STANDARD OPERATING PROCEDURE FOR ALGAE SAMPLE RECEIPT, SUBSAMPLING, AND DIATOM SAMPLE CLEANING, ABBREVIATED

ALG 10001A

Method Reference: See References Section.

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Great Lakes Environmental Center, Inc.
(GLEC)

Julianne Heinlein Technical Approver	10/11/2024 Date
Dennis J. McCauley President/Principal Research Scientist	10/11/2024 Date
Jennifer Hansen GLEC Quality Assurance Officer	10/11/2024 Date

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I. SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes the sample receipt, subsampling, and archiving of algae samples for both soft algae identification and diatom identification. It also describes the microwave digestion of the diatom subsample using the ETHOS UP microwave digester to clean organic matter from the diatom cells.

1.2 This SOP may be modified by mutual consent of Great Lakes Environmental Center (GLEC) and the client to achieve the objectives of a given study plan.

II. SUMMARY OF METHOD

2.1 Upon receipt of samples, their condition is assessed and sample labels are checked against the chain of custody form (COC). If a sample is missing, mislabeled, or in poor condition this is promptly reported to the client. NEON samples require documentation of sample receipt in an electronic form provided by NEON staff. See Section 9.1.4.

2.2 Algal samples may be subsampled for soft algae analysis and diatom analysis. resulting in two separate samples for storage.

2.3 The diatom subsample is digested in nitric acid in the ETHOS UP microwave digester to remove all organic matter prior to storage in scintillation vials. This subsample of cleaned diatom cells is later used to make mounted diatom slides for analysis, see GLEC SOP ALG 10002A.

2.4 This SOP draws from protocols outlined in the following documents:

2.4.1 The National Rivers and Streams Assessment 2023-2024 Laboratory Operations Manual;

2.4.2 “The protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program,” Report No. 02-06, the Academy of Natural Sciences, Patrick Center for Environmental Research-Phycology Section; and

2.4.3 Acker 2016 “Identification and enumeration of algae in phytoplankton samples”.

III. DEFINITIONS

3.1 Diatom algae – Diatoms are algae with distinctive, transparent cell walls made of silicon dioxide hydrated with a small amount of water.

3.2 Deionized (DI) water – Water that has had its mineral ions removed. It is produced by purifying tap water by reverse osmosis (RO) followed by passing it through carbon and de-ionization cartridges. It is a physical process using ion exchange resins, which bind to and filter out the mineral salts from water.

3.3 Digestion – Refers to the solubilization of organic material by strong acid oxidation, which may be aided by pressure and heat generated by microwaves.

3.4 Safety Data Sheet (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, flammability, and reactivity, including how it should be stored, handled, and disposed.

3.5 Soft algae – Soft-bodied, non-diatom algae.

3.6 Macroalgae - large macroscopic filamentous, colonial, tuft-forming, crustose, tissue-like or coenocytic eukaryotic algae and cyanobacteria that have forms recognizable with the naked eye (from Stancheva et al. 2015).

3.7 Subsample - Portion of the sample obtained by randomly extracting a volume from the composite sample collected by the field crew.

3.8 Microwave digester/digestion – The ETHOS UP is a microwave system which uses contactless microwave irradiation to heat material. This allows organic material in diatom samples to be digested, leaving the silicon dioxide valves in place.

IV. INTERFERENCES AND CAUTIONS

4.1 Samples preserved in alcohol should never be treated with nitric acid due to the risk of an explosive reaction. If you believe a sample may have been mistakenly preserved in ethanol, please set it aside and inform the lab manager.

4.2 The nitric acid digestion in the microwave digester may release corrosive fumes. The discharge vent hose should be secured in a functioning fume hood which should be running during active digestion.

4.3 Do not homogenize soft algae subsamples enough to break up microscopic colonies. This will make the soft algae identification more difficult.

