STANDARD OPERATING PROCEDURE FOR THE FILTERING OF CHLOROPHYLL-*a* SAMPLES

WILLARD SPUR 2011 MONITORING ACTIVITIES

State of Utah
Department of Environmental Quality
Division of Water Quality

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Utah Division of Water Quality (DWQ) Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical experts. The primary purpose of this document is for internal DWQ use. This SOP should not replace any official published methods.

Any reference within this document to specific equipment, manufacturers, or supplies is only for descriptive purposes and does not constitute an endorsement of a particular product or service by the author or by DWQ. Additionally, any distribution of this SOP does not constitute an endorsement of a particular procedure or method.

Although DWQ will follow this SOP in most instances, there may be instances in which DWQ will use an alternative methodology, procedure, or process.

REVISION PAGE

Date	Revision #	Summary of Changes	Sections	Other Comments
9/9/2011	1	Not applicable	Not applicable	New SOP. Began document control/revision tracking.

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1.0 SCOPE AND APPLICABILITY

This document presents the Utah Division of Water Quality's (DWQ) Standard Operating Procedure (SOP) for field processing of water samples from Utah's wetlands, lakes, and rivers/streams for chlorophyll-*a* analysis (phytoplankton method). This SOP does not cover chlorophyll-*a* samples collected for the UCASE Program (Utah Comprehensive Assessment of Stream Ecosystems). The UCASE chlorophyll-*a* method (periphyton method) is covered in a separate document.

This SOP gives procedures for the field filtering of water samples for chlorophyll-*a*; it does not discuss collection of water samples. Water sample collection should be performed in accordance with specific program SOPs or field manuals, or a specific project sampling and analysis plan (SAP). This SOP applies to all personnel collecting chlorophyll-*a* samples including DWQ monitors, non-DWQ State of Utah cooperators, and volunteer monitors.

Chlorophyll-*a* is a green photosynthetic pigment and concentrations of chlorophyll-*a* are used to infer algal biomass. The DWQ uses these data in several ways. As an important indicator for lake water quality assessment, chlorophyll-*a* concentrations are one component used to calculate the Carlson Trophic State Index, a measure of the degree of eutrophication in a lake/reservoir. Chlorophyll-*a* concentrations in rivers/streams are used to support nutrient criteria development. Chlorophyll-*a* is also included in a suite of water chemistry parameters evaluated as a component of a Multimetric Index (MMI) used to assess wetland condition.

2.0 SUMMARY OF METHOD

A filter apparatus comprised of a hand pump or Geopump™ (peristaltic pump), plastic tubing, a filter flask, and filter funnel with a filter stage is used to filter the water sample. A glass-fiber filter is placed on the filter stage. Sample water is poured into the filter funnel and then pumped through the filter. The filter (containing the residue to be analyzed) is removed from the filter stage and wrapped in foil or placed into an opaque sample container. A volume of 500 ml is typically filtered; however, turbid water may clog the filter, resulting in a lower sample volume. The total volume filtered is recorded and the sample is frozen until extracted and analyzed by the laboratory.

3.0 DEFINITIONS AND ACRONYMS

DI: deionized water

ml: milliliter(s)

mm: millimeter(s)

psi: pounds per square inch

Residue: The part of the sample remaining as a solid on the filter paper after the liquid passes through the filter.

RPM: revolutions per minute

µm: micrometer(s), also called micron(s)

4.0 HEALTH AND SAFETY WARNINGS

Not applicable to this SOP.

5.0 CAUTIONS

IMPORTANT: If a sample is turbid, filter only small volumes of the sample at a time. A turbid sample will not filter to 500 ml. Keep track of the volume filtered and record the final volume on the sample label and the lab sheet. Submit only one (1) filter to the laboratory, regardless of the final volume, unless otherwise directed by the project manager.

When using a hand pump or Geopump™, be careful to not exceed 7 psi (14 in. Hg) when applying pressure. High pressures can rupture algal cell walls allowing the chlorophyll-*a* pigment to pass through the filter and not be included in the analyzed sample, leading to underestimated concentrations or false negative results. In addition, when using a Geopump™, or other peristaltic pump, set the motor RPM to the lowest setting to reduce pressure on the filter.

6.0 INTERFERENCES

A dirty filter apparatus can lead to sample contamination. The apparatus should cleaned prior to a sampling trip and should be decontaminated between samples by rinsing the filter stage and filter flask 3 times with deionized (DI) water and shaking dry. The stage and flask should be inspected for any adhered particles. If noticeable buildup is on the stage or flask they should be soaked in soapy water and then scrubbed until buildup is removed. After cleaning, the stage and flask should be thoroughly rinsed with tap water followed by DI water and then air dried.

Highly turbid water may cause the filters to clog more frequently. If sample water is turbid, filter only a small volume at a time.

