# WHONDRS Protocol for Summer 2019 Sampling Campaign

This protocol describes sediment and river water sampling for the WHONDRS Summer 2019 Sampling Campaign. Please keep in mind that it is your responsibility to ensure compliance with any environmental regulations associated with the field sampling (e.g., permits to sample). Also note that **your sampling location must be within ~100 m of a location that measures river discharge, height, or pressure.** Data must be public and at a logging frequency of at least 2 hours; it is preferable to have 1 year of data. **Sampling must be completed on Sunday or Monday and samples shipped overnight on the following Monday or Tuesday.** If you cannot accommodate these dates, please contact WHONDRS@pnnl.gov. A video protocol is being developed. Once completed it will be available at: WHONDRS - YouTube. Please contact WHONDRS@pnnl.gov with any questions or concerns. Note that leaf collection is no longer being done.

## MATERIALS

| **In addition to the sampling kit provided by WHONDRS, you will need the following:** | **The sampling kit provided by WHONDRS should contain the following:** |
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| 1) Cooler with wet or blue ice for keeping samples cold in the field<br>2) Method for collecting latitude and longitude in decimal degrees in the field (smart phone is sufficient)<br>3) Method for taking pictures of the field site (smart phone is sufficient)<br>4) -20 C freezer for freezing the ice packs in advance of shipping (do not freeze the samples but be sure to freeze the ice packs at least 48 hours prior to shipping)<br>5) Refrigerator to store samples prior to shipping<br>6) Access to a FedEx office for shipping samples back overnight (WHONDRS pays for shipping)<br>7) A method to safely dispose of used needles<br>8) Thermometer to measure temperature in the water column<br>9) Safety glasses (some of the vials are pre-acidified and should be treated with care)<br>10) Meter tape for estimating distances among sampling locations (can also approximate by pacing off distances)<br>11) Optional: If you have a method of measuring dissolved oxygen, please provide dissolved oxygen data from 50% of the water column depth where you collect your water samples. You can submit this data when you upload your metadata or send to whondrs@pnnl.gov. Please provide information on the method used to collect the data. | 1) Hard copy data sheet + pencil<br>2) Shipping label that is pre-filled for sending samples back via FedEx overnight<br>3) Freezer "blue ice" packs (place these in a -20C freezer for at least 48 hours prior to shipping)<br>4) pH strip<br>5) Two pairs of nitrile gloves to minimize contamination<br>6) Small tape measure(may want to bring your own)<br>7) Flags (temporarily mark sediment sampling locations)<br>8) 1 sterile sediment scoop<br>9) Foil-wrapped metal scoopula to assist in distributing sediment from the sediment scoop<br>10) 3 18 gauge needles<br>11) 3 mL and 60 mL syringes<br>12) 3 sterile jars (125 mL) for sediment collection<br>13) 2 sterile 50 mL falcon tubes pre-filled with RNAlater<br>14) 3 4mL vials for water sample for stable isotopes<br>15) 8 clear glass vials for unfiltered river water<br>16) 2 15mL falcon tubes for unfiltered water<br>17) 3 **pre-acidified** amber vials for filtered samples<br>18) 1 un-acidified amber vial with bright yellow tape for filtered sample<br>19) 3 blue cap 15 mL falcon tubes for anions/SpC from filtered sample<br>20) 3 **pre-acidified** orange cap with star symbol 15 mL falcon tubes for cations from filtered sample<br>21) 1 125 mL plastic bottle for filtered sample<br>22) 2 sterivex filters (one is extra)<br>23) Epitube with 1 mL of RNAlater |

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|---|---|
| | 24) 2 luer-lok caps (plus extras) to seal filter after filled with RNALater |

**IDENTIFY SAMPLING LOCATIONS**

After confirming that you have all of the needed gear, identify three wadeable (i.e., in water) sampling locations for sediment collection in depositional zones, as defined in the NEON protocol (NEON.DOC.001193; Jensen, 2019): **"A depositional zone is defined as the area within a river where the energy regime is low and typically are found at the inside bend of a stream, riffle, pool lip, downstream from obstacles or simply shallow waters near the shore** (USGS, 1994) (Figure 1)". These are low water velocity locations that would allow fines to settle on the riverbed. As in the NEON protocol (NEON.DOC.001193; Jensen, 2019), "The sample strategy for sediment analysis focuses on fine-grained surficial sediments from natural depositional zones during low-flow conditions (USGS, 1994). **Surface sediment is considered to range from 1 to 3 cm in depth** (Golterman et al., 1983; Keith, 1991)." Sampling should avoid large debris greater than 4 mm in size.