V. EQUIPMENT AND SUPPLIES

5.1 Carboy for DI

5.2 Chemical fume hood

5.3 Erlenmeyer flask – 1L or 500 mL, to pour DI into beakers

5.4 ETHOS UP microwave digester - with nylon digestion tubes, with valves and screw caps, and carousel

5.5 Forceps

5.6 Lab coat

5.7 Laboratory labeling tape

5.8 Laboratory trays

5.9 Nitrile gloves

5.10 Permanent markers and pens

5.11 Project specific sample log

5.12 250 mL wash bottle

5.13 Safety glasses

5.14 Syphon - to remove supernatant when rinsing cleaned diatom samples

5.15 Tape - electrical or similar

5.16 Watch glasses, aluminum foil, or parafilm to cover cleaned diatom samples during rinsing period

5.17 5 mL pipette and tips - one tip per sample

5.18 20 mL scintillation vials

5.19 150 mL beakers, - labeled with sample ID, one per sample plus extras for reagents/water

VI. REAGENTS AND STANDARDS

6.1 Reagent Water – DI water

6.2 Reagents –

6.2.1 Concentrated nitric acid (HNO_3)

6.2.2 70% ethanol

6.2.3 10% hydrochloric acid (HCl)

6.2.4 Glutaraldehyde

VII. SAMPLE RECEIPT

7.1 Sample Receipt (Workflow Chart for Sample Receipt is provided below in Figure 1).

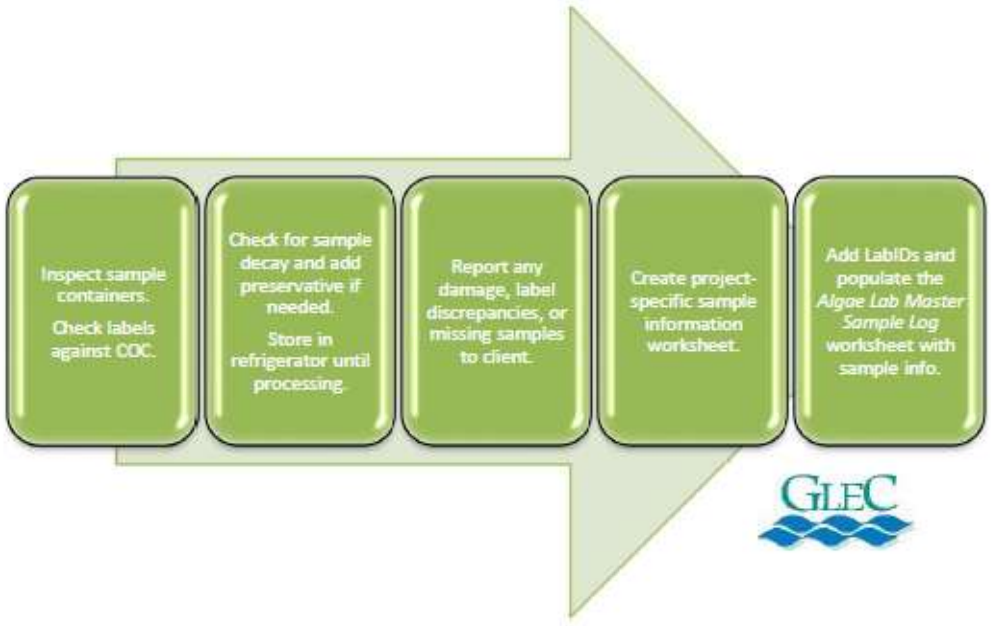


Figure 1. Workflow Chart for Sample Receipt

7.1.1 Chain of custody form (COC), listing a unique sample ID for each sample shipped, should be included with each shipment to GLEC.

Note: NEON samples are preserved in glutaraldehyde.

7.1.2 Upon sample arrival at GLEC, evaluate the condition of each sample container, checking for cracks, leakage, etc. Cross-check the sample information on the sample containers against the sample information listed on the COC form(s). Smell the sample to determine if any decay is

occurring. If decay is detected, add more of the original preservative to all original samples. This addition should be recorded in the sample information sheet. NEON sample receipt will be documented in an electronic receipt form provided by NEON staff.

7.1.3 In the event of any container damage, label discrepancies, and/or missing samples, the client should be contacted before processing the samples. For NEON samples, the technician should "Reply All" to the shipping notification email.

