Algal Identification Quality Control (QC) Procedures: Initial Demonstration of Proficiency, Ongoing/Individual QC, Group QC, and Annual QC

1. SCOPE AND APPLICATION

1.1 This SOP outlines quality control procedures for diatom and wet algal identifications: Initial Demonstration of Proficiency (IDOP); Ongoing/Individual QC; Ongoing Group QC; and Annual QC. Daily discussions are encouraged to facilitate training of new analysts and ongoing professional development, particularly about samples that have new or different taxa, or ones that prompt questions. This SOP is used for samples with LIMS test codes BLOOM-ID, ALGAL-ID, and those that begin with DTM, DTY, PKD, and PRN.

2. SUMMARY OF METHOD

2.1 For quantitative phytoplankton (PKD/DTY) and periphyton (PRN/DTM) IDOP, new analysts must successfully re-identify 10 samples (passing QC >70% similarity of taxa lists). After a total of 10 passing samples, IDOP is achieved. 10% of samples are then QC'd until a total of 20 samples have passed. IDOP does not necessarily have to be consecutive samples. After a total of 20 passing samples is achieved, ongoing QC is conducted on 5% of samples analyzed, minimum one sample per quarter.

2.2 For the qualitative BLOOM-ID and ALGAL-ID IDOP, new analysts must successfully re-identify 20 consecutive samples with 100% accuracy for the dominant taxon, co-dominant taxa, or the lack of dominance of any taxon. Ongoing QC for BLOOM-ID/ALGAL-ID is performed monthly.

2.3 Analysts participate in group QC exercises throughout the year (every 3-4 months) to ensure that they are making comparable identifications and continue to educate themselves on common and uncommon taxa in Florida waters.

2.4 After two or more full-time analysts are fully trained on quantitative ID, they participate in an annual QC, for which they each analyze 3 samples twice and taxa lists are compared to determine intra-sample and inter-analyst variability (goal is >70% similarity between lists).

2.5 If any QC measure fails to pass, the source of the error(s) is determined and corrected. Further training may be conducted, or samples may be re-analyzed.

3. SOFTWARE, DATABASES, AND TECHNICAL DOCUMENTS

3.1 Primer-E software and documentation. Refer to SOP AB-15 for Primer-E software accessibility and instructions.

3.2 Microsoft Excel software

3.3 Statewide Biological Database (SBIO)

3.4 DEP Laboratory Information Management System (LIMS)

3.5 Blank algal identification bench sheet, printed from LIMS

3.6 Original algal identification bench sheet

3.7 QC Spreadsheets, located in Lab5 → Biology → AlgalCommon → QCSpreadsheets

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3.7.1 Initial Demonstration of Proficiency (IDOP): QC-Wet/Diatom Training folder → analyst-specific folder; create sample-specific Excel files

3.7.2 Phytoplankton and Periphyton Algal Identification QC Log: QC-wet working; analyst-specific tab

3.7.3 Ongoing Individual Quality Control: QC-Wet/Diatom Working folder → appropriate Freshwater QC file

3.7.4 Group Quality Control: Quarterly QC → Group QC file

3.7.5 Annual QC Procedures: Save results in Annual QC folder

4. QUALITY CONTROL METHODS

4.1 **Phytoplankton and Periphyton (Quantitative) IDOP**

4.1.1 The new analyst must read and sign off on all Algal Biology SOPs.

Note: The trainer ensures the new analyst is provided identification keys/pictures and reference materials along with relevant SOPs. The trainer also ensures that the analyst has spent adequate time examining previously identified samples in conjunction with the associated training material and bench sheets before IDOP is initiated. New analyst is responsible for reviewing and learning the material and asking for help with identifications. A continued discussion is necessary between the trainer and the analyst for better understanding of the taxa observed and how to identify it accurately.

4.1.2 The new analyst re-identifies a minimum of 10 samples previously identified by a proficient analyst. Wet identification should be performed using SOP AB-03 for periphyton and SOP AB-05 for phytoplankton. Diatom identification should be performed using SOP AB-03.1 or AB-05.1. Due to the quantity of samples received, new analysts should be trained on phytoplankton before periphyton.

