

WHONDRS Surface Water Metabolite Biogeography Sampling Protocol

A video protocol is available at: <https://www.youtube.com/watch?v=2lRp14ow4tE&>

(Note: the video does not show the step wherein additional water is pushed through the filter but not collected)

Contact WHONDRS@pnnl.gov with any questions or concerns.

In addition to the sampling kit provided by WHONDRS, you will need the following:	The sampling kit should contain the following items:
<ol style="list-style-type: none"> 1) Cooler with wet ice or blue ice for keeping samples cold in the field 2) Celsius thermometer for taking water temperature 3) A method for collecting latitude and longitude in decimal degrees in the field (a smart phone is sufficient) 4) A method for taking pictures of the field site (a smart phone is sufficient) 5) A -20 C freezer for freezing the ice pack (ice pack should be frozen for 48 hours prior to shipping) 6) A refrigerator to keep the samples cool if there is a shipping delay 7) Access to a FedEx office for shipping samples back overnight 8) Access to stream gauge data (that can be made public) for at least the 3 months prior to collecting the samples. Data must be recorded at a minimum frequency of every two hours. 9) Optional: full size measuring tape 	<ol style="list-style-type: none"> 1) Hard copy data sheet 2) Pre-filled shipping label for sending samples back via FedEx overnight 3) Freezer pack (place this in a -20C freezer for at least 48 hours prior to shipping) 4) 20mL syringe 5) 60mL syringe 6) 3mL syringe 7) One pair of nitrile gloves 8) One filter with needle attached 9) One spare filter (keep sealed unless needed) 10) One needle to be used with 3mL syringe and epi tube of RNALater after sampling 11) One spare needle (keep sealed unless needed) 12) One clear 40mL vial. Fill this vial first. Pierce septum to fill. Do not unscrew cap. 13) Three amber 40mL vials. Pierce septa to fill. Do not unscrew caps. <ol style="list-style-type: none"> a. The amber vials each contain 10 uL of 85% phosphoric acid to aid in sample preservation. Fill these vials after the clear vial to minimize acid transfer to the clear vial. 14) One small plastic epi tube filled with RNALater, a preservative to use on the filter after sampling 15) Two luer lock caps to seal the ends of the used filter after it has been filled with RNALater 16) One small whirlpak bag to store the used filter in 17) Small measuring tape 18) Pencil for recording field metadata 19) pH strip and color matching chart

CHOOSE SAMPLING LOCATION AND COLLECT METADATA

- 1) Choose your location to be as close as possible to a stream gauge. Also try to sample in a location with natural sediments as opposed to locations with artificial substrates.
- 2) Attempt to sample ~1m from the shoreline. Record the distance from the shoreline where samples are collected.
- 3) Confirm there is downstream flow at the sampling location (e.g., drop in a leaf and make sure it moves downstream)
- 4) Record the date, local time, time zone, and your name on the data sheet
- 5) Measure the water depth at the sampling location and record it on the datasheet in cm

- 6) Measure the temperature of the water at **50% of the water column depth**; record it on the datasheet in degrees C
- 7) Record latitude and longitude in **decimal degrees**. For this, feel free to use a GPS or a smart phone app such as 'My GPS Coordinates' or Google maps.
- 8) Remove the pH strip from its protective plastic bag, making sure to keep the paper dry.
- 9) Dip the strip into the river water upstream of where you are standing.
- 10) Wait 15 seconds after wetting the pH strip and then compare the color to the color chart provided with the pH strip. Record the pH value on the metadata sheet.
- 11) Place the pH strip on a white background (the back of the metadata sheet) and photograph the pH strip. This picture will be uploaded with the metadata.

COLLECT SAMPLES

Note: While sampling, please **stand downstream of the sampling location**. This is important to avoid a non-representative sample.