7.1.4 Inform client of sample receipt. For NEON samples, this includes completing an electronic receipt form provided by NEON staff. When completed, inform the lab manager and they will upload this receipt form to the NEON Data Portal.

7.1.5 All Sample information should be entered into a project-specific Excel spreadsheet for tracking and protocol documentation. Use the bar code reader to directly populate the project-specific Excel worksheet.

7.2 Preserved samples should be kept in a sample refrigerator, at 4ºC, until processed. Long term storage of preserved materials may be at room temperature in a dark space. Cleaned diatom subsamples may be kept at room temperature.

7.3 Samples with live algae should be refrigerated, kept in the dark and processed or preserved within 2 days of receipt.

VIII. QUALITY CONTROL

8.1 Clean glassware, nylon digestion tubes and valves/screw caps, Nalgene bottles, and forceps in the following manner. Rinse with hot tap water and soak in Liquinox © 3% solution for a minimum of 4 hours. Rinse with tap water followed by triple rinse with DI water. Soak and rinse forceps or other utensils used while processing samples. Rinse siphon tube with DI water between samples. If discoloration is noted on the nylon digestion tubes, they may be cleaned with nitric acid.

8.2 Observed loss of material at any stage of this procedure compromises quantitative samples. Samples which experience such loss should be discarded immediately if sufficient material has been retained to allow for a fresh sample. If the compromised sample is the only material available from the original sample, the procedure should be completed, and the nature of spillage or loss should be documented clearly.

IX. PROCEDURE

9.1 Subsampling, Archiving, and Shipping

9.1.1 Check that each sample is intact and smell the sample to determine if any decay is occurring. If decay is detected, add more of the appropriate preservative to all the original samples and note this addition on the data sheets.

9.1.2 Measure the sample volume and note any deviations from the expected volume.

9.1.3 Multiple subsamples may be taken from the original algal sample. The following steps outline the process of subsampling for both soft algae analysis and diatom analysis. All steps may not be required for all contracts. A workflow chart for the archiving of NEON samples is provided in Figure 2 below.

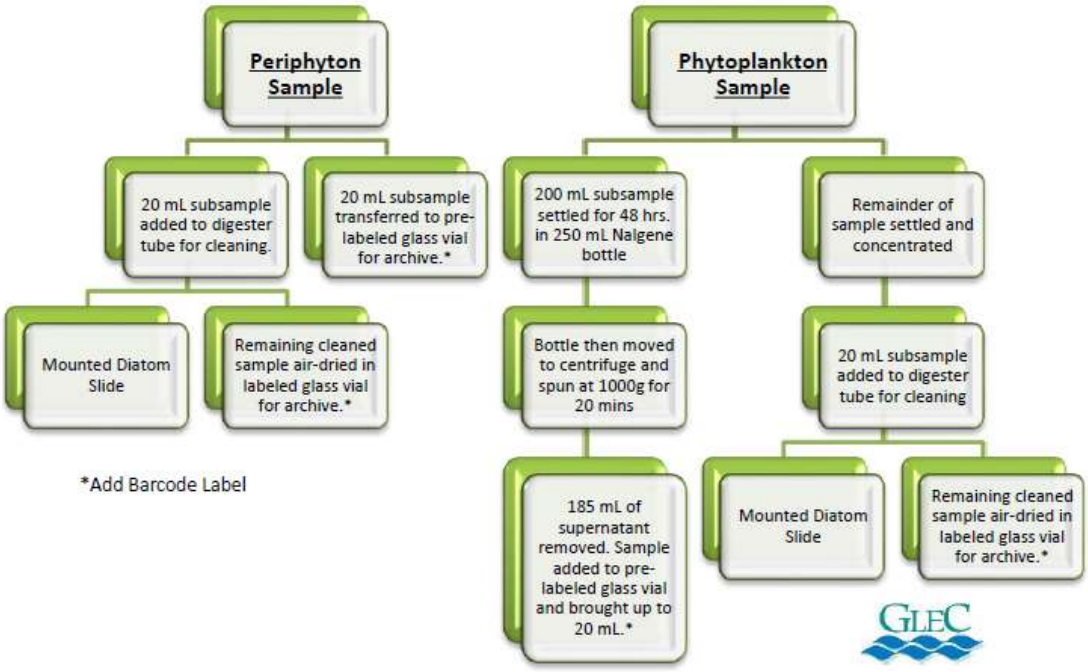


Figure 1. NEON Sample Archiving Workflow

9.1.3.1 If samples are to be digested for diatom slides, start a Diatom Slide Preparation Worksheet including the date of digestion, number of samples, amount digested (generally 20 mL, note deviations), and initials of technician.

