Phytoplankton Sample Receipt & Sample Handling for Taxonomic Analysis

1. SCOPE AND APPLICATION

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

1.2 This method applies to all phytoplankton samples with Laboratory Information Management System (LIMS) codes beginning with DTY or PKD. Portions of this method apply to samples with LIMS codes BLOOM-ID or ALGAL-ID.

2. SUMMARY OF METHOD

2.1 Sample preservation is checked and if samples are not preserved, preservative is added. 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 in the sample 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. Samples information is entered into the sample logbook.

2.4 The sample is concentrated (settled and vacuumed) in preparation for analysis. An archive and working samples are created from the concentrated 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 PKD/DTY.

3. APPARATUS AND EQUIPMENT

3.1 Samples with Laboratory Information Management System (LIMS) labels
3.2 Empty purple job folder, stored in B227A above diatom microscopes
3.3 Computer with Sato large label printer
3.4 Computer with Sato small label printer
3.5 Beakers, 1-L (or other size as appropriate)
3.6 Graduated cylinders, 1-L (or other size as appropriate)
3.7 Aluminum foil to cover beaker
3.8 Volumetric pipette, 10-mL, connected to vacuum
3.9 Centrifuge tubes, plastic, 50-mL, 2 each
3.10 Permanent marker
3.11 Dilu-Vials®, 20-mL

4. REAGENTS AND CHEMICALS

4.1 Lugol's solution, prepared per Standard Operating Procedures (SOP) AB-07
4.2 100% Buffered Formalin, prepared per SOP IZ-10

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4.3 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 or SOP AB-16.

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].

Note: For Everglades samples without labels on samples, scan barcodes of sample labels in folder. Then apply corresponding labels to the appropriate samples according to the paperwork in the folder.

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 samples for Lugol's preservation by observing the color and odor of the sample. If the sample is brown and has an iodine odor, it has been preserved in the field. However, since some samples are tannic water, color may not always indicate if the sample is preserved or not. If in doubt, drop a small amount of sample onto a paper towel. Lugol's in the sample will turn the paper towel dark blue. If the sample has not been preserved, add 3 mL of Lugol's to the sample.

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 Lugol's. No other action is needed.
- If the time since collection is greater than 24 hours and the sample was held at 4°C or below, contact the customer to see if they still want the sample analyzed.

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- 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.

- 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 6.1.12 if time is limited or workload heavy. 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 inside 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: Equipment Blank and Field Blank samples for phytoplankton identification are not analyzed. Using the Laboratory/Technical/Prep Worksheet Manager utility in LIMS, create and complete a prep worksheet for the sample using the date received. Make sure the time day for prep is after the time it was received. This changes the status from "W" to "V". Using the Laboratory/Technical/Results Editor utility in LIMS, enter a "no result" with O qualifier, per SOP BG-03.

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.

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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].

Note: For Everglades samples without labels on samples, scan barcodes of sample labels in folder. Then apply corresponding labels to the appropriate samples according to the paperwork in the folder.

6.1.10 From the "Print Prep Labels" window, print out 3 Algal Logbook Labels, bench sheets, 1 Algal Vial Label (using the small SATO printer) and 2 Algal Bottle Labels (using the large SATO printer). Place all labels in job folder until affixed to their appropriate destination.

Note: Equipment Blank and Field Blank samples for phytoplankton identification will not be analyzed. Print only one logbook label and one bench sheet. The label will be placed in the logbook and the bench sheet in the folder, both with a note, in order to keep track of what happened to the samples in case of questions later.

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". Press [OK] to print.

Note: Of the 3 labels, one label is 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 phytoplankton, diatom phytoplankton, 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.

Note: The bench sheets of samples that will be reported as “no result” need to be placed in the folder and the reason for “no result” needs to be written on the bench sheet.

Algal Vial Label:

Choose “Options\Algal Vial Label”. Scan barcodes on the samples to be prepped and/or analyzed. Open “File”, “Print”. When the "Print" window appears, choose the SATO label printer (small labels). Leave "Number of copies:" at "1". Press [OK] to print.

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

Algal Sample Bottle Labels:

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 "2". Press [OK] to print.

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

Note: One label will be used for the beaker during settling. The other is placed in the folder until after the settling process is complete.

