

VI. Field Sampling and Preservation Methods

A. Overview

The following water sampling requirements are taken from the Wisconsin State Lab of Hygiene protocols. All water samples collected for analysis need to comply with the equipment and procedures that follow.

B. Equipment

- 250 mL polyethylene bottle(s) (one per site)
- Extension pole (PVC pipe or stick) with rubber band to use to fasten bottle
- 1.0 mL vial of sulfuric acid (H₂SO₄)
- Nitrile or latex gloves
- Safety glasses
- Waterproof pen or marker
- Lab slips (also called “Test Request – Inorganic Surface Water & Microbiology” form) (one lab slip for each sampling site per visit; each lab slip is unique and should *never* be photocopied)
- Cooler(s) (one for every 2-3 samples)
- 2 gallon Ziploc bags filled with ice cubes (not ice packs)
- Waders or shoes that can get wet (not needed if sampling is done from shore)

C. Considerations/ Precautions

To avoid bias, the monitoring should be conducted at a sampling location as follows:

- **Avoid disturbing the sample.** If the sample is collected by wading in the stream, walk upstream to the sample location and take the sample facing upstream. Also avoid surface water and your hands from touching the rim of the bottle or inside of the cap.
- **Collect water sample 3 to 6 inches below surface, rinse sample bottle three times and fill it to its neck the fourth time.** Surface samples tend to have debris and other things floating on the surface and should be avoided.
- **Do not collect sample immediately downstream of a wastewater or storm sewer outfall pipe.**
- **Ensure sample is representative of the upstream conditions.** Stream reaches with major springs or major sediment deposits, such as former millpond beds, may create much localized conditions that aren’t reflective of the upstream conditions and should be avoided. Also avoid reaches immediately downstream of where cattle are in the stream.

- **Ensure sample is collected in an area with thorough mixing of stream water.** Stream reaches immediately downstream from tributaries or major springs may not have complete mixing and should be avoided.
- **Collect sample in portion of stream with greatest or strongest flow².** This may or may not be in the middle of the stream. In general, relatively straight reaches of the stream are preferred. However, if a meander section of the stream is selected for sampling, the sample should be collected in the portion with greatest flow at the outside of the meander. Slow flow areas along the banks, in eddies or immediately downstream of islands should be avoided. Note: If sampling with an extension pole, reaching out from shore to an area of flow with some movement (and not necessarily to an area of strongest flow) is sufficient. Your safety is most important!
- **Don't trespass on private lands to collect sample.** Use a public access point, such as a road right of way, or seek permission from the landowner or operator to cross their land for the purpose of collecting the samples.

D. Collection method

1. **On a 250 ml sample bottle circle the word “nutrients,” check the H₂SO₄ box, and write the field number and sample location on the bottle** (the latter two are listed on your lab slip as the “Field Number” and the “Sample Address or Location Description”) (Figure 1).
2. Locate a sampling location that is at least 10 to 20 feet away from a bridge crossing, in the middle of the stream channel, and is at least knee deep*.
Enter the stream and walk upstream towards the sampling location. This ensures the sample is not contaminated by sediment that has been dislodged from the substrate.
3. Facing upstream, **rinse the 250 mL polyethylene bottle three times with water 3 to 6 inches below the water's surface. The fourth time, fill the bottle to its shoulder and cap.** Whenever possible, and especially when stream flow is swift or water levels are

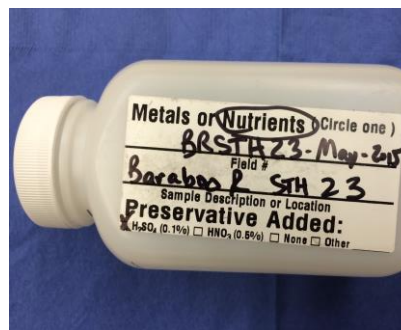


Figure 1. 250 mL polyethylene sample bottle.

