Homogenization Procedure for Periphyton Samples

1. SCOPE AND APPLICATION

   1.1 This method details the process of homogenizing periphyton samples to produce a more uniform sample for sample analysis.

   1.2 This method is used for quantitative or qualitative periphyton samples that have not been homogenized prior to receipt in the Biology Laboratory.

   1.3 This method can be used for samples with Laboratory Information Management System (LIMS) test codes beginning with "PRN"; however, if samples were processed prior to shipping to the laboratory or if the samples do not contain clumps of algae, the procedure is not necessary.

2. SUMMARY OF METHOD

   2.1 A portion of the periphyton sample is poured from the original sample container into another container.

   2.2 This aliquot of sample is then blended with a hand blender to break up large chunks of algae. As a part of the blending process, the blender and any glassware used must be rinsed, which further dilutes the sample. All dilution factors must be recorded on the container so that total sample dilution can be taken into account during analysis.

3. APPARATUS AND EQUIPMENT

   3.1 Beaker, 250 mL
   3.2 Graduated cylinder, 100 mL
   3.3 Maxim™ hand blender
   Note: The blender is stored beneath the algal settling area in B205 but may be located in the chlorophyll filtering and prep area since it is also used to process periphyton chlorophyll-*a* samples. After using to homogenize formalin-preserved periphyton, be sure to rinse thoroughly to remove any formalin residue that might affect chlorophyll results.
   3.4 Milk dilution bottle, 160 mL
   3.5 Safety equipment (see section 9.1)

4. REAGENTS AND CHEMICALS

   4.1 Squeeze bottle of deionized (DI) water

5. SAMPLE COLLECTION AND PRESERVATION

   5.1 Samples are collected, preserved, and delivered to the lab according to FS 7000 – *General Biological Community Sampling*.

6. SAMPLE PREPARATION PROCEDURE

The sample is initially prepped per Standard Operating Procedure (SOP) AB-10.1 or is received prepped from the collecting organization. This SOP is used if further homogenization of the sample is required for analysis

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due to clumps of algae (algal mats, filamentous algae, chunky algae from periphytometers) that do not break up by vigorously shaking the sample.

6.1 Sample Check Out

6.1.1 Do not check out the sample using the LIMS Custody utility if the sample has already been transferred to another container during sample preparation (SOP AB-10.1). The LIMS Custody utility is only used to track the original sample container. Proceed to step 6.2.

If the sample is in the original container in which it was received, check out the samples using the LIMS Custody utility following step 6.1.1.1. Log out of LIMS and log off the computer. Proceed to step 6.2.

6.1.1.1 In LIMS, select Laboratory/Receiving/Custody, choose CheckOut [Ctrl+O]. Using the hand-held barcode reader, scan the barcodes on sample labels on sample bottles. Click [OK]. Scan the barcode on the back of the employee ID badge two times. Close window using Custody\Exit [Alt+F4].

Note: Using a computer that has a barcode reader attached makes this process quicker, but if the barcode reader is unavailable or not functioning, manually enter the LIMS number of the sample.

6.2 Homogenization Procedure

6.2.1 The sample should be contained in a 160-mL milk dilution bottle and contain 5% formalin preservative. See SOP AB-10.1

6.2.2 Mix the contents of the sample by shaking the bottle 100 times over a sink. Immediately pour 20 mL of the well-mixed sample into the 100 mL graduated cylinder.

Note: The amount of sample to pour into the 100 mL graduated cylinder will depend on the density of the sample and the total volume of sample available. Make sure that you leave approximately 10-50 mL for diatom clearing (SOP AB-02.1).

Note: Since the samples contain formalin and the milk dilution bottles sometimes leak, this procedure should be performed under a fume hood. Gloves should be worn. Line the area under the hood with paper towels to catch any sample that might leak out.

6.2.3 Put the original milk dilution bottle with the remaining sample aside to be used for the diatom clearing process. It should already be properly labeled from the initial slide scraping process (SOP AB-10.1).

6.2.4 Pour the aliquot of sample from the graduated cylinder into the 250 mL beaker. Rinse the cylinder with an equal amount of DI water and add that to the beaker.

Note: If necessary, you may rinse the cylinder more than once. Be sure that each rinse amount is equal to the original sample aliquot amount so that it will be easier to keep up with the dilution factor. For instance, if your original sample aliquot was 20 mL, you should rinse the cylinder with 20 mL of DI at a time.