Chlorophyll-*a* pigment is broken down by exposure to sunlight (photodecomposition). Therefore the filtering procedure should be carried out in subdued lighting conditions, if feasible. The filter apparatus should be set up prior to collection of the sample, the sample bottle should be protected from sunlight once collected, and the sample should be filtered as soon after sampling as possible. In addition, processed and frozen filters should also be protected from light during storage by using an appropriate opaque sample container (such as aluminum foil).

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

Samplers are required to read this SOP annually and acknowledge they have done so via a signature page (see **Appendix**) that will be kept on-file at DWQ along with the official hard copy of this SOP. Personnel collecting chlorophyll-*a* samples must be trained by an experienced DWQ employee. Demonstration of capability may be required for cooperators and volunteer monitors on a yearly basis. Yearly review sessions will be held by the State of Utah to review procedures and should be attended by all personnel intending on sampling for chlorophyll-*a*.

8.0 EQUIPMENT AND SUPPLIES

_____Copy of this SOP

_____Field notebook, lab sheet

_____Pens, pencils

_____Sample labels (**Figure 1**)

_____Sample containers for filters (aluminum foil is preferable, another clean opaque container is an acceptable alternative)

_____Polypropylene graduated cylinder, 500 ml

_____Glass fiber pre-filters (47 mm diameter, 0.7 µm pore size)

_____Plastic disposable filter forceps, length: 4 ½" (2 pair aids in filter folding)

_____DI water in squeeze bottle

_____Cooler and dry ice (if unable to put samples in freezer immediately after processing)

- Hand pump Method (preferred)
  - _____Hand pump (25" Hg, 15cc pumping rate/stroke)
  - _____Tygon or similar tubing (approximately 24"), diameter needs to fit filter flask arm
  - _____Nalgene or similar polypropylene filter flask, 500 ml
  - _____Nalgene analytical test filter funnels
  - _____#7 filter flask stoppers with ½ -inch hole
- Geopump™ Method (alternate)
  - _____Geopump™ with quick-release pump head, fitted with approximately 3 feet of Masterflex tubing attached to a Swinnex-style filter holder (47 mm diameter) secured to tubing using a hose clamp

Figure 1. Chlorophyll-*a* sample label
(U:\WQ\PERMITS\MONITORS\Labels\Chlorophyll-a WaterColumn (5162).doc)

Chlorophyll -*a* Water Column (Frozen)
SITE ID: _____
_____STORET: _____
DATE: _____ SAMPLERS:_____
SUB SAMPLE VOL (ml): _____
COMPOSITE VOL (ml): _____
_____

9.0 PROCEDURE

The preferred procedure is the Hand Pump Method. This method allows for easier decontamination between samples because the water sample does not contact any tubing. Also, sample volume is more accurately measured using the hand pump method. Lastly, vacuum pressure is more easily controlled using the hand pump method. Use the Geopump™ method only as an alternative to the hand pump method.

9.1 Hand Pump

Start each sampling trip with equipment that has been cleaned with soap (liquinox) and water, rinsed 3 times with tap water, rinsed 3 times with DI water, and allowed to dry.

1) Attach tubing onto hand pump and filter funnel.

2) Insert the funnel adapter into the #7 stopper; wetting the adapter may aid in this. Once the adapter is in the stopper it need not be disassembled unless a thorough cleaning of the entire apparatus is required.

3) Insert the filter funnel assembly into the top of the filter flask until it seals tightly. **Figure 2** provides a photograph of the entire filter apparatus assembly attached to a hand-pump.

4) Remove the top of the filter funnel from the filter stage.

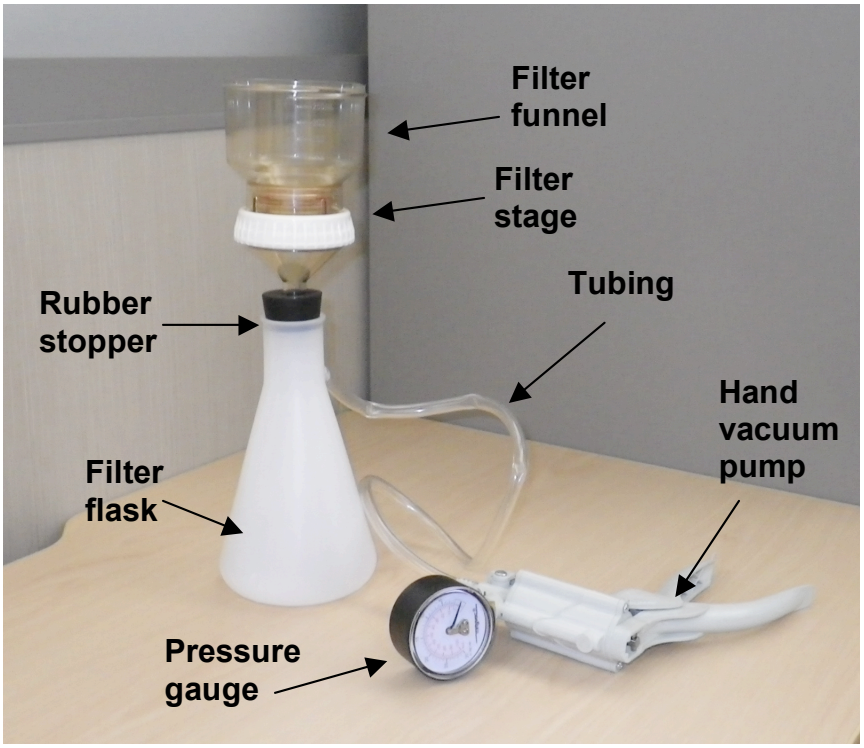
5) Using the forceps place the glass fiber filter on the filter stage. Take care not to touch the filter or inside of the filter funnel with your hands to avoid contamination.