Sediments will be collected from 3 sites (Figure 2). Ideally, all sites will be within ~100m of the stream gauge. Do your best based on local conditions, but try to make the 3 sites at least 10m apart from each other. The first step is identifying 3 sites (flags is provided). Then collect metadata, then conduct water sampling at the most downstream site (**water is only collected at the downstream site**), and then collect sediments (**collect water before sediments**). The most downstream location should use the vials marked with "D" (e.g., S19S_0001_D). The midstream location should use the vials marked with "M", and the upstream location should use vials marked with "U". **Sediment collected should be under water at the time of sampling. Water should be collected at 50% depth of the water column, directly above where the downstream sediment will be collected.** Once locations are chosen, collect the metadata as follows.

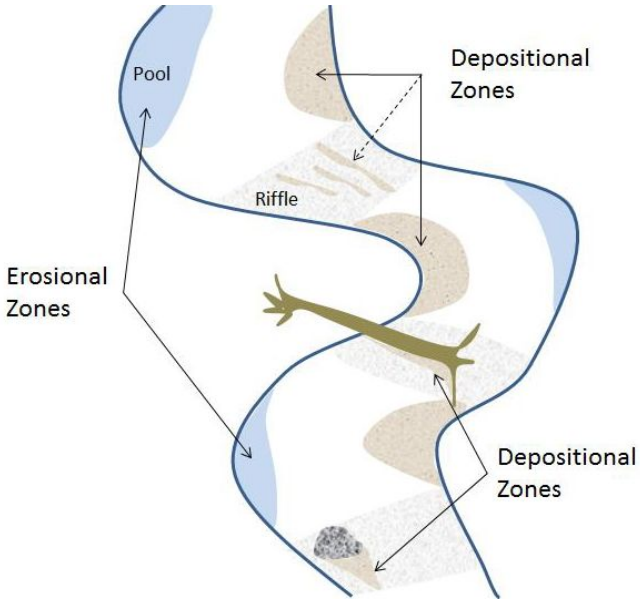


**Figure 1**. Examples of depositional zones. Collect sediments from depositional zones and avoid erosional zones. Image from NEON protocol (NEON.DOC.001193; Jensen 2019)

**COLLECT METADATA**

1) Record the general vegetation type, basic geomorphology, sediment type, types of depositional zones to be sampled, and coverages of algae, submerged plants, and overhead canopy (at each sediment sampling location) using the checklists/categories on the metadata form, and please note general weather conditions (e.g., raining, sunny, etc.).

2) For each sampling location record latitude and longitude in **decimal degrees**. You can use a GPS or a smartphone app such as 'My GPS Coordinates' or Google maps.
3) Estimate and record the distance among the three sampling locations in meters. You can use a field measuring tape or just pace off the distance.
4) At each sediment sampling location, measure the water depth where sediments will be collected and record it on the datasheet in cm.
5) With a smartphone or camera, take a photo of the undisturbed sediment at each sampling location. Ensure a measuring tape **extended to 30 cm** is visible in each of the photos. Note that these will be uploaded along with the metadata. Record which picture goes with which site.
6) At the most downstream sampling location, take the following photos (note these will be uploaded along with metadata). For the last two pictures (b, c), lay out a **measuring tape to 30cm** as a reference for scale and make sure it can be seen in the photos. The first picture (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
7) At the most downstream location measure the pH of the surface water
    a) Remove the pH strip from its protective plastic bag, making sure to keep the paper dry.
    b) Dip the strip into the river water upstream of where you are standing.
    c) 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.
8) If you have a dissolved oxygen sensor/probe, take a measurement at 50% water column depth at the most downstream location, and record the value in percent saturation and mg/L.
9) At the most downstream sampling location, measure the temperature of the water at 50% of the water column depth; record it on the datasheet in degrees C
10) 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. You will fill in the rest of the metadata via an online form.

