Periphyton Sample Receipt & Sample Handling for Taxonomic Analysis

1. SCOPE AND APPLICATION

1.1 This method describes the process of handling and tracking periphyton samples once they have been received in the Biology Laboratory. This includes checking preservation and preparing an archive of the sample.

1.2 This method applies to all periphyton samples with Laboratory Information Management System (LIMS) codes beginning with DTM and PRN. Portions of this method apply to samples with LIMS code BLOOM-ID or ALGAL-ID.

2. SUMMARY OF METHOD

2.1 Sample preservation is checked, and if samples are not preserved, they are either preserved as is or prepped (slides scraped) and preserved, whichever is appropriate for the method. Preservative is not added to BLOOM-ID or ALGAL-ID samples until the sample has been viewed by an analyst since these samples are designed for quick turn around and only the dominant species is identified.

2.2 Submittal sheets and related paperwork for all samples in a LIMS job are printed and placed into a job folder. Sample information is checked against the submittal paperwork and sample containers for accuracy. Any errors are corrected.

2.3 Sample information is entered into a workload tracking spreadsheet. Labels and bench sheets are printed and placed into the corresponding job folder. Sample and preservation information is entered into a sample logbook.

2.4 An archive and working samples are created from the original sample and recorded in an archive logbook. For BLOOM-ID or ALGAL-ID, an archive can be prepared and preserved, but the original sample is not handled as for DTM/PRN.

3. APPARATUS AND EQUIPMENT

3.1 Samples with Laboratory Information Management System (LIMS) labels
3.2 Empty purple job folders, stored in B227A above diatom microscopes
3.3 Computer with Sato small label printer
3.4 Computer with Sato large label printer
3.5 Beaker, 250 mL
3.6 Graduated cylinder, 100 mL
3.7 Several single edge razor blades
3.8 Milk dilution bottle, 160 mL
3.9 Dilu-Vial®, 20-mL

4. REAGENTS AND CHEMICALS

4.1 Formaldehyde test kit
4.2 Deionized (DI) water with squeeze bottle

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4.3 100% buffered formalin, prepared per Standard Operating Procedure (SOP) IZ-10

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. METHOD

6.1 Sample Workload

6.1.1 Receiving Room staff log samples into the Laboratory Information Management System (LIMS) and affix a LIMS sticker to each sample. Biology staff should check the backlog in LIMS periodically throughout the day for new samples. Often the algal samples need to be preserved and this should be done promptly after log-in.

Note: If samples are picked up from the Receiving Room after log-in, samples must be checked out using an employee ID card; this transfers sample custody to Biology. If someone does not pick up the samples from Receiving, the samples are assigned to Biology's custody and placed in the Biology B227 refrigerator.

6.1.2 Open the Laboratory/Receiving/Custody utility in LIMS. When the "DEP Central Laboratory Custody" window opens, choose Custody/CheckOut [Ctrl+O] or the [CheckOut] button. Using the hand-held barcode reader; scan barcodes on sample bottles. Click [OK]. Scan ID card and rescan ID card. Close window when finished by choosing Custody/Exit [Alt+F4].

Note: If the scanner is unavailable or not functioning, enter the LIMS number of the sample using the computer keyboard and press [Enter].

6.1.3 Create a purple folder for all samples within a LIMS Job. Job labels will be attached to the top of the sample bottles in Receiving. Place the label on the outer, upper left corner of an empty purple folder.

6.1.4 Check all samples for formalin preservation using the formaldehyde test kit (stored in the B227 refrigerator). If the sample is preserved, proceed to step 6.2. If the sample is not preserved, check the collection date and time against the current date and time and do one of the following:

Note: Do not preserve BLOOM-ID or ALGAL-ID samples until they have been viewed by an analyst to determine the dominant species.

- If the time since collection is less than or equal to 24 hours and the sample was held at 4°C or below, preserve the sample by adding 3 mL of buffered formalin. No other action is needed.
- If the time since collection is greater 24 hours and the sample was held at 4°C or below, contact the customer to see if they still want the sample analyzed.
  - If they do, preserve the sample and create an Non-conformance Report (NCR) in LIMS. Results should be reported with a "Y" qualifier.
  - If they do not, dispose of the sample. Create a NCR in LIMS and enter the result in LIMS as "No Result" with an "O" qualifier.