9.1.3.2 Using preprinted labels, label 20 mL scintillation vials for the soft algae subsamples. If applicable, label corresponding scintillation vials for the cleaned diatom subsample and set aside. Using lab tape, mark 150 mL beakers with the sample ID. These beakers will be used for digestion of the diatom subsamples. If archiving NEON phytoplankton samples, use label tape to mark clean 250 mL Nalgene bottles for settling and centrifugation.

9.1.3.2.1 NEON archive subsamples should be labeled according to the NEON protocol outline in "Exhibit A_SOW Macroalgae Taxonomy and Algae Archiving" pages 4-5

For all subsampling, suspend and homogenize the algal sample by inverting or swirling the original sample immediately before removing sample.

9.1.3.3 Soft Algae

9.1.3.3.1 For Periphyton Samples: Using a 5 mL pipette with a clean tip, before the material settles again, transfer a 10 mL aliquot to the pre-labeled 20 mL glass sample vial. Invert the sample again to homogenize and remove another 10 mL of sample. This soft algae subsample will be kept in a sample refrigerator, at 4ºC until taxonomic analysis.

9.1.3.3.2 For Phytoplankton Samples: Decant 200 mL of homogenized sample into the pre-labeled 250 mL Nalgene bottles. Allow the samples to sit, unmoved for 48 hours to allow algal cells to settle to the bottom. Carefully transfer these settled samples to the centrifuge. Centrifuge at 1000g for 20 minutes.

9.1.3.4 Diatoms

9.1.3.4.1 For Periphyton Samples: Invert the original sample to homogenize and, before the material settles again, transfer a 10 mL aliquot to a nylon ETHOS UP digester tube using a 5 mL pipette

with a clean tip. Invert the original sample again to homogenize and remove another 10 mL of sample to the tube. Proceed to Microwave Digestion Procedure outlined in 11.2.

9.1.3.4.2 For Phytoplankton Samples: Allow the remainder of the phytoplankton sample (800 mL) to settle, undisturbed, for 48 hours. Concentrate the sample ~10X by removing an appropriate volume of the supernatant. Invert the remaining sample to homogenize and, before the material settles again, transfer a 10 mL aliquot to a nylon ETHOS UP digester tube using a clean 5 mL pipette tip. Invert the original sample again to homogenize and remove another 10 mL of sample to the tube. To obtain the required frustule density on the prepared slides, very sparse samples may require the digestion of more than 20mL of the concentrated phytoplankton sample. Proceed to Microwave Digestion Procedure outlined in 11.2.

9.1.3.4.3 Digested diatom samples can be stored at room temperature. Archive diatom samples are to be air dried for long-term storage. After a diatom slide been made and QC reviewed for quality, the cleaned diatom samples are prepared for air drying. Allow samples to settle, undisturbed, in the 20 mL glass vials, for 48 hrs. After this time, carefully remove ≥ 15 mL of the overlying water using a 5 mL pipette, with a clean tip, without disturbing the diatom cells settled on the bottom. Cover, leaving cap slightly ajar to allow the remaining water to dehydrate.

9.1.4 Barcode labels should be added to all NEON archive samples. Follow the labeling protocol outlined in “Exhibit A_SOW_Macroalgae Taxonomy and Algae Archiving” pages 4-5.

9.1.5 NEON Sample Return: All samples prepared for archiving will be shipped to the NEON Biorepository at Arizona State University following the guidelines in Appendix 1 in “Exhibit

A_SOW_Macroalgae Taxonomy and Algae Archiving" pages 4-5. Leftover sample may be discarded after archive samples have been received and approved by NEON.