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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 Find the appropriate Algal Sample Tracking Log in B227A. Affix an Algal Logbook Label in the logbook in numerical order and mark if the sample was received with Lugol's preservative or not. If the sample was received unpreserved, record that preservative was added by the lab and record the quantity if it differs from 3 mL. Date and initial the entry.

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 a particular sample number within a job is 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.

6.2 Sample Preparation: Settling

6.2.1 Rinse a 1-L graduated cylinder well with DI water.

6.2.2 To ensure that the sample is mixed, pour half the sample into the 1-L graduated cylinder and recap sample bottle. Mix the sample thoroughly by inverting the capped sample bottle several times and pour the rest of the sample into the cylinder, up to 1 L. Record the original sample volume on both of the Algal Sample Bottle Labels that are located in the folder.

Note: All samples to be analyzed should be settled except for BLOOM-ID or ALGAL-ID samples. It is easier and less time consuming to over-concentrate and dilute prior to analysis than to count algae at the density in the original sample.

Note: The original volume should be no more than 1 L; it is okay for it to be under but not over 1 L.

6.2.3 Transfer sample to a 1-L beaker (or other size as appropriate). Rinse the graduated cylinder well with DI water after each sample before measuring the next sample to be settled.

Note: Sample volume should be measured in a graduated cylinder first and then transferred to a beaker for settling because the beakers are not volumetrically correct. Beakers, however, work better for settling because they are more stable and easier to work with when there are many samples to settle at one time. Graduated cylinders are more easily knocked over and broken.

6.2.4 Cover the beaker with foil. Affix one label to the beaker and put the other label in the job folder. Without putting a hole in the foil, carefully write the date that the sample was poured into the beaker for settling, analyst initials and sample number on the aluminum foil to keep track of settling time. Carefully move the beakers to the settling area. Let the beaker settle for 5 full days (120 hrs).

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Note: 5 days of settling is sufficient to meet the recommended settling time of 1 hr per millimeter of column depth according to Standard Methods.

(Settle Start to Settle End =120 hrs)

6.2.5 Place the job folder in the appropriate location in the settling area.

6.2.6 Rinse the brown bottles to remove any remaining sample and Lugol's. Place the bottles in the appropriate area on the shelf outside B227A. Receiving will perform bottle discard.

6.2.7 Open the Laboratory/Receiving/Custody utility in LIMS. 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 press [Enter].

Note: The LIMS Custody utility tracks the handling of the original sample bottle. Once the sample bottle has been emptied, the Custody utility is no longer used to follow the sample aliquot through the lab.

6.2.8 Using the Laboratory/Technical/Prep Worksheet Manager utility in LIMS, create a new prep worksheet for each sample or batch of samples processed per this SOP, AB-11. Log out of LIMS and log off the computer.

Note: BLOOM-ID or ALGAL-ID samples do not require prep and do not have to be assigned in Prep Worksheet Manager.

6.3 Post-Settling Sample Preparation

6.3.1 After settling is complete (on the sixth day), use the pipette attached to the vacuum (see SOP TA-08.04) and siphon water from the top of the beaker until there is a volume that is between 100-mL and 150-mL left in the beaker. During the vacuuming process do not disturb the algae, which have settled to the very bottom of the beaker. If algae on the bottom of the beaker are disturbed, stop vacuuming and keep a larger concentrated volume.

Note: Try to stay within the 100 to 150- mL range if possible. Only leave the amount at a higher volume if the sample is too dense and you are disturbing the settled sample on the bottom of the beaker. This concentration regime will result in a workable counting density in most cases. If algal populations are expected to be extremely sparse, the sample can be further settled after the formalin preservative has been added in Step 6.3.3.

Note: If algae are still observed floating in the beaker after the sixth day of settling, do not vacuum. Consult taxonomy supervisor.

6.3.2 Discard the portion of the sample that was siphoned off in the sink and flush with plenty of tap water.

Note: All acid/Lugol's waste can be disposed of in the sink with ample amounts of water. Due to upgrades to the Lab's plumbing, it is no longer necessary to neutralize the acid with 2 heaping teaspoons of sodium bicarbonate for every four samples that have been siphoned.