6.2.5 Using the Maxim^{TM} hand blender, blend the sample until you no longer see any large clumps of periphyton material and sample appears homogenous.

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Note: Blending should be done at low speed to keep from splashing sample. High speed makes it hard to control the mixer. Also, be aware that a vacuum will be created that will suck the mixer down to the bottom of the beaker if you do not hold the blender carefully. The blender blades should not hit the bottom of the beaker.

6.2.6 Pour the blended sample mixture into another 160 mL milk dilution bottle and affix the appropriate Algal sample bottle label. Use another 20 mL of DI to rinse any periphyton material off the blender blades into the 250 mL beaker. Use a final volume of 20 mL of DI to rinse this material and any periphyton material left in the beaker into the milk dilution bottle.

Note: If necessary, you may rinse the cylinder more than once. Be sure that each rinse amount is equal to the original sample aliquot amount so that it will be easier to keep up with the dilution factor. For instance, if your original sample aliquot was 20 mL, you should rinse the cylinder with 20 mL of DI at a time.

6.2.7 Record the sample volume and number of slides scraped in the appropriate spaces on the Algal sample bottle label. Calculate the dilution factor of the blended sample by dividing the total number of milliliters in the diluted subsample by the number of milliliters of concentrated material removed from the original sample.

Note: For instance, if you started with 20 mL of sample and added 80 mL of DI for all rinsing and blending steps, your final dilution factor will be 5 (100 mL total ÷ 20 mL original sample).

6.2.8 This diluted and homogenized sample will be saved for the wet periphyton identification and may be further diluted to achieve the appropriate counting density.

6.3 Sample Check In

6.3.1 If the sample was checked out of LIMS in step 6.1.1 (in the original container in which it was received), check in the sample using LIMS Laboratory/Receiving/Custody/CheckIn [Ctrl+I]. Log out of LIMS and log off the computer.

7. SAMPLE ANALYSIS PROCEDURE

7.1 Samples are analyzed per SOP AB-03.

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Use a pen with black ink for all logbook and benchsheet entries.

8.2 DEP Laboratory Information Management System (LIMS)

8.3 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error and initial and date the correctio. If the correction requires an explanation, write a comment and reference the error.

8.4 If a problem occurs during sample processing, submit a nonconformance report (NCR) describing the problem and the corrective action. To create a NCR, open LIMS and go to Laboratory/Administrative/Non-Conformance Report. For a decision tree regarding issuance of NCR's, refer to SOP LB-002.

8.5 Refer to the Laboratory Quality Manual

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9. SAFETY

9.1 Lab coat, latex or vinyl gloves, and eye protection are required when working with samples containing formalin.

9.2 Avoid breathing formalin fumes or exposing co-workers to them. Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of this chemical. Work in a well-ventilated area.

9.3 Refer to the Material Safety Data Sheet (MSDS) for information about reagents and chemicals in this SOP.

9.4 Refer to the DEP Laboratory Health and Safety Plan.

9.5 Refer to the DEP Laboratory Quality Manual.

10. POLLUTION PREVENTION AND WASTE MANAGEMENT

10.1 Handling of waste formalin is performed per SOP IZ-10 and SOP IZ-38.

10.1.1 Formalin disposal containers are located under the sinks in the main laboratory (B205).

10.2 Refer to the DEP Laboratory Health and Safety Plan

11. REFERENCES

11.1 DEP Laboratory Quality Manual, http://www.dep.state.fl.us/labs/library/lab_sops.htm

11.2 DEP Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

11.3 DEP SOP FS-7000, *General Biological Community Sampling*

11.4 SOP AB-10.1, *Periphyton Sample Receipt & Sample Handling for Taxonomic Analysis*

11.5 SOP AB-02.1, *Diatom Preparation for Periphyton and Phytoplankton*

11.6 SOP AB-03, *Periphyton Non-Diatom Taxonomic Analysis*

11.7 SOP IZ-10, *Preparation and Proper Disposal of Buffered Formalin*

11.8 SOP IZ-38, *Formalin Assay, Recycling and Buffering*

Appendix of Significant Changes

08/24/2010 Reformatted document. No significant method changes.

07/01/2015 Formatted for 508 compliance. Updated links. Reformated References.

08/10/2017 Standardized SOP name format.

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