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 the preservation, concentrating the sample, 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. If DTY/PKD samples are not preserved, then preservatives are added. Chemical preservatives are not added to BLOOM-ID or ALGAL-ID samples until an analyst has viewed the sample. These samples are designed for quick turnaround, with only the dominant species 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 the LIMS Prep Worksheet Manager.

2.4 The DTY/PKD sample is concentrated (settled and vacuumed) in preparation for analysis. Working samples and archive samples are created from the concentrated sample and recorded in the LIMS Prep Worksheet Manager and an archive logbook. Archive samples are not prepared for BLOOM-ID or ALGAL-ID samples.

3. APPARATUS AND EQUIPMENT

3.1 Samples labeled with Laboratory Information Management System (LIMS)
3.2 Empty purple job folder, stored in B227A
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 DEP SOP 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 periodically check the backlog in LIMS throughout the day for new samples. Often, the algal samples need to be preserved; this should be done promptly after log-in.

6.1.1.1 If samples are picked up from the Receiving Room after log-in, samples must be checked out using an employee ID card; this procedure 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 this window when finished by choosing Custody/Exit [Alt+F4].

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

6.1.2.2 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, then it has been preserved in the field. However, since some samples are tannic water, color may not always indicate prior sample preservation 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, check the collection date and time against the current date and time and do one of the following:

Note: BLOOM-ID and ALGAL-ID samples should not be received chemically preserved. Refer to SOP AB-18 for BLOOM-ID/ALGAL-ID preservation and hold times.

Note: Any Lugol's solution needed will be added during the settling portion of sample prep (6.2.2).

6.1.4.1 If the time since collection is less than or equal to 36 hours and the sample was kept on ice, the sample will be preserved by adding 3 mL of Lugol's during the settling portion of sample prep. No other action is needed.

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6.1.4.2 If the time since collection is greater than 36 hours and the sample was kept on ice, contact the customer to see if they still want the sample analyzed.

6.1.4.2.1 If the sample is analyzed, preserve the sample during the settling portion of sample prep and create a Non-conformance Report (NCR) in LIMS. Results should be reported with a "Y" qualifier and a comment to see the associated NCR.

6.1.4.2.2 If the sample will not be analyzed, properly dispose of it and create a NCR in LIMS. Enter the result in LIMS as "No Result" with an "O" qualifier and a comment to see the associated NCR.

6.1.4.3 If the time since collection is greater than 36 hours and the sample was not preserved with either Lugol's or ice, then notify the customer that the sample will not be analyzed. After obtaining a response, properly dispose of the sample. Create a NCR in LIMS. Enter the result in LIMS as "No Result" with an "O" qualifier and a comment to see the associated NCR.

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.

6.1.5.1 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 (DEAR\Labs\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.

6.1.6.1 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.

6.1.7.1 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.

6.1.7.2 Using the Laboratory/Technical/Prep Worksheet Manager utility in LIMS, create a prep worksheet for the sample using the date received. This changes the status from "W" to "V".

6.1.7.3 Equipment Blank and Field Blank samples for phytoplankton identification are not analyzed. These will be reported as "No Result" with an O-qualifier. Using the

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Laboratory/Technical/Results Editor utility in LIMS, enter a “no result” with O qualifier, per SOP BG-03.

6.1.8 In the Algal Common Tracking for Algal Samples folder (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 Use a Biology Lab computer connected to a small SATO label printer to open the Laboratory/Receiving/Print Preparation Labels utility in LIMS. Using the hand-held barcode reader; scan barcodes on sample bottles.

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

6.1.9.2 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 2 Algal Logbook Labels, 1 Algal Vial Label (using the small label SATO printer),2 Algal Bottle Labels (using the large label SATO printer), and bench sheets. Place all labels in the 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.

6.1.10.1 Algal Logbook Labels:

6.1.10.1.1 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 small label SATO printer.. Increase "Number of copies:" to "2". Press [OK] to print.

6.1.10.1.2 One label will be used for the algal logbook and the other for the archive sample.

6.1.10.2 Bench sheets:

6.1.10.2.1 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 the appropriate type of bench sheet to print. Bench sheets will automatically print. Be sure to print both diatom and wet ID bench sheets.

6.1.10.2.2 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.

6.1.10.3 Algal Sample Bottle Labels:

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6.1.10.3.1 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 large label SATO printer.Leave "Number of copies” at "2". Press [OK] to print.

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

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

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

6.1.11.1 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.

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. If the sample was not preserved in the field, add 3 mL Lugol’s solution to the cylinder. Mix the remaining original sample thoroughly by inverting the capped sample bottle several times, then pour the sample into the cylinder up to 1 L. Record the original sample volume on both Algal Sample Bottle Labels that are in the folder.

6.2.2.1 All samples to be analyzed should be settled. 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.

6.2.2.2 It is preferable to add Lugol’s to the sample in the cylinder than to add it to the original sample container. Sample containers that have had Lugol’s in them are unable to be recycled. Any bottle that contained Lugol’s should be marked with a piece of red tape on the lid and discarded appropriately.

6.2.2.3 The sample’s original volume should measure no more than 1 L; it is acceptable for it to fall under but not over the 1 L limit.

6.2.3 Transfer the 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.

6.2.3.1 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 large label to the beaker and keep the other label in the job folder. Without breaking through the aluminum foil, carefully write the date that the sample was poured into the beaker for settling, analyst initials, and sample number on the foil to keep track of settling time. Carefully move the beakers to the settling area. Let the beaker settle for 5 days.