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6.3.3 After vacuuming is complete, determine the volume of formalin to be added (see below). Rinse a disposable pipette. Using the disposable pipette, remove that volume of sample from the beaker and discard the liquid in a waste container. Wearing the appropriate safety equipment and working under a fume hood, add the appropriate volume of formalin:

- 3 mL formalin to a sample that is siphoned down to around the 100 mL mark on the beaker.
- 4 mL formalin to a sample that is siphoned down to between the 100 and 150 mL marks on the beaker.
- 5 mL formalin to a sample that is siphoned down to between the 150 and 200 mL marks on the beaker.

Note: Be careful not to disturb the algae because it is settled in the bottom portion of the beaker.

Note: Formalin is added to aid in long-term storage because Lugol's will allow bacteria and fungus to grow after about thirty days of storage, especially if the sample is not stored in the dark.

Note: Although the actual volume is unknown until it can be measured in a graduated cylinder, it can be estimated using the marks on the beaker. Do not measure the volume first as that will disturb the algae and prevent removal of any liquid without resettling the sample.

Note: Rinse the pipette well between samples to avoid cross-contamination.

Note: The amount of formalin added, as described above, should result in a minimum of about 2.5% of the sample.

6.3.4 Rinse a 250-mL graduated cylinder (or other size as appropriate). Swirl the remaining sample in the beaker to mix, and pour into the cylinder. Record the actual volume of concentrated sample plus formalin on the Algal Sample Bottle Label. Pour the sample back into the labeled beaker, making sure to keep the sample well mixed and not allowing time for settling in the graduated cylinder.

Note: Rinse the graduated cylinder well with DI water after each sample before measuring the next sample's concentrated volume.

6.3.5 Create an Archive sample: Swirl the remaining sample in the beaker 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.

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

6.3.7 Create the working samples: Swirl the remaining sample in the beaker to resuspend and mix the algae. Pour equal portions of the remaining, well-mixed sample into two 50-mL centrifuge tubes. Transfer the Algal Sample Bottle Label from the beaker to the centrifuge tube that will be used for wet algal identification. This will be the working sample from which an aliquot will

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be settled for wet algal identification. Use the other label printed in step 6.1.7 and located in the folder to label the second 50-mL tube, which will be used for diatom processing and identification.

Note: Any excess sample (after all archives and working aliquots have been stored) needs to be discarded into the formalin waste container.

Note: Put only 50 mL or less in the centrifuge tubes. If overfilled they can leak.

Note: If the sample is not to be prepped and analyzed for diatoms, this second tube does not have to be prepared.

6.3.8 Complete the prep worksheet for each sample using the Laboratory/Technical/Prep Worksheet Manager utility in LIMS. 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 prepared. Log out of LIMS and log off the computer.

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

7. SAMPLE ANALYSIS PROCEDURES

7.1 Samples are analyzed per SOP AB-05 and AB-05.1.

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Use a pen with black ink for all logbook and bench sheet 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, 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.

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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.

10.3.1 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.floridadep.org/labs/library/progplan.htm

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

11.2. Standard Methods SM10200 (2011)

11.3 SOP FS 7000, *General Biological Community Sampling*

11.4 SOP AB-05, *Phytoplankton Non-diatom Taxonomic Analysis*

11.5 SOP AB-05.1, *Phytoplankton Diatom Identification and Species Proportional Counts*

11.6 SOP TA-08.04, *Filtering Algal Growth Potential and Limiting Nutrient Samples*

11.7 SOP AB-07, *Preparation of Lugol's Solution*

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

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

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

Appendix of Changes

12/17/2009	Added BLOOM-ID sample handling. Changed number of labels to be printed since barcodes are now printed in the benchsheet header. Changed Custody to track original sample container, not subsequent aliquots created after sample is poured out of original container for settling.
07/01/2010	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 tracking log. Removed step 6.2.5, the folder color-coding system no longer used.
07/10/2012	Added ALGAL-ID test code.

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07/02/2014 Revised Prep Worksheet Manager instructions, step 6.3.9. Updated reference to SOP IZ-10.

07/01/2015 Formatted for 508 compliance. Updated links. Created forms AB-LOG-(version #) and AB-ARCHIVE-(version #).

09/21/2017 Standardized SOP naming formats. Changed recording Lugol's quantities for unpreserved samples and added note about floating algae after settling.

10/01/2018 Clarified Section 6.2.4. (Sample Prep: Settling); added Standard Methods 10200 to the References section.

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