² In some instances, water depth may be very shallow. When sampling during low flow conditions, take extreme care to ensure that the sample is not contaminated with sediment dislodged from the substrate. Also, at such times, it is more important to collect the sample in the area of strongest flow than the deepest location. Contact WAV staff if the water levels are low and you require a syringe to collect the water sample.

D. Considerations/ Precautions

Samples should be shipped as soon as possible, ideally within 1-2 days after being collected. Holding times are longer than one week, but the clock begins immediately after sampling and the lab needs them quickly in order to process samples on time. **However, do not mail samples on Fridays or Saturdays because lab staff is usually not present on weekends. If the ice melts completely, sample data will be flagged and unusable.** If the weather is extremely warm, if you collect a sample from more than one site, and/or if you collect QA/QC samples in addition to your regular sample you should add extra ice or use multiple shipping coolers to submit your samples to the lab. **Refrigerate the samples or keep them on ice until they are shipped.**

E. Questions?

Volunteers should feel free to contact Ilana Haimes (608-266-3599) or Peggy Compton (608-342-1633) with questions or concerns about any aspect of the project.

VIII. Field QC Duplicate Samples

a. Overview

To document the accuracy and precision of the field data collected by volunteers, ten percent of the samples that are monitored for total phosphorus (TP) are chosen each year to participate in collection of additional quality assurance/quality control (QA/QC) samples. The samples are randomly selected from the list of stations that are monitored. These QA/QC tests document the accuracy and precision of the data collected and look at natural variability and sampling error.

Two types of QA/QC samples will be collected by volunteers: field blank and field replicate (duplicate) samples for total phosphorus. If your site(s) have been selected at random for QA/QC sampling, you will be notified. To carry out sampling, please read the following instructions.

The result of this additional sampling is that you will be mailing a total of three water samples to the lab following one of your site visits: one regular sample, one duplicate, and one blank. Each sample should be placed in a separate Ziploc baggie. Refrigerate or ice the samples until you are ready to ship, ideally within 1-2 days, like with your regular sample.

B. Equipment

- 250 mL polyethylene bottle
- Extension pole (PVC pipe or stick) with rubber band to use to fasten bottle
- 1.0 mL vial of sulfuric acid (H₂SO₄)
- Nitrile or latex gloves and safety glasses
- Waterproof pen or marker
- Lab slip marked as “duplicate”
- Cooler with ice (from regular sampling)
- Waders or shoes that can get wet (not needed if sampling is done from shore)

C. Collection Method

Please prepare this sample at the same time as your regular samples, as follows:

Collect, preserve, and ship a second sample from the same location following the directions on pages 4-8 of this manual, **but write “DUPLICATE” on the bottle after the field number.** Find the lab slip that is pre-marked “DUPLICATE” near middle of the page, fill in the required fields, and place it in the Ziploc bag containing lab slips **Remove the sticker with the expiration date from your vial of acid and affix it to the top of the lab slip.**

Collected By Name		Telephone	Email
Where the sample was collected			
Station ID (STORET #)	Sample Address or Location Description		
573076	Baraboo River at STH 23-Bridge in Reedsburg		
County	Waterbody ID (WBIC)	Point / Outfall (or SWIMS Fieldwork Seq No)	
57 - Sauk	1271100	81878578	
Sample Details			
Sample Description / Device Description			
Enforcement? <input type="radio"/> Yes <input type="radio"/> No	If Field QC Sample (select one):		Depth of Sample: _____ <input type="radio"/> ft <input type="radio"/> m <input type="radio"/> in <input type="radio"/> cm
If yes, include chain of custody form.	<input checked="" type="radio"/> Duplicate <input type="radio"/> Blank <input type="radio"/> _____		Or Top and Bottom of Sample Interval:
Is Sample Disinfected? <input type="radio"/> Yes <input type="radio"/> No	Grant or Project Number		_____ - _____ <input type="radio"/> ft <input type="radio"/> m <input type="radio"/> in <input type="radio"/> cm
If yes, how? _____			

Figure 6. Lab slip for the duplicate sample.