**COLLECT UNFILTERED WATER SAMPLES**

1) After collecting the metadata, proceed to the most downstream sampling site and collect water samples from the water column above where sediments will be collected as follows [**If water was disturbed while identifying upstream sampling locations, let the water clear before sampling**]
2) Put on the provided nitrile gloves (do your best if they don't fit well)
3) Locate the 8 clear glass vials and 2 15mL falcon tubes marked "RAW"
4) One at a time, unscrew the vial cap and with the opening pointing upstream and submerge the vial in the river water (to approximately 50% depth of the water column). For the glass vials, fill at least to the pre-marked line (approx. 30 mL), leaving some headspace in the vial. For the 15mL falcon tubes, fill to ~12ml. Store all on ice.

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**NOTE ABOUT FILTERED WATER SAMPLE COLLECTION**: It is important to collect at least one replicate of each sample type prior to collecting all replicates of a given sample type. This will ensure that we can generate all data types. As such, please first collect one replicate of each type (indicated by sub-sections below). Then go back through the protocol to collect the second replicates and repeat once more to collect the third replicates. If your filter clogs and you are unable to collect all replicates, please note that on the metadata sheet.

Also, it is highly recommended to do the filter-based sampling with 2 people. One person can operate the syringe and the other can hold the filter/needle assembly (while syringe is being (re)filled) and sample vials. This will keep everything clean and save a lot of time.
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**COLLECT FILTERED WATER SAMPLES - (CLEAR 4mL VIALS)**

5) Locate the three small (4mL) vials
6) Open the filter package that has a needle attached to the filter but **do not touch the inlet to the filter** and leave the needle/filter assembly in the package (when the video protocol is available, it will have a demonstration)
7) Open the 60 mL 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.
8) While sampling, please **stand downstream of the sampling location and point the opening of the syringe upstream**. This is important to collect a representative sample.
9) Fill the syringe with river water, collecting water from 50% of the water column depth. Expel the syringe contents into the river (downstream of the sampling location) and repeat this two more times. You only need to flush the syringe as described when you first open the syringe package. You do not need to flush repeatedly when collecting additional syringe volumes.
10) After flushing the syringe 3 times, fill the syringe again from 50% of the water column depth; this is the sample water to be collected.
11) 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. It is okay to touch the cylindrical filter housing.
12) Push 5 mL of water through the filter/needle assembly. This water is not collected.
13) Place the tip of the needle near the bottom of the vial and push water through the filter. Keep the needle in the vial and push about 15ml, letting it overflow. This will exchange the vial volume a few times for a robust sample. It's important to **keep the vial completely full and seal the vial** with the lid so there is no headspace. It may be easiest to do this by placing the vial on a flat surface and ensuring that surface tension produces a dome of water above the top of the vial. Then carefully seal the vial tightly. Store the vial on ice.
14) Move on to the next sample type (don't collect all replicates at once). After collecting a replicate of the other filtered sample types (see below) repeat steps 8 through 14. Repeat the collection of 1 replicate of each sample type until all replicates are collected

**COLLECT FILTERED WATER SAMPLES - AMBER 40mL VIAL WITH YELLOW TAPE**

15) There should be sufficient water in the syringe to collect the following sample. If more water is needed, unscrew the filter/needle assembly from the syringe (don't pull air into the filter). If sampling with 2 people, have one person hold the assembly by the filter housing (don't touch the needle) while the other person refills the syringe. If sampling alone, carefully place the filter/needle assembly back into the plastic needle cover that is still in the filter package and refill the syringe. After refilling, screw the syringe back onto the filter/needle assembly and **expel a small volume of water (**this water is not collected).
16) **Note** that **one filter and one needle should be used to collect all the filtered water samples**. 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, they should be shipped unopened when the samples are shipped.**

17) Locate the 40mL **amber** vial with yellow tape (there is only 1 of these). Unlike the other amber vials, this vial has no acid. Fill it to the premarked fill line. **Do not unscrew the top of the vial**. Instead, 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. Only remove the white disc that rests on top of the cap and conceals the septum.
18) Pierce the septum with the needle and fill the vial to the premarked fill line (approximately half full).
19) After injecting sample water into the vial, withdraw the needle from the septum. There is only 1 replicate of this sample type.
20) Check how much water is in the syringe. If needed, refill the syringe (make sure to keep the needle and filter inlet clean (do not submerge the filter in the river); see above)

**COLLECT FILTERED WATER SAMPLES - BLUE CAP FALCON TUBES**

21) Locate the plastic, blue-capped falcon tubes.
22) Fill one of the **blue cap falcon tubes to 15ml**. **Do not touch the inside of the vial with the needle, filter housing or anything else.** Cap the tube and place it in the cooler.
23) Move on to the next sample type (don't collect all replicates at once). Repeat the collection of 1 replicate of each sample type until all replicates are collected.