4.1.2.1 Successful passing of IDOP does not require re-identification of 10 samples in succession; however, the new analyst should not fail three re-identifications in a row, and the number of passing samples should be greater than the non-passing samples.

4.1.2.2 The new analyst's supervisor or designated trainer will choose samples for wet or diatom re-identification. Samples for wet identification should contain less than about 40% diatoms.

4.1.2.2.1 Trainer needs to ensure that samples to be re-identified for wet identification or diatom identification should not have very low densities or taxa counts or be "problem" samples, such as very dirty samples. These samples are difficult to achieve 70% agreement on due to factors other than analyst proficiency.

4.1.2.3 If the sample is a phytoplankton or periphyton wet ID, the second analyst will prepare and settle a new aliquot of the sample, using the same dilution as the first analyst. A counting chamber with the same cell depth as that of the first analyst is used.

4.1.2.4 If the sample is a diatom sample, use the permanent slide mount.

4.1.3 For each sample, open the Wet/Diatom-Training QC Template Excel file. Go to File → Save as… and rename it with new analyst, sample, and date information. Fill in the spreadsheet with

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the taxonomic identifications and unit counts, either by typing the names and unit counts or by retrieving data from SBIO (see note).

4.1.3.1 Name the QC file using the following format: New Analyst's Initials_Original Analyst's Initials_Quantitative/Qualitative_ Phytoplankton/Periphyton/Diatom_LIMS#_analysis date.

Example: A file for an initial proficiency re-identification performed by Jane A. Doe on a sample originally identified by John B. Deer on sample #123456, a qualitative phytoplankton sample, on March 17, 2008 would be named JAD_JBD_QL_PKD_123456_031708.xls.

4.1.3.2 Re-type the file name into the first cell of row one (A1). In the "Taxa" column, enter the taxa identified. "Count A" will be the unit counts of the new analyst. "Count B" will be the unit counts of the proficient analyst (trainer). Enter zeros where there is no count. Save the spreadsheet.

Note: To help with data entry, the taxonomic data (with phylogeny) can be retrieved from SBIO. For each sample, save the taxa list as an Excel file using the LIMS# as the file name. This provides most of the taxa and counts that can be used to create an aggregation file (also used in step 4.1.4). Some taxa names, unit counts, and phylogeny may need to be added to the file. Save the phylogeny as a separate file "_Agg" before the .xls extension or as worksheet within the QC file.

4.1.3.3 If a wet identification sample has cell counts, enter those into a separate spreadsheet. These counts will be used to assess if cells are being counted correctly but will not be used for purposes of assessing identification proficiency.

4.1.4 Analyze each QC sample separately using PRIMER-E (per SOP AB-15). Conduct Bray-Curtis similarity analyses on these data, first with species level, non-transformed data.

4.1.4.1 If the Bray-Curtis similarity is >70%, the QC analysis has been completed satisfactorily (passed).

4.1.4.2 If the similarity value is <70%, repeat the analysis with a square root transformation. Square root transformation lessens the effect of differences in counts (large numbers of Alga #1 in sample A vs. B) when there are large differences in the number of units among the taxa present.

4.1.4.2.1 If the similarity is still < 70%, repeat the analysis at genus-level using a taxa aggregation file.

4.1.4.2.2 If the similarity is still < 70%, conduct the genus-level analysis with square root transformed data.

4.1.4.3 Record all similarity values, whether the QC passed at species or genus level, and the file name (.xls or _Agg.xls) on the QC bench sheet (paper), in the Excel taxa/unit file, and in the QC Summary Excel file.

4.1.5 If the QC has passed at the genus level but not at the species level, compare the taxa lists from the two analysts. If differences exist between taxa lists, give additional training to bring the analyst up to the same level of proficiency.

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4.1.6 If the QC does not pass at any level of comparison or transformation, the sample cannot be counted towards the ten successful re-identifications needed to establish proficiency.