IX. Field QC Blank Samples

A. Equipment

- Distilled water
- 250 mL polyethylene bottle
- 1 mL vial of sulfuric acid (H₂SO₄)
- Waterproof pen or marker
- Lab slip marked as “blank”

B. Collection Method

Please prepare this sample after the collection of your regular samples, as follows:

With a waterproof marker, circle the word “nutrients” on a 250 ml bottle, check the H₂SO₄ box, and write the field number and sample location on the bottle (these are listed on your lab slip as “Field Number” and “Sample Address or Location Description”). **To the right of the field number, write “BLANK”.**

To prepare the sample, rinse the bottle three times with distilled water, then fill it to its neck with distilled water. Preserve the sample with acid and then securely cap it. Mix the sample by inverting the bottle several times. Ship the sample along with the regular and duplicate samples following the directions in Section VII of this manual.

To complete the lab slip, find the lab slip that is pre-marked with the words “BLANK” near the middle of the page, fill the required fields, and place it in the Ziploc bag containing the regular and duplicate sample lab slips. Remove the sticker with the expiration date from your vial of acid and affix it to the top of the lab slip.

Collected By Name	Telephone	Email
Where the sample was collected		
Station ID (STORET #) 573076	Sample Address or Location Description Baraboo River at STH 23-Bridge in Reedsburg	
County 57 - Sauk	Waterbody ID (WBIC) 1271100	Point / Outfall (or SWIMS Fieldwork Seq No) 81878578
Sample Details		
Sample Description / Device Description		
Enforcement? <input type="radio"/> Yes <input type="radio"/> No If yes, include chain of custody form.	If Field QC Sample (select one): <input type="radio"/> Duplicate <input checked="" type="radio"/> Blank <input type="radio"/>	Depth of Sample: _____ <input type="radio"/> ft <input type="radio"/> m <input type="radio"/> in <input type="radio"/> cm
Is Sample Disinfected? <input type="radio"/> Yes <input type="radio"/> No If yes, how? _____	Grant or Project Number	Or Top and Bottom of Sample Interval: _____ - _____ <input type="radio"/> ft <input type="radio"/> m <input type="radio"/> in <input type="radio"/> cm

Figure 7. Lab slip for the blank sample (filled with distilled water).

X. Equipment Cleaning Procedures

Anytime you monitor, even if just at one stream site:

BEFORE leaving the stream:

- INSPECT equipment and REMOVE sediments, plants and animals
- SCRUB equipment with a stiff brush (including crevices) to remove sediment and debris
- RINSE equipment with tap water (using a spray bottle is useful for this)
- DRAIN all water from equipment

If you are moving to another stream site:

AFTER leaving the stream and BEFORE entering another stream, if you have entered the stream, switch to a completely new set of gear or do one of the following disinfection steps:

- SOAK waders and other equipment in bleach solution (1 tbsp per gallon of water) FAR AWAY FROM SURFACE WATER for 20 minutes in tote, then rinse. Wear gloves when handling bleach; or
- FREEZE for 8 hours.

ADDITIONAL RECOMMENDATIONS:

- After mixing with water, the bleach solution is only viable for one day. A new batch will need to be mixed before every sampling effort.
- Bleach solution will not be effective against certain invasive species, such as the New Zealand mudsnail. Please take additional measures when sampling in a region affected by resilient AIS to clean and disinfect equipment effectively.
- Do not use felt-soled boots. They are more likely to harbor small-bodied AIS and are very difficult to disinfect completely.
- If visiting multiple sites along the length of a stream or river, begin at the top of the watershed and work downstream.

XI. Total Phosphorus Monitoring Videos

Training videos showing the total phosphorus monitoring processes described in this protocol can be found on the **Water Action Volunteers Video Training webpage:**

<http://watermonitoring.uwex.edu/wav/monitoring/video.html>

Please contact WAV staff with any questions throughout the monitoring season!