6) Wet the sides of the filter funnel and filter with DI water from the squeeze bottle to create a good seal on the filter.

7) Mix the water sample in its container multiple times to homogenize, and pour the sample into a graduated cylinder to measure 500 ml (or a smaller volume if sample is turbid).

8) Pour 250 ml (or a smaller volume) of sample water into the filter funnel and use the pump to pull the sample through the filter.

Figure 2. Filter apparatus.



9) Continue filtering measured volumes of sample, being careful not to exceed 7 psi of vacuum pressure during filtration. When up to 500 ml of sample water has been filtered, or the filtration rate has slowed, or the filter has turned a green/brown color, sufficient sample has been filtered.

NOTE: *If the sample is turbid, only filter small volumes of sample at a time. Try to estimate how much turbid water you can put through a filter by paying attention to how the pressure increases as you pull the water through the filter. If you overestimate the volume of water that can pass through the filter and water is left remaining on top of a clogged filter, filtering must be repeated with another volume of sample and a new filter.*

10) Rinse down the sides of the filter funnel with DI water and use the pump to pull the rinse water through the filter. You can stop filtering when the filter is still slightly wet; the filter need not be completely dry.

11) Remove the filter funnel being careful not to disturb the filter stage or filter.

12) Using forceps, remove the filter from the filter stage, being careful not to rip the filter or scrape off any green/brown residue.

13) Fold the filter in quarters with the residue facing the inside.

14) Place the folded filter onto a piece of aluminum foil and fold the foil to make a package for the filter. Alternatively, place the folded filter into a clean opaque sample container (such as a plastic film canister).

15) If using foil, place the foil-wrapped sample into a small ziptop bag.

16) Fill out the sample label and either drop into the ziptop bag with the sample or stick onto the ziptoc bag containing the sample. If using an opaque container, attach the label to the container using clear tape.

17) Place the sample into a larger ziptop bag and store the sample immediately on dry ice**.**

Decontamination Procedure: *Between samples, triple rinse the graduated cylinder, filter funnel, filter flask, and foreceps with DI water and shake dry as best as possible.*

9.2 Geopump™

Start each sampling trip with equipment that has been cleaned with soap (liquinox) and water, rinsed 3 times with tap water, rinsed 3 times with DI water, and allowed to dry.

1) Geopump™ assemblies are already set up in field vehicles for water chemistry sample processing.

2) Open the filter holder.

3) Using the forceps place the glass fiber filter on the filter stage. Take care not to touch the filter or inside of the filter holder with your hands to avoid contamination.

4) Mix the water sample in its container multiple times to homogenize, and pour 500 ml of sample into the clean graduated cylinder; place the clean intake tubing down into the graduated cylinder.

5) Begin pumping the sample through the filter. Use the lowest pumping speed on the Geopump™ to reduce pressure on the filter.

6) When 500 ml of sample water has been filtered, or the filtration rate has visibly slowed, sufficient sample has been filtered. Do not try to force more sample through the filter; this increases pressure on the filter.

NOTE: *If the sample is turbid, only filter small volumes of sample at a time. Try to estimate how much turbid water you can put through a filter by paying attention to how the flow rate changes as you pull the water through the filter.*

7) Pump the last remaining sample to get all water out of the tubing. Turn off the pump.

8) Open the filter holder being careful not to disturb the filter stage or filter.

9) Using forceps, remove the filter from the filter stage, being careful not to rip the filter or scrape off any green/brown residue.

10) Fold the filter in quarters with the residue facing the inside.

11) Place the folded filter onto a piece of aluminum foil and fold the foil to make a package for the filter. Alternatively, place the folder filter into a clean opaque sample container (such as a plastic film canister).

12) If using foil, place the foil-wrapped sample into a small ziptop bag.