4.1.7 Continue with re-identifications and analysis (steps 4.1.2 through 4.1.7) until 10 samples have been successfully re-identified.

4.1.8 After 10 samples have been successfully re-identified, the IDOP is complete. The new analyst should continue to have 10% of samples QC'd by another analyst until 10 additional samples have passed the 70% QC threshold.

4.1.9 After a total of 20 samples have passed QC, the standard 5% of samples analyzed will be QC'd, minimum one per quarter (see section 4.3 Ongoing Quality Control below).

4.1.10 Record the date of IDOP for the assigned Method in the LIMS training module.

4.2 BLOOM-ID and ALGAL-ID (Qualitative) IDOP

4.2.1 The new analyst must read and sign off on all Algal Biology SOPs. If the person(s) training the new employee determines that the new analyst has spent adequate time re-examining samples, reviewing literature and reference materials, and studying microscope photos and slides of common bloom forming and algal mat forming/periphytic species, then the new employee should begin independent identification of samples previously identified by a proficient analyst.

4.2.2 In the QC-wet training folder, use the Bloom-Algal_ID Training QC Template to create an analyst-specific Excel spreadsheet to document all passed and attempted re-identifications.

4.2.2.1 The file should contain the LIMS sample number, the sample location, date analyzed/re-identified, the new analyst's identification, the reported identification, and any relevant comments.

4.2.3 Twenty consecutive samples should be prepared and re-identified for dominance (per SOP AB-18) with 100% accuracy. For each sample, the new analyst must correctly identify the dominant taxon, co-dominant taxa, or the lack of dominance in the water or periphyton sample. If a sample is incorrectly identified or partially identified, the sequence should be restarted until 20 consecutive samples are identified.

4.2.3.1 <u>BLOOM-IDs and ALGAL-IDs are not preserved</u>. New analysts must re-identify samples as they arrive at the lab at the same time the official analysis is taking place. Analysts must take care not to share information about the dominance identification until the testing analyst can record their identification.

4.2.3.2 There are several common bloom-forming algal species in Florida waters that are frequently observed in BLOOM-RESP samples. Because these species are so common, incorrectly identifying any of them could indicate that more training and exposure to different samples is needed. Correctly identifying the dominant taxon/taxa in twenty consecutive samples ensures the analyst is strong and efficient with their identifications.

4.2.4 Other taxa present should also be identified by the new analyst and reviewed with the trainer.

4.2.5 When 20 samples in a row have passed for dominance identifications, then a demonstration of proficiency has been shown for BLOOM-ID/ALGAL-ID analyses. Record the date of IDOP for the assigned method in the LIMS training module.

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4.3 Phytoplankton and Periphyton Ongoing/Individual QC

4.3.1 Each taxonomist records in his/her assigned Phytoplankton and Periphyton Algal Identification QC Log (Wet or Diatom) all the samples he/she has analyzed.

4.3.2 When 20 samples of a particular analysis type have been analyzed, another proficient analyst randomly chooses a sample from the list. For each analyst, at least one sample per quarter should be QC'd. If only one person performs that type of identification, he/she must QC his/her own sample.

4.3.3 The second analyst then identifies the randomly selected samples following steps 4.1.2.3 and 4.3.3.1. Data are entered into a QC-working Excel spreadsheet.

4.3.3.2 Save the individual QC spreadsheets using the naming format of First Analyst's Initials_Second Analyst's Initials_Quantitative/Qualitative_ Phytoplankton/Periphyton/Diatoms_LIMS#_analysis date.

Example: A file for a QC performed by Jane A. Doe and John B. Deer on sample #123456, a qualitative phytoplankton sample, on March 17, 2008 would be named JAD_JBD_QL_PKD_123456_031708.xls.