**COLLECT FILTERED WATER SAMPLES - AMBER 40mL VIALS WITHOUT YELLOW TAPE**

24) Locate the three 40mL amber vials that <u>are not marked</u> with yellow tape. These contain a very small volume of phosphoric **acid** (safety glasses recommended). Refill the syringe if needed (see above for details on avoiding contamination).
25) Using the same approach detailed above (i.e., pierce the septum with the needle), fill one of the acidified **amber** glass vials (that does not have yellow tape) **to the premarked line** -- do not unscrew the vial cap and don't touch the septum.
26) Shake the vial gently to incorporate the acid into the sample. Store vial on ice. There is no need to replace the flip top cap that previously covered the septum.
27) Move on to the next sample type (don't collect all replicates at once). Repeat the collection of 1 replicate of each sample type until all replicates are collected.

**COLLECT FILTERED WATER SAMPLES - ORANGE CAP FALCON TUBES WITH STAR SYMBOL (these contain <u>acid</u>)**

28) Locate the plastic, orange-capped falcon tubes. These contain acid. Ensure safety glasses and gloves are worn (they should be worn throughout the entire sampling procedure).
29) Fill one of **orange cap falcon tubes to 15mL**. **Note** these falcon tubes contain acid, use safety glasses and dispense the volume carefully. Turn over gently to mix acid into sample. Place tube in the cooler.
30) Go back to collect the next replicate of the 4ml vial (top of page 4). Repeat the collection of 1 replicate of each sample type until all replicates are collected. After collecting all of the filtered water replicates for each sample type, remove the needle **but not the filter** and safely dispose.

**After collecting all of the replicates above, collect one more filtered water sample:**

**COLLECT FILTERED WATER SAMPLES - 125mL PLASTIC BOTTLES**

31) Locate the 125mL bottle (there is only 1 of these).
32) Refill the syringe if needed (see above for details on avoiding contamination).
33) Fill to at least to the 80ml mark (too much is okay, too little is not). Place bottle in the cooler.

****Collect all filtered water samples before preserving the filter****

**PRESERVE THE USED FILTER**

34) After filling the vials, you will need to push more water volume through the filter to ensure enough microbial material is captured.
    a) Refill the 60mL syringe (ensuring you do not touch the outlet of the syringe or the inlet of the filter).
    b) 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 the filtration rate slows down significantly and it becomes difficult to push water through the filter, it is okay to stop filtering and move to the next step, as there is likely enough microbial biomass on the filter.
35) After filtering the additional water, you will need to preserve the filter.
    a) Detach the 60mL syringe from the filter, expel remaining water, 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) Take one of the small luer lock caps provided, dip it into the stream to rinse it and then **attach it to the open (discharge) end of the filter**. Note that the filter has a "male" side and a "female" side and therefore there are two types of caps provided.
    d) From the provided supplies, take out the small plastic epi tube filled with RNALater. This is the preservative for the filter. Also take out a 3mL syringe and a new needle.
    e) Connect the new needle to the 3mL syringe, carefully open the small epi tube, and fill the syringe with the RNALater by simply putting the needle down into the liquid.
    f) After filling the syringe with RNALater, invert the syringe so that the needle is facing up. Safely discard the needle.
    g) Take the used filter off the 60mL syringe, taking care not to touch the inlet of the filter.
    h) Attach the 3mL syringe to the filter and rotate so that the 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.
    i) Detach the syringe and place back into syringe wrapper. You can ship this used syringe back with your samples. Locate the remaining luer lock cap, dip it into the stream to rinse it, and then attach to the filter. Gently shake the sealed filter to distribute the RNALater.
    j) Put the capped filter into a small whirlpak bag, tie up to seal, and place in the cooler. Please ensure no water enters the whirlpak bag.