13) Fill out the sample label and either drop into the ziptop bag with the sample or stick on the ziptoc bag containing the sample. If using a container, attach the label to the container using clear tape.

14) Place the sample into a large ziptop bag and store the sample immediately on dry ice**.**

Decontamination Procedure: *Between samples, flush (using the pump) the entire filter holder/tubing assembly with at least 500 ml of DI water, and also be sure to rinse the exterior of the intake tubing and the forceps thoroughly with DI water. Triple-rinse the graduated cylinder with DI water and shake dry as best as possible.*

10.0 LABORATORY ANALYSIS

Filters are extracted and analyzed for chlorophyll-*a* by Standard Method 10200 H (homogenization followed by spectrophotometry). Samples should be analyzed within 3 weeks of collection. The methodology and quality assurance and quality control procedures for this analysis and analyzing laboratories can be obtained from:

Sanwat Chaudhuri, Chemical and Environmental Services Bureau Director
Unified State Laboratories: Public Health, Utah Department of Health
4431 South 2700 West
Taylorsville, UT 84119
(801) 965-2400
UPHL@utah.gov

NOTE: *The sample processing procedure described in this SOP can also used to prepare filters for ash free dry mass (AFDM) analysis. Filters are analyzed by the State Laboratory using Standard Methods (American Public Health Association, 1999, section 10400.D.3). See* ***Figure 3*** *for AFDM sample labels.*

Figure 3. AFDM sample label
(U:\WQ\PERMITS\MONITORS\Labels\ DRY WEIGHT Water Column(5162))

DRY WEIGHT Water Column (Dry-Ice) State of Utah Lab

SITE ID: _____
_____ STORET: _____
DATE: _____ SAMPLERS:_____
SUB SAMPLE VOL: _____COMPOSITE VOL: _____

11.0 DATA AND RECORDS MANAGEMENT

The volume of sample water filtered must be recorded in two places: on the lab sheet and on the sample label (**Figure 1**). Once the laboratory has received the samples, the lab sheets are photocopied and copies sent back to DWQ. Data management staff review these sheets on a biweekly basis to ensure all expected laboratory results are received.

12.0 QUALITY ASSURANCE AND QUALITY CONTROL

Field quality control samples to be performed with chlorophyll-*a* sampling include blanks and duplicates.

For blanks, decontaminate the filtering apparatus as described in **Section 9.0**. Then filter 500 ml of DI water through the filter apparatus and process as a regular sample. At a minimum one blank should be performed per sampling trip to check for cross-contamination between samples. Some other frequency may be required according to a program or project specific quality assurance plan or sampling and analysis plan.

For the field duplicate, filter a second volume of water from a separate sampling container collected concurrently or immediately after the regular chlorophyll-*a* sample was collected. Filter and process the field duplicate as a regular sample. The filtering apparatus should be decontaminated as described in **Section 9.0** between processing the regular and duplicate sample. At least one field duplicate should be performed for every 10 regular samples, or at a frequency indicated in a program or project specific quality assurance plan or sampling and analysis plan.

13.0 REFERENCES

Hambrook Berkman, J.A., and Canova, M.G., 2007, Algal biomass indicators (ver. 1.0): U.S. Geological Survey Techniques of Water-Resources investigations, book 9, chap. A7, section 7.4, August, accessed__*date*__ from http://pubs.water.usgs.gov/twri9A/.

Lazorchak, James M., Donald J. Klemm, and David V. Peck. 1998. Environmental Monitoring and Assessment Program – Surface Waters: Field Operations and Methods For Measuring the Ecological Condition Of Wadeable Streams. Washington D.C.: U.S. Environmental Protection Agency.

Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998, Method 10200H “Chlorophyll”.

USEPA. 2010. Sampling and analytical procedures for GLNPO’s Open Lake Water Quality Survey of the Great Lakes. EPA 905-R-05-001. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, Illinois. (http://www.epa.gov/glnpo/monitoring/sop/index.html)

14.0 APPENDIX

SOP Acknowledgment and Training Form (front and back)

SOP Acknowledgement and Training Form

This SOP must be read and this form signed annually. This form must be kept with the current version of the SOP.

Document Title:	
Document Revision Number:	
Document Revision Date:	

Please sign below in accordance with the following statement: "I have read and understood the above referenced document. I agree to perform the procedures described in this SOP in accordance with the document until such time that it is superseded by a more recent approved revision."

Printed Name	Signature	Date

SOP Acknowledgement and Training Form (continued)

Trainee: Sign below to acknowledge that training on this SOP was received, understood, and all questions/concerns were addressed by the trainer.

Trainer: Sign below to acknowledge that training on this SOP was completed for the individual listed and that trainee is competent to perform the procedures described within.

Date of Training	Trainee Printed Name	Trainee Signature	Trainer Printed Name	Trainer Signature