit sideways motion. The samples must be chilled at  $< 6^{\circ} \text{C} \pm 2$ .

### III. Sampling Best Practices

1. A two-person team is recommended; one team member handles the samples, and the other records the samples in the COC form etc.
2. Samplers should select and use the same clothes to conduct PFAS sampling. If items have been previously laundered with fabric softener or dryer sheets, wash six times with unscented laundry detergent and hang dry prior to sampling.
3. Do not get gas and/or stop for fast food prior to sampling, as this can contaminate samples.

<b>Field Equipment</b>	
<b>Best Practices</b>	<b>Do not use</b>
Loose paper (non-water resistant)	Teflon materials
Aluminum or Masonite clipboards	Waterproof materials (field books, Labels, etc.)
Pens	Plastic clipboards, binders, post-it notes etc.
Regular ice	No chemical (blue) ice packs
<b>Clothing and appurtenances</b>	
<b>Best Practices</b>	<b>Do not use</b>
Well-laundered clothing, defined as clothing that has been washed six or more times after purchase, made of synthetic or natural fibers	New Clothes or water resistant, waterproof, or stain-treated clothing.
Cotton clothing preferable	Clothes laundered with fabric softener and/or dryer sheets.
Boots made with polyurethane and polyvinyl chloride (PVC)	No Tyvek or Gore-Tex materials
Organic sunscreens that are free or natural	No fragrances, cosmetics, moisturizers, hand creams, hair products or other personal care products

# Standard Operating Procedure

## Sample Collection Procedure for *Cyanotoxins/Harmful Algal Blooms (HABs)*

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**PURPOSE:** Use the following procedure for the collection of Cyanotoxins/HABs samples for Eurofins.

### I. General Preparation

1. LDH/OPH will supply kits and ice chests.
2. Eurofins Laboratory uses EPA method 545 + 546 and in house method L231.

### II. Sampling Procedure

1. For each surface water system, collect samples from the raw water tap and the finished water Point of Entry tap. Remove any attachment from the tap such as hoses, filters, screens or aerators.
2. Flush the water tap until the temperature has stabilized (approx. 3 to 5 min), and then reduce water flow to a smooth (laminar) stream about the size of a pencil, if possible.
3. Sampling Kits:
  - a. Collect one sample using 250 mL (8 oz) amber bottle with sodium bisulfate + ascorbic acid (EPA 545),
  - b. Collect one sample using 250 mL (8 oz) bottle with sodium thiosulfate (EPA 546)
  - c. Collect two samples using 40 mL amber vials with ascorbic acid + 2-chloroacetamide + EDTA + trizma (Eurofins L231).
4. Fill sample bottles, making sure not to flush out sample preservatives. Sample bottles and/or vials **do not** need to be completely full.
5. For sample bottles marked method 545, agitate after collection until reagent dissolves.
6. For sample bottles marked method 546, invert sample several times to mix sample.
7. Place sample bottles in the supplied bubble wrap bags and seal for shipment. Pack bottles for each water system in the supplied zipper bags.
8. Package with loose ice on top of samples to keep colder. **Please fill cooler to the top with ice.** Do not bag ice. (Must not exceed 10°C).
9. Ship **overnight priority** to the designated laboratory