4.3.3.3 Record the diatom ID QC in QC-diatom working → Freshwater Peri/Phyto Diatom _QC_NEW

4.3.3.4 Record the wet ID QC in QC-wet working → Freshwater Peri/Phyto Wet_QC_NEW

4.3.4 PRIMER-E will be used to conduct QC analysis and record similarity values per steps 4.1.4 through 4.1.8.

4.3.4.1 Identifications determined to be incorrect are corrected on the bench sheet and in SBIO. If the supervisor determines that the sample should be re-identified by both analysts, the re-identifications of any QC sample should be put through the steps outlined in this SOP to determine if the re-identification passed QC standards and if problem areas have been corrected. If a sample is re-analyzed and the second set of analyses passes the QC, the second result should be reported in SBIO.

4.3.4.2 Within a sample, low algal density or high taxa evenness are two examples of why similarity may not meet the 70% threshold.

4.3.4.3 If the supervisor and analysts determine that the differences in the taxa lists are due to variability within the sample or level of experience rather than identifications, the sample does not need to be re-identified.

4.4 BLOOM-ID and ALGAL-ID Ongoing/Individual QC

4.4.1 Ongoing/Individual QCs are conducted monthly. All analysts will have at least one BLOOM-ID/ALGAL-ID sample QC'd to identify the dominant taxon, co-dominant taxa, or lack of a dominant taxon. Results are saved in Bloom ID-Algal ID Ongoing QC → QC-BLOOM-ALGAL ID.

4.4.1.1 Because BLOOM-ID/ALGAL-ID samples are not preserved, it is preferable that all QCs should be performed the same day as the original analysis; however, during peak bloom season, there may not be enough time for this. In these situations, any recently received sample still containing healthy, non-degraded algae may be used for QC.

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4.5 Ongoing Group QC Exercises

4.5.1 Each quarter, one quantitative sample is selected and the wet algae examined by all of the analysts, together. The sample should contain a variety of taxa, new or different taxa, or taxa that prompt questions, such as those taxa that may be easily confused with other taxa. Some projects, however, contain very few taxa or contain a high quantity of the same taxa. While these may be used for the QC exercise, other projects may be more appropriate. Professional judgement will determine which samples are appropriate. If necessary, multiple samples may be used to reach the target of 25 different taxa.

4.5.2 All laboratory analysts set aside time on an agreed upon day. Sitting around a single microscope, the analysts take turns choosing a specimen (i.e. algal unit) to identify. Without discussion, each analyst writes down his or her identification of the chosen specimen, identifying it to the lowest level of which s/he is capable.

4.5.2.1 Using a single microscope removes variability associated with differences in scopes and gives all analysts the same view of each taxon.

Note: Each analyst should wipe down the microscope eye piece and focus knobs with ethanol after each respective identification.

4.5.3 After everyone has identified all the specimens needed to reach the target of 25 different taxa, the analysts compare identifications and discuss any differences. All analysts' original identifications and final identifications are entered into Quarterly_QC → Group QC. For each Group QC event, create a new tab and name it with the date of the QC.

Note: If possible, 25 different taxa should be identified; however, if there are not 25 different taxa in the selected samples to re-identify, make a note in the spreadsheet with the reason why. (ex: algae were sparse; all samples were dominated by one taxon)

4.5.3.1 Group QC sessions are an opportunity for all analysts to share their expertise with each other. While there is no pass or fail, analysts' results should agree the majority of the time. If there are more disagreements than agreements, the reason should be determined and noted. Further training may be needed..

4.5.3.2 The Group QC spreadsheet is used to track taxa examined each quarter. By reviewing the taxa in the spreadsheets, analysts can ensure that a wide range of taxa are identified in Group QC sessions over time. The spreadsheet also identifies uncommon taxa that may appear in our samples, taxa that repeatedly cause confusion, and taxa that are common in our samples. The Comments column should be used to record the resolution of any discrepancies in identifications and any tips for identifying uncommon or confusing taxa.

4.5.3.3 "Correct" is the best identification as agreed upon by the group. In some cases, it may be agreed that the most correct answer is to back up to a higher taxonomic level such as genus or family.

4.5.3.2.1 If a consensus cannot be reached, the analysts will note the lowest level at which they agree as the "correct" identification. These taxa are tracked so that those, for which this occurs frequently, can be submitted to outside experts for resolution.

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4.5.3.4 In some cases, the identifications and discussion take considerable time, especially with three or more analysts. This exercise should be completed in one day.