9.2 Diatom Microwave Digestion

9.2.1 Load samples into their respective tubes.

9.2.2 Calculate the necessary amount of nitric acid for the run (10 mL per sample) and carefully pour this volume into a labelled glass beaker in the hood.

9.2.3 Using the 5 mL pipette and a single pipette tip, add 10 mL nitric acid to each nylon tube. Let sit with tubes open for 45 minutes to 'predigest' in fume hood. Cap each of the sample tubes with the two-part screw on caps.

9.2.4 After pre-digestion, set the carousel with location number one at the front. Close the ETHOS UP door and load the 'diatom digestion method.' Click the green start arrow on the home screen to begin the digestion. Enter the number of samples in the carousel (1-32 out of 44).

9.2.5 Ensure that the vent tube is secured inside the fume hood, which should remain on for the duration of the digestion protocol.

9.2.6 Once the protocol is complete, the door will remain locked until the vials are fully cooled to less than 80°C.

9.3 Diatom Rinsing and Sample Transfer

9.3.1 Label a 150 mL glass beaker for each sample digested and place on a black lunch tray inside the fume hood, which should be on for all subsequent steps.

9.3.2 Open the ETHOS UP, remove the carousel, and set in the fume hood.

9.3.3 Decant each sample into the appropriate labelled beaker. Rinse the tube several times with a DI filled rinse bottle, pouring the rinsate into the beaker. Visually check that all sediment has been rinsed out of the tube into the beaker.

9.3.4 Once all samples are decanted, carefully fill the beakers with DI water. Check 'DI Top Off' box on the Worksheet.

9.3.5 Diatom samples must be allowed to settle at least eight hours between each rinse. Set the tray to the side of the laboratory sink and cover samples with aluminum foil, parafilm, or a watch glass to avoid contamination.

9.3.6 After settling, syphon off the supernatant from each beaker. Rinse the syphon tip with tap water between each sample. Do not remove more supernatant than can be removed comfortably without disturbing the settled diatom layer.

9.3.7 Once the supernatant has been removed from all samples, refill the beakers with DI water and note the date, time, and initials of the rinse on the Worksheet. Repeat syphoning and rinsing six times, or until the overlaying water is approximately pH neutral (6-8 using pH paper). If more than 10 mL of nitric acid was used in the digestion, additional rinses may be necessary. If less than 10 mL of nitric acid was used in the digestion, rinsing could be less than six times.

9.3.8 After the final rinse and settling period, prepare 20 mL glass scintillation vials labeled for each sample and syphon off as much supernatant as possible without disturbing diatom layer.

9.3.9 Gently swirl each beaker to resuspend the diatoms in the remaining DI water and quickly pour this slurry into the appropriate vial. Rinse the sides and bottom of the beaker and pour this into the vial. Be conservative with the DI water, as the glass vials cannot be overfilled, total volume should be 20 mL or less.

9.3.10 After all the sample contents are transferred from the 150 mL beaker to the 20 mL vial, bring the final volume of the glass vial up to 20 mL with DI water. The vials hold 20 mL at the shoulder.

X. DATA ANALYSIS AND CALCULATIONS

Not Applicable.

XI. EQUIPMENT MAINTENANCE

Glassware, digester tubes, and Nalgene bottles are cleaned after each use by soaking in Liquinox © 3% solution for a minimum of 4 hours and rinsing with DI water.

XII. REFERENCES

12.1 Acker, F. 2016. Identification and enumeration of algae in phytoplankton samples. Procedure No. P-13-52. 8 pages.

12.2 EPA-841-B-07-010. National Rivers and Streams Assessment: Laboratory Methods Manual, Nov. 2009. http://www.epa.gov/sites/production/files/2013-11/documents/nrsa200809_labmethodsmanual_20091229_2_web.pdf

12.3 ETHOS UP User Manual. (2018). Milestone.

12.4 GLEC SOP ALG 10002A. Permanent Diatom Slide Preparation.

12.5 Report No. 02-06 the Academy of Natural Sciences, Patrick Center for Environmental Research-Phycology Section. "The protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program," May 2012. http://water.usgs.gov/nawqa/protocols/algprotocol/algprotocol.pdf

12.6 Stancheva, R., Busse, L., Kociolek, J.P. & Sheath, R. 2015. Standard operating procedures for laboratory processing, identification, and enumeration of stream algae SWAMP BioAssessment Procedures 2015: 1-100.