- 1) Put on the provided nitrile gloves (do your best if they don't fit well)
- 2) Open the filter package that has a needle attached to the filter but leave the needle/filter assembly in the package
- 3) Open the syringe package and remove the syringe. Please **don't touch the outlet of the syringe and do not fully remove the plunger from the syringe body**. This is important to avoid contamination.
- 4) Fill the syringe with river water, collecting water from 50% of the water column depth. Expel the syringe contents into the river and repeat this two more times.
- 5) After flushing the syringe 3 times, fill the syringe again from 50% of the water column depth; this is the sample water for the first replicate.
- 6) Screw the syringe onto the filter that has a needle connected to it. Remove the plastic cover that is protecting the needle while the needle is still in the filter package. Please **don't touch the outlet of the syringe, the inlet of the filter, or any part of the needle**. This is important to avoid contamination. Also, retain the plastic needle cover. Note that it is okay to touch the cylindrical filter housing.
- 7) Push 2-3 mL of water through the filter/needle assembly. This water is not collected.
- 8) Beginning with the **clear** glass vial that has '-1' at the end of its sample identification number, remove the flip top cap on the glass vial. Please **don't touch the septum** that is exposed after removing the flip top cap. This is important to minimize contamination. **Do not unscrew the top of the vial**. Only remove the white disc that rests on top of the cap and conceals the septum.
- 9) Pierce the septum with the needle and **fill the vial to the premarked fill line** (approximately half way full). Please don't fill more than the pre-marked fill line.
- 10) After injecting sample water into the vial, withdraw the needle from the septum and carefully place the filter/needle/syringe assembly back into the plastic needle cover that is still in the filter package (see the video protocol for demonstration of this method).
- 11) Place the vial containing the sample into the cooler with wet or blue ice. Note there is no need to record any information on the sample label. There is no need to replace the flip top cap that previously covered the septum.
- 12) Unscrew the syringe from the filter/needle assembly, expel any remaining liquid into the river, and refill the syringe from the same sampling location, again at 50% depth of the water column.
- 13) Screw the syringe back onto the filter/needle assembly, **expel a small volume of sample water** through the filter/needle assembly, and repeat the above sampling procedure for the amber vials with '-2' and '-3' and "-4" at the end of their sample identification numbers. These are the second, third, and fourth replicates. Note that **one filter and one needle should be used to collect all four replicates**. The second filter and second needle sent with the kit should only be used if there is an issue with the first filter or needle. If an extra filter or needle is needed, please do not touch the inlet or outlet of the filter, and please don't touch the inlet of the

needle or any part of the metal needle (it is okay to touch the plastic needle cover). If the extra filter or needle are needed, please record the reason on the paper metadata sheet. **If the extra needle or filter are not needed, ship unopened when the samples are shipped.**

- 14) After filling the amber vials, shake each one gently to incorporate acid into the sample. Then place back into cooler.
- 15) After filling all of the vials, you will need to push more water volume through the filter to ensure enough microbial material is captured.
 - a. Detach 20 mL syringe from filter, ensuring you do not touch the inlet of the filter.
 - b. Locate 60mL syringe, fill the syringe from 50% of the water column depth.
 - c. Attach 60mL syringe to the filter, ensuring you do not touch the outlet of the syringe or the inlet of the filter. Expel volume of water through the filter downstream of the sampling location (**do not collect water**).
 - d. Push another 10 full syringe volumes of water through the filter. The goal is to filter about 700 mL of water through the filter unit. Do not collect the filtered water. If your filter clogs at any point, you can assume you have enough microbial material and can stop pushing additional water through the filter.
- 16) After filtering the additional water, you will need to preserve the filter. If more than one filter is used, only preserve and send back the first filter (unless the second was used due to contamination of the first)
 - a. Detach the 60mL syringe from the filter, expel any remaining water onto the ground, and fill the syringe with air.
 - b. Attach the air-filled syringe to the filter and push the air through the filter. The goal is to expel as much water from the filter as possible. Repeat 2 or 3 times if needed.
 - c. Safely discard the needle attached to the filter.
 - d. Take one of the small luer lock caps provided and attach it to the open end of the filter. Note that the filter has a “male” side and a “female” side and therefore two types of caps provided.
 - e. Locate the small plastic epi tube filled with RNALater. This is the preservative for the filter. Also locate the 3mL syringe and new needle.
 - f. Connect new needle to the 3mL syringe, carefully open the small epi tube, and fill syringe with the RNALater by simply putting the needle down into the liquid.
 - g. After filling syringe with RNALater, invert syringe so that the needle is facing up. Discard the needle.
 - h. Detach used filter from 60mL syringe, taking care not to touch the inlet of the filter.
 - i. Attach 3mL syringe to the filter and rotate so that syringe is facing down and the filter is below it. Push the plunger to slowly fill the filter with RNALater. Fill until you feel some resistance or until you have used all the RNALater.
 - j. Detach the syringe. Cap the filter using one of the small luer lock caps provided and shake the sealed filter gently.
 - k. Put capped filter into a small whirlpak bag, tie up to seal, and place in the cooler. Please ensure no water is entering the whirlpak bag.