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- If the sample is not preserved and has not been kept at 4°C or below, advise the customer that the sample will not be analyzed. After obtaining a response, dispose of the sample. Create a NCR in LIMS and enter the result in LIMS as "No Result" with an "O" qualifier.

6.1.5 After preservation has been checked and/or completed, processing can be interrupted at any point during steps 6.1.6 through 6.1.13 if time is limited. Custody of the sample however, must be tracked in LIMS, with samples being checked "in" if pausing the process and back "out" when the process continues. Pausing the process allows time for Receiving to scan all submittal forms and make any log-in corrections.

6.1.6 In the Receiving folder, \\fldep1\DEAR\Labs\Lab3\Receiving, use the Event ID on the job label to find the sample submittal forms. Print the submittal forms and any related paperwork scanned at log-in that contain information pertinent to the analysis (e.g. emails, notes, etc). Place the printed pages in the purple job folder.

Note: It is not necessary to print other documentation such as copies of shipping labels, backs of submittal forms, copies of LIMS requests, etc. as these are available on the computer and usually wind up being duplicated in the final data folder because they were also printed by other analytical groups.

6.1.7 Compare the sample container labels with the field sheets in the job folder to make sure all samples were received and logged into LIMS properly. Check the date collected and field ID. Report any discrepancies to Receiving **immediately**. Errors should be corrected before proceeding to step 6.1.8.

Note: Occasionally, a LIMS # is created (can appear on the Job label) but no sample exists. This is due to the way samples were originally scheduled in LIMS. Receiving will cancel the "missing" sample. If the error still appears after half or 1 day, contact Receiving and have it corrected. Likewise, a sample may be collected but not recorded on the submittal form. Receiving must log in all samples received.

Note: Check Everglades samples very carefully to make sure the correct test has been assigned. If the sample information contains a number of slides, the sample should be logged in as quantitative.

6.1.8 In the AlgalCommon Tracking for Algal Samples folder, \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\Tracking for Algal Samples, record each sample's information on the Workload_Time_Tracking spreadsheet. The needed sample information can be retrieved and saved electronically using the Laboratory/Technical/Backlog Reports module in LIMS, then copied and pasted directly into the spreadsheet.

6.1.9 Open the Laboratory/Receiving/Print Preparation Labels utility in LIMS. Using the hand-held barcode reader; scan barcodes on sample bottles.

Note: Use a Biology lab computer with the small SATO label printer.

Note: If the scanner is unavailable or not functioning, enter the LIMS number of the sample using the computer keyboard and hit [Enter].

6.1.10 From the "Print Prep Labels" window, print out 3 Algal Logbook Labels, bench sheets, and 1 Algal Vial Label (using the small SATO printer). If the sample is an Everglades scrape

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sample, print 1 Algal Bottle Label (using the large SATO printer). Place all labels in job folder until affixed to their appropriate destination.

Algal Logbook Labels:

To print labels, go to the sample Log-in station, open LIMS and select “Laboratory”, “Receiving”, “Print Preparation Labels” and de-select the two selected label icons. Choose “Options”, “Algal Logbook Label” and scan the barcodes on the sample bottles. Select “File”, “Print”. When the "Print" window appears, choose the SATO label printer (small labels). Increase "Number of copies:" to "3" (exception: Everglades 15 mL centrifuge tubes will only need “2” copies). Press [OK] to print.

Note: One of the labels will be for the logbook, the second for the archive sample, and the third for the archive logbook.

Bench sheets:

While the sample numbers are still listed, choose “Bench Sheets” from the top menu. This will drop down a list of bench sheets to choose from (Everglades periphyton, diatom periphyton, etc). Click on a type of bench sheet to print. Bench sheets will automatically print. Be sure to print both diatom and wet ID bench sheets.

Algal Vial Label:

Choose “Options\Algal Vial Labels”. If Everglades pre-scraped sample in the 15 mL centrifuge tube, skip to Documentation (step 6.5). Scan barcodes on the samples to be prepped and/or analyzed. Open “File”, “Print”. When the "Print" window appears, choose “SATO label printer” from the "Name:" drop down list. Leave "Number of copies:" at "1". Press [OK] to print.