**COLLECT SEDIMENT**

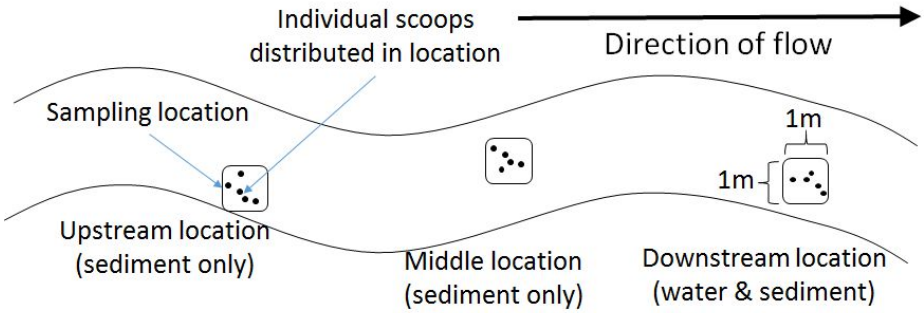


**Figure 2.** Idealized layout of sampling locations, each within a depositional zone. Each location should be ~1m$^2$, and in each location collect a number (~5-10) of spatially distributed scoops to fill one 125mL jar. Start at the downstream location and work upstream.

1) Refer to the stream diagram above (Figure 2) for examples of possible sampling schemes.

2) Starting at the downstream location, put on a new pair of nitrile gloves to be used for all sediment locations.
3) At each sediment site:
    a) Identify an approximately 1 $m^2$ region within the pre-identified depositional zone that is **under water**. This will be the location used to collect multiple scoops of sediment (Figure 2) that will be composited.
    b) Remove large debris from the sediment sampling locations (>4mm). Remove large cobbles that make up an armored layer, if applicable, to reach finer sediments.
    c) For each of the three sampling locations you will locate a 125mL sediment collection jar. Each jar is specific to the sampling location (i.e., there is a jar labeled for downstream and different jars labeled for the middle and upstream sites). For the **downstream location only** you will also locate the 2 50mL falcon tubes pre-filled with RNALater.
    d) Remove the 30mL metal scoop from packaging; ensure you only touch the scoop handle and not the spoon (the same scoop will be used for all sediment sites). Locate the metal scoopula wrapped in foil, which will help distribute sediment from the scoop. Unwrap the scoopula and ensure you only touch the scoopula handle. Wash the scoopula by dipping it in the stream.
    e) Use the scoop to collect surface sediments (**1-3 cm depth**) from the streambed within the ~1$m^2$ area at a number of locations (5-10) needed to fill the 125mL jar. Sampling should avoid large debris greater than approximately 4 mm in size to allow for the collection of enough sediment for analysis.
    f) For the downstream site, use the scoopula to put ~90% of the sediment from each scoop into the 125mL jar. Put the remaining 10% of sediment from each scoop into the RNAlater tube. Be careful to not splash RNAlater out of the tube. **The goal is to have composite samples** in the 125mL jar and in the RNAlater. Add sediment the fill line indicated on the RNAlater tube (**overfilling will diminish preservation of RNA**). Take care not to spill the RNAlater at the sampling site. After adding sediment, gently mix with the RNAlater by inverting the tube 5-10 times. For the middle and upstream sites, there are no RNAlater tubes, so place all sediment in the 125mL jars.
    g) From the 125ml jar, decant any excess water (we need sediment, not water for this piece).
    h) Store the sealed sediment/RNALater tubes and 125mL jar in the cooler.
    i) Repeat the sediment sampling protocol at the next two sediment sampling locations. Be sure to **rinse the sediment scoop** in river water between sites to reduce cross-site contamination.

**TRANSPORT AND STORE SAMPLES AFTER COLLECTION**

After all samples are collected, transport them to a refrigerator, keeping them on wet or blue ice during transit:

1) Place all samples (all vials/bottles/jars and the filter preserved in RNAlater) in a **refrigerator**. Please don't use a freezer as this will render samples unusable.