TAKE SITE PHOTOS

- 1) Take the following photos with a smart phone or camera (note these will be uploaded along with metadata). For the last three pictures (b, c, d), lay out a **measuring tape to 30cm** on the shoreline as a reference for scale and make sure it can be seen in the photos. The first figure (looking across the river) doesn't need to contain the measuring tape.
 - a. Looking across the river to give a sense of how broad the river is
 - b. Looking upstream, showing the river surface, shoreline sediments, and vegetation
 - c. Looking downstream, showing the river surface, shoreline sediments, and vegetation
 - d. Looking straight down onto a location with representative shoreline sediments

- 2) Please confirm that all fields on the paper metadata sheet are filled out prior to leaving the field, and take a picture of the data sheet. This picture will also be uploaded.

STORE SAMPLES

- 1) After sampling, transport samples on wet or blue ice. Then place all samples (all vials and the filter preserved in RNAlater) in a refrigerator. Don't use a freezer for these as they may burst.
- 2) After storing the samples in a refrigerator, place the ice pack only in the freezer if you did not place it there prior to sampling

FILL OUT ONLINE METADATA

- 1) After the samples are in the refrigerator, please enter metadata into the digital form that can be found at <https://whondrs.pnnl.gov>. If there is difficulty in accessing the form, please email WHONDERS@pnnl.gov for a copy of the form.
- 2) Please enter as much information as possible. The form indicates what information is required and what is optional.
- 3) The form also provides instructions on how to submit the field photos and the stream gauge data. Please name all files with the Sample ID. For the photos, please follow the naming convention described in the metadata form (e.g., S000001-across, S000001-up, S000001-down, S000001-sed, S000001-data, S000001-pH).
- 4) For the stream gauge data, if the closest stream gauge provides downloadable real-time data, it is acceptable to provide the url and the PNNL WHONDERS team will download and format the data. If stream gauge data is not available for download, please contact whondrs@pnnl.gov to send data files or submit via the online metadata form.

SHIP SAMPLES

- 1) All shipments should be stored in the refrigerator until they are **shipped on a Monday or Tuesday**. It is critical that overnight shipments be made on Monday or Tuesday. Please **don't ship later in the week**. This is important in case there are shipping delays.
 - a. Remove materials from the refrigerator and pack the cooler as close as is reasonable to the time FedEx will ship the package. This minimizes warming.
- 2) When you're ready to ship,
 - a. Place glass vials back in the provided vial holder. Place the vials and the vial holder into provided ziplock bag and into cooler. Please do not send back broken vials; instead find some material to place in the vial holder to take the place of the missing vial.
 - b. Pack the unused filter, unused needle, measuring tape, and absorbent padding that came with the supplies.
 - c. Place the frozen ice pack on top of the materials.
 - d. Put the lid of the cooler on and then place the paper metadata sheet on top of the lid. This will keep it dry.
 - e. Please **don't return** gloves or used needles. Please dispose of the used needles in an appropriate sharps container.
- 3) Tape outer box closed with sufficient packing tape to protect during shipping.
- 4) Adhere shipping label to the outside of the box by removing the backing from the plastic sleeve that contains the shipping label.
- 5) Drop package off at FedEx or have it picked up by FedEx.
- 6) **On the same day you ship the package**, notify WHONDERS@pnnl.gov that you shipped the package, and include the FedEx tracking number in your email. This is critical to ensure sample integrity and timely delivery. The subject line of the email should be "SHIPPED SAMPLES – [unique sample ID #]". If you wait until the day after you have shipped the samples, the samples may arrive prior to our notification, which will increase the likelihood of delivery issues and warming.