Note: This will be used on the top of the archive sample.

Note: Everglades pre-scraped samples do not need Algal Archive Logbook Labels because they are kept in their original tubes as the archive.

Algal Sample Bottle Label:

Deselect the two selected label picture buttons then choose “Options” “Algal Sample Bottle Labels”, scan the barcodes on the logbook labels of samples to be prepped and/or analyzed. Open “File” “Print”. When the "Print" window appears, choose the SATO label printer (for large labels) . Leave "Number of copies at "1". Press [OK] to print.

Note: Scanning the labels is simpler than bringing the samples with you.

6.1.11 From the "Print Prep Labels" window, choose File\Exit. Log out of LIMS and log off the computer.

Note: It is very important to log off the computer when work is complete. The community computers in the laboratory are set to time out after 15 minutes. If that happens while someone is logged in, only that person or an Administrator from Lab Data Support can unlock the computer. This makes it very difficult for someone else to do his or her work on that computer.

6.1.12 In the Algal Sample Logbook in B227A, affix an Algal Logbook Label in numerical order and mark if the sample was received with formalin preservative or not. Record quantity of preservative added if the sample is received unpreserved. Date and initial the entry. For Everglades scrape samples, the final volume and number of slides scraped should be recorded in the comments section of the logbook.

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Note: Overall, the sample numbers in the logbook will not be in consecutive order because samples are not always received into Biology in consecutive order. For a given job, keep those labels in numerical order. If sample numbers within a job are not consecutive, it may be that a sample was added to that job after the fact. Keep that sample number with the other samples in that job even though it may appear to be out of sequence.

6.1.13 If the sample contains slides, proceed to the Slide Scraping (step 6.2). If the sample is non-Everglades qualitative periphyton (PRN-QL-C), which does not require scraping proceed to step 6.3.

6.2 Slide Scraping

6.2.1 If the sample needs scraping, transfer slides and water packed with the slides from centrifuge tube into a clean 250 mL beaker. Rinse the inside of the tube into the beaker twice with DI water. Scrape algae from slides into the beaker using a razor blade, holding the slides by the edge. Rinse remaining algae from slide into beaker with DI water being careful not to lose sample on fingers or razor blade. Try to keep the final sample volume as close to 95 mL as possible. In order to do that, allow for the DI water that will be used to rinse the beaker and graduated cylinder in step 6.2.2.

Note: Scrape all edges of the slides as well as each side. Since the slides have been immersed in the water in the tube and algae will be dislodged by fingers while holding the slides, it has been determined that the entire slide should be scraped. Remove any twigs or other debris before scraping and place into beaker.

Note: If the sample has already been preserved with formalin, scraping should be performed under a fume hood and gloves should be worn.

Note: Rinse the beaker and graduated cylinder with DI between samples to avoid transferring algae to and contaminating the next sample.

Note: It is acceptable if the final sample volume exceeds 95 mL, but it should not be less than 95 mL to make addition of the formalin preservative more consistent and easier to calculate (see Step 6.2.3). If the sample volume is less than 95 mL after the sample has been completely processed, bring it up to 95 mL with DI water.

6.2.2 Pour scraped material into the 100 mL graduated cylinder (If the sample is more than 100 mL, measure the sample in a larger graduated cylinder to avoid having to measure and rinse more than once and subsequently increase the volume by having too many rinse steps). Rinse the beaker with DI water and add the rinse water to the graduated cylinder. Record volume and then pour into milk dilution bottle. Rinse any adhered material from the cylinder into the milk dilution bottle with DI water.

Note: Note the amount of DI water used to rinse from the graduated cylinder to the milk dilution bottle and add that amount to the recorded volume.

Note: If the final sample volume is less than 95 mL, bring it up to 95 mL with DI water.

Note: Although there is only one space on the Algal Sample Bottle Label for sample volume, the interim volumes can also be recorded there so that the information doesn't get separated from the sample as long as the final volume is clearly noted.

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6.2.3 If the sample has not been preserved, add 5mL of buffered formalin, or an amount sufficient to result in a 5% solution. Include this amount in the total volume measurement recorded.

