A. Scope

This method pertains to the collection of surface water chemistry grabs for the determination of the concentration of nutrients (forms of nitrogen and phosphorus). While nutrients are generally grouped as nitrogen or phosphorus for field sampling protocols it is more important to consider if the sample should be preserved on non-preserved. This SOP will also cover the rare circumstance where field staff may be asked to filter samples in the field (Section F). However, for nearly all DNR sampling protocols samples that need to be filtered before analysis will be filtered at the Wisconsin State Lab of Hygiene. There is a video available on sample preservation for DNR monitoring: http://intranet.dnr.state.wi.us/int/es/science/ls/videos/Sample_Preservation.wmv

1. Preserved Samples

Preserved samples have a known quantity of acid added to the sample immediately after collection. Holding time for samples between sample collection and analysis is **28 days**. Acid preservative for samples is provided in vials by the Wisconsin State Lab of Hygiene (WSLH). Constituents that are preserved before analysis include:

a. Total Phosphorus
b. Total Dissolved Phosphorus
c. Total Nitrogen
d. Total Dissolved Nitrogen
e. Kjeldhal Nitrogen (organic nitrogen plus ammonia)
f. Nitrate + Nitrate (most common together, can be ordered individual)
g. Ammonia (NH3 and NH4)

2. Non-Preserved Samples

Non-Preserved samples do not have acid added to the sample which dramatically reduces the holding time. Non-preserved samples have a holding time of only **48 hours**. Constituents that are not preserved before analysis include:

a. Total Dissolved Phosphorus
b. Dissolved Ortho-Phosphorus

B. Summary of method:

Prior to sample collection all sampling equipment and sample containers must be thoroughly cleaned. Sample bottles for nutrients from the WSLH will be pre-cleaned and ready for use. In general, the possibility for contamination for non-filtered and lab filtered nutrients are low if simple sampling techniques are used. However, contamination is a major concern with field filtered nutrient samples due to the extra equipment used that has the chance to contaminate the sample. Generally, DNR field staff will be sampling for non-filtered or lab filtered nutrients for baseline monitoring programs.

In stream systems the sampler should wade into the water moving upstream and sample near the thalweg making sure that the area is free of recently disturbed sediments. Samples should be collected 3-6 inches below the surface of the water to avoid any surface scums or particles. Samples that require a preservative must be preserved with 1mL H_2SO_4 per 250 mL sample bottle and stored on ice before analysis. Samples that are not preserved have a **48 hour** holding time. Preparations must be made to send these samples to the WSLH before the holding time expires.

i. **Exception:** Samples for Total Dissolved Phosphorus require a different preservative than the other preserved nutrient samples. Total Dissolved Phosphorus requires a preservative of 0.48 mL H_2SO_4 12.5% and is collected in a 60 mL sample bottle and still has a holding time of **28 days**. Contact the WSLH for needed sampling equipment for Total Dissolved Phosphorus

1. Standard QA/QC practices

In general, one field blank and one duplicate sample for nutrients is recommended for every ten nutrient samples (i.e. 10% rule). For a field blank de-ionized (DI) water is transported into the field in a separate container. While in the field, a crew member fills a nutrient bottle with DI water and transports it on ice with the other samples. A field duplicate is taken in the field in the same location as the single sample. For each QA/QC sample the appropriate preservative should be added to the sample in the same many as any other grab sample. In general, a field blank is used to determine if there is any cross contamination or interference in the sample collection. A duplicate is used to determine how interferences in laboratory analysis or inherent variability in the concentration of the waterbody.

C. Safety:

Safety precautions of a general nature should be recognized. Life jackets should be worn if sampling from a boat or in areas of swift current. Collecting samples in cold weather, especially around cold waterbodies, carries the risk of hypothermia, and collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Preserving nutrient samples requires the use of small amounts of acid. Caution should be used to avoid contact with skin or eyes when acidifying the sample. A first aid kit should always be carried with the field crew for general safety considerations.

D. Equipment:

- 250 mL polyethylene bottle(s) (Preserved samples)
- 60 mL polyethylene bottle (Non-Preserved samples and Total Dissolved Phosphorus)
- 1.0 mL vial H_2SO_4 (Preserved samples)
- 0.48 mL vial H_2SO_4 (Total Dissolved Phosphorus only)
- Waterproof pen or marker
- Lab slip
- Ice
- Cooler
- Instruments to measure flow, temperature, dissolved oxygen, pH and specific conductivity

E. General Collection procedures

1. Label the bottle with the appropriate field number and sampling location and, if appropriate, check the box on the label indicating that H_2SO_4 has been added as a preservative. Circle "Nutrients" indicating the bottle has been sampled for nutrients.

2. Locate a sampling location that is at least 10 to 20 feet upstream from a bridge crossing, in the middle of the stream channel, and is at least knee deep. In cases where stream depth is shallow it is more important to collect the sample in the area of strongest flow (thalweg) than the deepest location. Walk upstream to the sampling location.This ensures the sample is not contaminated by sediment that has been dislodged from the substrate.

    a. If sampling using collection equipment (i.e. from a bridge) be sure to triple rinse equipment with DI and stream water. After first rinse, be sure to manually inspect equipment and wipe of any adhered dirt or debris.