STANDARD OPERATING PROCEDURE FOR PERMANENT DIATOM SLIDE PREPARATION, ABBREVIATED

ALG 10002A

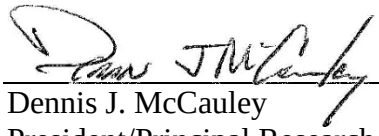
Method Reference: Not Applicable. Method is based on National Rivers and Streams Assessment 2013-2014 Laboratory Operations Manual and "The protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program," Report No. 02-06, the Academy of Natural Sciences, Patrick Center for Environmental Research-Phycology Section

October 11, 2024

Great Lakes Environmental Center, Inc.
(GLEC)

10/11/2024

Maggie Petersen — Date
Technical Approver



10/11/2024

Dennis J. McCauley — Date
President/Principal Research Scientist

10/11/2024

Jennifer Hansen — Date
GLEC Quality Assurance Officer

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I. SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes the mounting of high-quality diatom slides.

1.2 Diatom community composition is used to assess water quality in many local, state, and federal bioassessment programs. Diatom species identification is currently based on the morphology of the diatom cell walls (valves). To accurately identify diatoms to species level, all cytoplasm is removed from the diatom cells and the cleaned diatoms are dried onto glass coverslips and then mounted onto microscope slides using a mounting medium with a high refractive index. These slides are then sent to a taxonomist for identification. They are permanent slides which may be archived for later use. This SOP focuses on the production of the permanent diatom slides. Diatom sample cleaning is addressed in GLEC SOP ALG 10001A.

1.3 This SOP may be modified by mutual consent of Great Lakes Environmental Center (GLEC) and the client in order to achieve the objectives of a given study plan.

II. SUMMARY OF METHOD

2.1 A small amount of cleaned diatom sample is deposited onto a new, clean microscope coverslip using an adjustable micropipette. The coverslips are allowed to dry overnight or until all water is evaporated. Dried coverslips are checked for a desirable diatom density and distribution. If the density and distribution are appropriate, the coverslips are mounted onto glass microscope slides using a mounting medium, which is heated on a hot plate to remove the solvent, toluene. Mounting of coverslips must be done in a chemical fume hood, to avoid toluene fumes. For diatom cleaning see SOP ALG 10001A.

2.2 This SOP is based on National Rivers and Streams Assessment 2013-2014 Laboratory Operations Manual and “The protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program,” Report No. 02-06, the Academy of Natural Sciences, Patrick Center for Environmental Research-Phycology Section.

III. DEFINITIONS

3.1 Subsample – Portion of the sample obtained by randomly extracting volume from the composite sample collected by the field crew.

3.2 Frustule – The siliceous cell wall of a diatom, composed of two parts, each called a valve. One valve is slightly larger than the other and fits over the other as a top on a box.

3.3 Valve – One part of the two-part diatom frustule. You can check whether you have one or two valves by focusing up and down on a cell in the microscope.

3.4 CDF –Concentration/Dilution Factor.

Calculations:

Dilution Factor = $\frac{Subsample\ vol. + Vol.of\ water\ added}{Subsample\ vol.}$

Concentration Factor = $\frac{Subsample\ vol. - Vol.of\ water\ removed}{Subsample\ vol.}$

3.5 Drip volume – Volume of cleaned diatom sample dripped onto coverslip before mounting onto a slide.

IV. INTERFERENCES AND CAUTIONS

4.1 All coverslips and microscope slides should be clean and free of oily residue.

4.2 Vibrations in the area where coverslips are left to dry can cause currents in the diatom sample and cause an uneven distribution across the coverslip that make a proper count impossible.

4.3 Solutes in the sample may also cause an uneven distribution across the coverslip that make a proper count impossible. Samples may need to be rinsed again in DI water if problems occur.