Note: If the sample has been preserved in the field with 5 mL of formalin and the final volume after scraping does not exceed 100 mL, it is not necessary to add more formalin. If the final volume exceeds 100 mL, it will be necessary to add more formalin to result in a 5% solution.

6.2.4 Using permanent marker, record the final volume and number of slides scraped in the appropriate spaces on the Algal Sample Bottle Label.

Note: Mark through any interim volumes recorded on the bottle and circle the final volume.

6.2.5 Open Laboratory\Receiving\Custody. When the "DEP Central Laboratory Custody" window opens, choose Custody\Check In [Ctrl+I] or the [Check In] button in the "Custody" window. Scan barcodes of sample labels. (Follow prompts) Scan ID card and rescan ID card, Choose File\Exit. Log out of LIMS and log off the computer.

Note: If the scanner is unavailable or not functioning, enter the LIMS number of the sample using the computer keyboard and hit [Enter].

6.2.6 Rinse the original centrifuge tube. Place the tubes in the appropriate area on the shelf outside B227A. Receiving will perform bottle discard.

6.3 Archiving and Storage

6.3.1 Create an Archive sample: Swirl the sample to resuspend and mix the algae. Place a portion (usually 20 mL) of the sample into a 20 mL Dilu-Vial®. Affix the appropriate Algal Logbook Label to the side of a 20 mL Dilu-Vial®. Cut the barcode off the Algal Vial Label and discard; affix the numbered part on the lid of the vial. Place this sample archive into the appropriate drawer located in the sample settling area.

Note: The drawers are labeled for the appropriate archive storage boxes.

Note: Everglades pre-scraped samples that come in the 15 mL conical bottom centrifuge tubes will not need to be archived because the remainder of the sample left after analysis will be archived in the tube in which it was received.

6.3.2 Affix an Algal Logbook Label in the Algal Archive Logbook (in the settling area in B205). Initial and date in the appropriate spot. Record the box and tray number in which the archive was placed.

6.3.3 Using the Laboratory\Technical\Prep Worksheet Manager utility in LIMS, create a new prep worksheet for each sample or batch of samples.

6.3.4 Using the Laboratory\Technical\Prep Worksheet Manager utility, complete the prep worksheet for each sample or batch of samples. To complete the prep worksheet: open prep worksheet logbook, highlight the appropriate worksheet ID, open the logbook for that worksheet, highlight the samples in the list, set the correct prep person and prep start and completion dates/times, and then click Complete. Do this for all samples you have prepared. Log out of LIMS and log off the computer.

6.3.5 For periphyton samples other than Everglades scrape samples, custody is maintained until the sample processing is complete. At that point, samples will be checked in using the Custody utility in LIMS.

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6.3.6 Put folder and samples in the appropriate places in the Algal lab.

7. SAMPLE ANALYSIS PROCEDURES

7.1 Samples are analyzed per SOP AB-03 and AB-03.1.

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 Algal Sample Logbook, form AB-LOG-(version #), \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\Log Books

8.4 Algal Archive Logbook, form AB-ARCHIVE-(version #), \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\Log Books

8.5 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 correction. If the correction requires an explanation, write a comment and reference the error.

8.6 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.

8.7 Refer to the DEP Laboratory Quality Manual.

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.

10.3 Refer to the DEP Laboratory Health and Safety Plan.

10.4 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.

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11. REFERENCES

11.1 DEP Laboratory Quality Manual, http://www.floridadep.org/labs/library/progplan.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-03, *Periphyton Non-Diatom Taxonomic Analysis*

11.5 SOP AB-03.1, *Periphyton Diatom Species Proportional Counts*

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

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

11.8 LB-002, *Non-conformance Reporting System*

Appendix of Changes

07/01/2010	Reformatted the layout following SOP LB-001. Reformatted and re-organized steps. No significant method changes.
07/12/2011	Entered the new name for the sample tracking logbook. Added direction to step 6.2.4 to initial and date preservation check in the tracking log. Removed step 6.2.5, the folder color-coding system no longer used.
07/02/2014	Revised Prep Worksheet Manager instructions, step 6.4.4. Updated reference to SOP IZ-10.
06/30/2015	Formatted for 508 compliance. Updated links. Created forms AB-LOG-(version #) and AB-ARCHIVE-(version #).
08/10/2017	Standardized SOP name format.

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