3. Facing upstream, rinse a polyethylene nutrients bottle three times with the water to be sampled. Rinse with water 3 to 6 inches below the water surface.

4. Avoid touching the inside of the bottle or inside of the cap.

5. Fill the bottle completely, 3 to 6 inches below the surface.

F. Collection Procedures Preserved Nutrients:

1. See Section E 1-5 for General Collection Procedures

2. Use a **250 mL** polyethylene nutrients bottle for sample collection

3. Add **one 1.0 mL vial of H_2SO_4**, cap, and invert the bottle several times.

a. For Total Dissolved Phosphorus use a 60 mL sample bottle add 0.48 mL 12.5% vial of H_2SO_4

4. Holding time before analysis is **28 days** if sample is stored refrigerated.

G. Collection Procedures Non-Preserved Nutrients:

a. In general, DNR staff will collect samples for Non-Preserved nutrients that will be filtered in the lab by the WSLH. This greatly reduces the chances of contamination but substantially decreases the holding time of the sample.

b. See Section E 1-5 for General Collection Procedures.

c. Use a **60 mL** polyethylene nutrients bottle for sample collection

d. Note, holding time for Non-Preserved nutrients is **48 hours**, much shorter than preserved samples.

3) Sampling at Depth

When sampling at depth is performed it is very easy to compromise the cleanliness of a sample as more hardware is involved in obtaining a sample (lake, nonwadeable river, etc.). One way to do this is to rigorously clean any equipment (i.e. Kemmerer sampler) used to obtain the sample. Secondly, be sure to thoroughly triple rinse collection equipment with ambient water.

E. Documentation:

Standard documentation procedures should be followed for the collection of samples for nutrient analysis. However, it must be very clear whether the samples were acid preserved in the field or not. Be certain samples are received by the lab well in advance of the holding time as multiple days will be required due to shipping and time needed for organization and sample analysis at the WSLH. As of 2017 the WSLH requires that yellow batch label on each vial of preservative is attached to the lab slip. This ensures that expired acid is not being used to preserve samples.

F. Field Filtered nutrients

For certain projects it may be required for DNR staff to filter nutrient samples in the field using field filtering equipment. This type of sampling is inherently more susceptible to cross contamination and approved Quality Assurance Project Plans (QAPPs) must be approved before the project begins. It may be required for employees to pass a certification of competence test for field filtered nutrients. In general, this would require a crew member processing two field blanks on site that must come

back from the lab as non-detect. In this case the crew member has shown the ability to perform the task.

I. Equipment:

- 60 mL polyethylene bottle
- Transfer bottle
- Waterproof pen or marker
- 50 mL plastic syringe, peristaltic pump or other filter apparatus
- Filter housing
- Membrane filter 0.45 µm pore size
- Lab slip
- Ice
- Cooler
- Instruments to measure flow and temperature (optional)

II. Field Filtering QA/QC

Cross contamination is much more likely for field filtered nutrients and as such a more extensive QA/QC plan is required. All duplicates and field blanks should be taken in accordance with standard nutrient QAQC collection procedures above. In addition, for every 10 samples taken one sample blank should be taken. For filtered nutrients a sample blank is taken by filtering DI water in the same manner as the original sample using the same cleaned filtering equipment.

III. Collection Procedures for Field Filtered Nutrients

a. See Section E 1-4 for General Collection Procedures.

b. Use a 60 mL polyethylene nutrients bottle for sample collection

c. Remove the plunger from the 50 mL plastic syringe. Attach a filter by pushing or screwing it onto the syringe tip. Note that it will only fit one correct way.

d. Pour non-preserved sample from the transfer bottle into the syringe and fill to the top of the barrel.

e. It is important to filter a known amount so it can be properly acidified, 50 ml is recommended.

f. Re-insert the plunger, place the filter over a 60-ml polyethylene bottle opening, and slowly push the plunger down until you reach the 50ml mark.

g. Use this excess filtrate to rinse the 60-ml bottle, and discard. The filtered nutrients bottle may only be rinsed with pre-filtered water, never with ambient stream water.

h. Place the plunger over the bottle opening and push the plunger down to filter the remaining sample (50ml). It may seem difficult, but most samples will only require 10-30 seconds to filter. The filter may rupture if too much pressure is applied. Inspect the filter and if it is ruptured discard the filter and syringe and start over. Ideally a second filtered sample bottle would be used to collect the new sample. However, if there are not extra bottles handy be sure to thoroughly, triple rinse the container with Filtered stream water.
    i. This is very time consuming, it is advised that an extra few seconds of patience filtering the sample can avoid a rupture filter and save these steps.

i. Add **one 1.0 mL vial of H_2SO_4**, cap, and invert the bottle several times.

j. Check the box on the bottle label that indicates the sample has been preserved and label with the appropriate field number.

k. Write on the lab slip that the 60 mL bottle has been field filtered and preserved with H_2SO_4.

l. Store bottles on ice during transport to a refrigerator or the WSLH.

G. Updates and Tracking

Version Number	Date	Sections	Name	Approval
3.2	05/15/2014	All	Shupryt/Turcotte/ Arneson/LTT Workgroup	Shupryt 5/26/2015
3.3	04/02/2019	All, minor editorial changes	Shupryt	Shupryt 04/02/2019

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