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Note: For a sample that has been preserved with Lugol's, 60 hrs (2.5 days) of settling is sufficient to meet the recommended settling time of ½ an hour per millimeter of column depth. However, while a minimum of 60 hours is required, this lab's standard practice is to extend the settling time to 5 days. This duration allows for better separation and concentration, as some algae species are more naturally buoyant and tend to remain suspended.

6.2.4.1 Settling time starts when the beaker has been covered and left in the settling area. Settling time ends when the beaker has been moved for vacuuming (Section 6.3).

6.2.4.2 It is acceptable to allow the sample to settle for longer than 60 hours, but not less.

6.2.4.2 For a sample not preserved with Lugol's, *Standard Methods* recommends a settling time of 1 hour per millimeter of column depth. Using the 1-L beaker, that equals 120 hours (5 days) of settling time.

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 any Lugol's. Place the bottles in the appropriate area on the shelf outside B227A until LIMS releases the sample for discard. (Refer to SOP LB-023 for sample 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.

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

6.2.8 Using the Laboratory/Technical/Prep Worksheet Manager utility in LIMS, complete the prep worksheet for each sample or batch of samples processed per this SOP, AB-11.

6.2.8.1 Make sure the prep date and time are set for after the time it was received.

6.2.8.2 Highlight the samples in the list, set the correct prep person, prep start, and completion dates/times. Click Complete. Do this for all samples you have prepared.

6.3 Post-Settling Sample Preparation

6.3.1 After settling is complete, use the pipette attached to the vacuum (see Tox SOP TA-08.01 for vacuum apparatus set-up) 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.

6.3.1.1 If algae are still observed floating in the beaker after at least 60 hours of settling, do not vacuum. Consult taxonomy supervisor.

6.3.1.1 If algae on the bottom of the beaker are disturbed, stop vacuuming and keep a larger concentrated volume.

6.3.1.2 Try to leave a volume 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.

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6.3.1.2 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.

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

6.3.2.1 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.

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 equivalent volume of sample from the beaker (without disturbing the settled algae) 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.

6.3.3.1 Be careful not to disturb the settled algae.

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

6.3.3.3 Although the actual volume is unknown until it can be measured in a graduated cylinder (step 6.3.4), 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.

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

6.3.3.5 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. 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.

6.3.4.1 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 appropriately labeled drawer located in the sample settling area.

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6.3.6 Affix an Algal Logbook Label in the *Sample Archive Logbook* (in the settling area, B205). Initial and date in the appropriate space. 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 be settled for wet algal identification. Use the other large label printed in step 6.1.7 and located in the purple folder to label the second 50-mL tube, which will be used for diatom processing and identification.

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

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

6.3.7.3 If the sample is not to be prepped and analyzed for diatoms, the 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:

6.3.8 Open prep worksheet logbook, highlight the appropriate worksheet ID, open the logbook for that worksheet.

6.3.9 Highlight the samples in the list, set the correct prep person, prep start, and prep completion dates/times. (Make sure the prep date and time are after the time it was received.) Click Complete. Do this for all samples prepared. Log out of LIMS and log off the computer.

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

7. SAMPLE ANALYSIS PROCEDURES

7.1 Samples are analyzed per SOP AB-05, AB-05.1, and AB-18.

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 Archive Logbook, form AB-ARCHIVE-(version #), https://fldeploc.dep.state.fl.us/sop/sop2.asp?sect=BIOLOGY&ssect=ALGAL+BIOLOGY&A1=Submit

8.4 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.

8.5 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

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Laboratory/Administrative/Non-Conformance Report. For a decision tree regarding issuance of NCR's, refer to SOP LB-002.

8.6 Refer to the DEP Laboratory Quality Manual for sample handling and QC.

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 for proper handling of reagents.

9.5 Refer to the DEP Laboratory Quality Manual for sample handling and QC.

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 for proper handling of reagents.

11. REFERENCES

11.1 DEP Laboratory Quality Manual, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

11.2 DEP Laboratory Health and Safety Plan, https://floridadep.sharepoint.com/dear/SitePages/Home.aspx

11.3 *Standard Methods 10200: Plankton* (2017)

11.4 SOP FS 7000, *General Biological Community Sampling*

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

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

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

11.8 SOP AB-18, *Analysis of Algal Bloom and Algal Identification Samples*

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

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

11.11 SOP LB-002, *Non-conformance Reporting System*

11.12 SOP LB-023, *Maintaining the Custody and Storage of Environmental Samples in the DEP Laboratory*

11.13 SOP TA-08.01, *Initial Algal Assay and Limiting Nutrient Sample Handling*

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Appendix of Significant 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.
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.
12/16/2019	Removed references to BLOOM-ID and ALGAL-ID samples in the sample prep section. Corrected sample hold time and preservation information. Section 5.1 removed reference to SOP AB-16—it no longer exists. Lugol's solution added at a later step than in previous versions. Reformatted. Updated links. Updated sample settling times based on *SM 10200C* (2017).
12/06/2021	Updated Links.
12/09/2022	Eliminated above diatom microscopes in 3.2.
11/17/2023	Updated and removed hyperlinks. Edited the number of algal logbook labels from 3 copies to 2 copies, because there is no longer a sample receipt logbook.
12/14/2023	Removed reference to sample logbook in multiple sections.
12/06/2024	Corrected minor grammatical errors, rephrased for clarity. Added our generally practiced settling time in step 6.2.4.1 and removed settling time "formula." Clarified settling start and end times in a separate step. Removed "on the third day" from 6.3.1.
12/06/2025	Removed the 6.2.8.1 duplicate 6.2.8.2. Added "(without disturbing the settled algae)" to 6.3.3 to be more direct. Section 6.1.10.3 was eliminated.

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