V. EQUIPMENT AND SUPPLIES

5.1 Adjustable micropipette - 100µL-1mL

5.2 Beaker, 150 mL

5.3 Ceramic squares, 1 ¾ or 2 inch unglazed

5.4 Ceramic-top hot plate with temperature control

5.5 Chemical fume hood

5.6 Coverslips

5.7 Diamond tipped scribe pen

5.8 Diatom Slide Preparation Worksheet, if applicable for project

5.9 Forceps

5.10 Laboratory tape

5.11 Mounting medium

5.12 Pipette tips for adjustable micropipette

5.13 Razor blade

5.14 Rinse bottle for DI

5.15 Single-edged razor blades

5.16 Slide labels

5.17 Slide boxes

5.18 Solid glass rod to apply mounting medium

5.19 Tissues (e.g., Kimwipe®, or similar)

5.20 22x22 mm -1 Glass coverslips

5.21 25x75x1 mm plain glass microscope slides

5.22 70-90% ethanol

VI. REAGENTS AND STANDARDS

6.1 Reagents –

6.1.1 Acetone

6.1.2 Ethanol – 70-90%

6.1.3 10% Hydrochloric Acid (HCl)

6.1.4 Mounting medium

VII. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Mounted and cleaned diatom slides should be properly labeled and kept in slide boxes marked with project title, number, and year. They may be stored at room temperature indefinitely.

VIII. QUALITY CONTROL

8.1 All finished slides should be checked for density, distribution, proper coverslip adhesion/placement, and cleanliness. Slides that are not optimal for counting should be remade.

8.2 New slides should be made at the request of the taxonomist.

IX. PROCEDURE

9.1 Dripping Coverslips

9.1.1 Use ceramic tiles as a drying base for sample coverslips.. The technician should use as many samples as they are comfortable working with at one time and that the space will allow with ease of manipulation.

9.1.2 Place one or two coverslips on each tile. Assign a different pipette tip to each sample and one to the DI water so that there is no inter-sample contamination.

9.1.3 Select concentrations that will be used for each sample and use label tape to mark each tile with the correct volume.

9.1.3.1 A good slide should have between 15 and 30 valves in one field of view at 400X magnification with a fairly even distribution. There will inevitably be sparse areas around the edges of the coverslip and higher density in the center but otherwise the distribution should be as uniform as possible.

Note: Because too much silt/sand can obscure the diatom cells, with very silty samples that are also low in diatoms a compromise must be found between diatom density and amount of silt.

9.1.3.2 Each coverslip holds up to approximately 1.5 mL of liquid. In order to distribute the diatoms around the whole coverslip, 1.3-1.5 mL total volume (DI volume plus sample volume)

should be used. Based on the default sample volume, calculate the amount of DI water needed to bring the total volume to 1.3-1.5 mL. Add this volume of DI water to the coverslip.

9.1.3.3 The volume of sample required to get a good density and distribution on the slide will depend on the density of the diatom sample and the concentration of sediment.

9.1.3.3.1 There are some samples that may need to be concentrated or diluted before making the coverslips. For example, if 0.05 mL is still too dense, dilute a subsample of the cleaned diatom sample and use the diluted subsample to make the slide. If a sample is obviously very sparse and multiple mL may need to be dripped, concentrating the sample before dripping may be appropriate. All dilutions and concentrations should be recorded in the diatom slide preparation sheet and added to the NEON ingest table. To concentrate a sample, see Section 9.1.3.4; to dilute a sample, see Section 9.1.3.5.

9.1.3.4 To concentrate a sample before dripping:

9.1.3.4.1 Allow the vial to settle for at least 8 hours.

9.1.3.4.2 Use a micropipette to remove the needed amount of water from the vial without removing any of the settled diatoms. The amount will vary depending on desired concentration level. Keep track of volume removed from the vial and label vial with concentration amount.

9.1.3.4.3 Record the subsample volume and the volume change in the diatom processing sheet..

9.1.3.5 To dilute a sample before dripping:

9.1.3.5.1 Shake vial well and quickly remove a subsample of the cleaned diatom material and transfer to another labeled vial.