**METADATA**

1) After the samples are in the refrigerator, please enter metadata into the digital form that can be found at https://forms.gle/xUwP3qrzLiG7oBfc7. If there is difficulty in accessing the form, please email WHONDRS@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 Sampling kit ID as follows:
    1. Use your kit ID in the first part of all file names (e.g., S19S_0001).
    2. S19S_0001-across (photo looking across stream at downstream water sampling location)
    3. S19S_0001-up (photo looking upstream at downstream water sampling location)

4. S19S_0001-down (photo looking downstream at downstream water sampling location)
5. S19S_0001-seddown (photo of undisturbed sediments to be sampled at the downstream location, looking down at the sediments)
6. S19S_0001-sedmid (photo of undisturbed sediments to be sampled at the middle location, looking down at the sediments)
7. S19S_0001-sedup (photo of undisturbed sediments to be sampled at the upstream location, looking down at the sediments)
8. S19S_0001-data (photo of the data sheet, taken after sampling complete)

4) For the stream gauge data, if the closest stream gauge provides downloadable real-time data, provide the url in the metadata form. If the data are not available online, use the online metadata form or contact whondrs@pnnl.gov for instructions to submit data files.

**SHIPPING**

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. Contact WHONDRS@pnnl.gov *before* you sample if this is not possible. **It is vital to ship within 24 hours following sampling.**
2) It can be useful to remove materials from the refrigerator and pack the cooler as close as is reasonable to the time FedEx will ship the package. This maximizes the time samples stay cold.
3) When you're ready to ship, place the glass vials back in the vial holder located in the cooler and place the vials and the vial holders into a ziplock bag. If there is a broken vial, please do not send it back; instead find some material to place in the vial holder to take the place of the missing vial.
4) **Also inside the cooler, include the unused filter, unused needle, measuring tape, scoop, spatula, and absorbent padding that came with the supplies**. Pack those materials into the cooler and then place the frozen ice pack on top.
5) Put the lid of the cooler on, fill out the field on the paper metadata sheet for the time and date the box is packed, and then place the paper metadata sheet on top of the lid. This will keep it dry.
6) Please **don't return** gloves or used needles. Please dispose of the used needles in an appropriate sharps container.
7) Tape the outer box closed. Please use enough packing tape to make sure it won't come open during shipping.
8) Adhere the shipping label to the outside of the box by removing the backing from the plastic sleeve that contains the shipping label.
9) Drop the package off at FedEx, or have it picked up by FedEx.
10) **On the same day you ship the package**, **notify WHONDRS@pnnl.gov that you shipped the package, and include the FedEx tracking number in your ema**il. This is critical to ensure sample integrity and timely delivery. The subject line of the email should be "SHIPPED SAMPLES – [Sample kit ID # (e.g., S19S_0001)]". 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 loss of sample integrity.

**REFERENCES:**

Jensen, B. 2019. AOS Protocol AND Procedure: Sediment Chemistry Sampling in Wadeable Streams. NEON.DOC.001193, Rev. G.

**MSDS:**

RNALater: https://100sb204.cims.tw/attachments/2014/7/2f52c04f27ba94d6.pdf

20% nitric acid: http://www.labchem.com/tools/msds/msds/LC17750.pdf

70% phosphoric acid:
http://www.compasschemical.com/wp-content/uploads/2014/07/Phosphorous-Acid-70-SDS-6-15-15.pdf

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**At the end of sampling you should have the following:**

**WATER**

- ❑ 8 - 40mL clear vials of unfiltered water (half full or slightly more)
- ❑ 2 - 15mL falcon tube of unfiltered water (full)
- ❑ 3 - 4mL clear vial of filtered water (totally full)
- ❑ 1 - 40mL amber vial of filtered water without acid (yellow tape) (half full)
- ❑ 3 - 40mL amber vial of filtered water with acid (half full)
- ❑ 3 - 15mL blue cap falcon tubes of filtered water without acid (filled to 15mL)
- ❑ 3 - 15mL orange cap falcon tubes of filtered water with acid (filled to 15mL)
- ❑ 1 - 125mL plastic bottle of filtered water (2/3 full)
- ❑ 1 - filter, used, filled with RNALater, capped, in a whirlpak bag

**SEDIMENT**

- ❑ 2 - 50mL falcon tubes with RNALater and sediment
- ❑ 3 - 125mL glass jars with sediment