9.1.3.5.2 Add the required amount of DI water to the subsample.

9.1.3.5.3 Record the subsample volume and the volume change in the diatom processing sheet. The "subsample volume" is the volume of the original cleaned sample that is transferred to a new vial. When diluting, the "volume change" will be positive.

9.1.4 Shake the sample vial to homogenize the diatom sample before subsampling.

9.1.5 Using the adjustable micropipette, quickly withdraw the pre-determined sample volume.

9.1.6 Next, carefully eject the sample volume into the DI water on the coverslip, slowly moving the tip around the coverslip to distribute the cells.

9.1.7 If done carefully, this will create a liquid dome because of the adhesion of water molecules. Use the pipette tip to carefully pull the sample/water mixture to the edges of the coverslip. Note that any slight overfill or too quick an addition of liquid will result in spillage.

9.1.7.1 If any of the liquid spills over the coverslip edge, the density calculations of the sample will be incorrect and the coverslip must be discarded and redone following steps in Sections 11.1.3.2 to 11.1.7.

9.1.8 Distribute diatoms around coverslip by shallowly pressing and releasing the pipette plunger while moving the tip gently around the coverslip. This creates tiny water currents that will help to spread out the cells.

9.1.9 Let coverslips dry overnight, or longer if necessary. To prevent dust from falling on drying coverslips, carefully suspend a large piece of tin foil over all tiles. Be very careful not to touch any sample.

9.1.10 Use microscope at 400X to carefully check the valve density and distribution of dry coverslips (diatom side up).

9.1.10.1 If distribution is bad, remake the coverslip.

9.1.10.2 If density is too low, add more sample following steps 11.4-11.10 to coverslip and let dry overnight, or make new coverslip with greater sample volume.

9.1.10.3 If density is too high, remake coverslip with lower volume.

9.1.11 Repeat until coverslips are good enough for mounting.

9.2 Mounting Coverslips

9.2.1 For coverslips that have a good distribution and density, record sample ID, the drip volume, and concentration/dilution information (if applicable).

9.2.2 Turn on fume hood fan and light, opening sash to marked level on the frame.

9.2.3 Turn on hotplate to 225-300°C.

9.2.4 When hotplate is ready, take first slide, move coverslip out of the way and add a small amount of mounting medium around the center of the slide.

9.2.5 Invert coverslip and place it on the mounting medium. This means that the coverslip will be diatom side DOWN in the mounting medium.

9.2.6 Place slide on hotplate and allow the mounting medium to boil until the bubbling slows down significantly. This process boils off the toluene solvent to harden the mounting medium.

Note: If heated for the right amount of time, the mounting medium should be hard by the time the slide cools. If not heated long enough, the medium will be too soft; if this happens, the slide can be put back on the hot plate to remove more solvent. If heated too long, it will yellow. This may take some practice.

9.2.7 Using forceps, remove the hot slide from the hotplate and place it back on its tile. Immediately, before the mounting medium hardens, using "open" forceps and even, light pressure, level, straighten, and place the coverslip just left of center on the slide.

9.2.8 Place slide and tile back in line and move to next slide.

9.2.9 After slides have cooled, use a razor blade to remove excess mounting medium. Clean slide with a bit of ethanol or acetone on a Kim Wipe. Assess the slide after mounting and if acceptable, place a printed label on the slide.

9.2.9.1 In addition to the standard slide label, NEON Diatom Slides should be scribed on the underside of the slide with the NEON sample ID and slide number as well as a unique barcode label. The suggested format is SITE.DATE.sample type.sample number.taxonomy.slide# (e.g. "ARIK.20160610.epithion. 1.taxonomy.slide1". A discussion of NEON labeling conventions can be found in "Exhibit A_SOW_Macroalgae Taxonomy and Algae Archiving".

9.2.10 Place slides in a slide box for future analysis.

X. DATA ANALYSIS AND CALCULATIONS

10.1 Be sure to record the sample volume dripped (drip amount) onto the finished slides.

10.2 Calculate a concentration/dilution factor (CDF) for each sample that was concentrated or diluted before dripping.

XI. EQUIPMENT MAINTENANCE

Not applicable.

XII. REFERENCES

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