4.5.4 Any incorrect identifications should be re-examined to identify the source of the problem. Adjustments, further training and/or outside consultation may be required.

4.5.4.1 Compare microscopes and adjustments to assure that all equipment is working properly. If analysts' microscopes differ, then their identifications may also.

4.5.4.2 Level of identification (genus or species) as a source of misidentification should be rectified with additional training, or with standardization of level of identification, as appropriate.

4.6 Annual QC Procedures

4.6.1 The Annual QC is designed to assess all variability (intra-analyst, inter-analyst, intra-sample, and inter-sample). This exercise may help identify potential areas of bias within taxonomic data generated by the Algal Taxonomy Workgroup.

4.6.2 Detailed instructions are designed to apply to QC exercises with three analysts for either diatoms or soft algae. The design of this exercise gives the desired 16-18 data sets for statistical comparison, for which number of analysts is unimportant.

4.6.2.1 If only two analysts participate, four samples must be analyzed as described.

4.6.2.2 If four analysts participate, only two samples need to be analyzed.

4.6.3 The Taxonomy Supervisor should choose and code three samples from the backlog for analysis.

4.6.3.1 Samples for diatom and soft algae analysis should be of the same general type (phytoplankton, periphyton, Everglades, etc.), but different samples may be used.

4.6.3.2 The supervisor setting up the exercise should retain a list with the identity of the samples. Analysts will not be told the identity of any sample analyzed in this exercise until the exercise is completed. They will be told the type of sample (periphyton, phytoplankton, etc.) as this determines how the sample is prepped and analyzed.

4.6.3.3 Diatom samples should be chosen from those with high diatom abundance; soft algae samples should be chosen from those with high soft algal abundance. This can be determined with a preliminary scan of some sample material using the inverted microscope.

4.6.4 For each sample, prepare one aliquot or permanent slide mount for analysis following SOP AB-03 for periphyton prep, SOP AB-02.1 for diatom prep, or SOP AB-05 for phytoplankton prep. Code aliquots or slides A-C so that the analyst cannot determine the identity of the individual sample. Aliquots and/or slides will be referred to as samples in the remainder of this SOP.

4.6.5 Every analyst identifies each of the three samples twice for a total of six analyses following SOP AB-03 for periphyton, SOP AB-05 for phytoplankton, and SOP AB-03.01 or AB-05.1 for diatoms. Record each analysis (identifications and counts) on a separate QC data sheet labeled by sample code and analysis number.

4.6.5.1 Perform analyses on each of the three samples before performing the second analysis of the three samples. Do not refer to the first analysis of the same sample when performing the second analysis. Each analysis is designed to be independent.

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4.6.6 In Excel, enter the data from the analyses by entering a cumulative list of taxa found in the first column and entering the unit counts from each analysis into a separate column.

4.6.6.1 Code each column at the top by sample, analyst and replicate, using a single cell. Each data row should contain a unique taxon.

4.6.4.1.1 Examples of a unique analysis code for column headings would be A-MH-1 or C-MC-2.

4.6.6.2 Enter zero (0) for each sample where a taxon is present in one analysis but is absent in another.

4.6.6.3 Insert a row at the top of the spreadsheet and enter a title detailing the date and the exercise in the top left cell (A1).

4.6.4.3.1 Choose a title and file name that clearly identifies the QC exercise. An example of a title cell might be: Annual QC: samples EVGSLOU1234, WakSprg123 and RIVOAKTST, Analysts Hua, Chen and Dasher, May 23, 2003.

4.6.6.4 Using the title created, save the spreadsheet to: QCSpreadsheets → Annual QC

4.6.6.5 Add additional information as needed.

4.6.7 Conduct Bray-Curtis similarity analyses in PRIMER-E (per SOP AB-15) with species- and genus-level taxa lists with and without square root transformation to determine the optimum percent similarity between counts.

4.6.7.1 Similarity between replicate counts of a single sample by a single analyst (intra-sample variability) and similarity between counts of the same sample by each pair of analysts (inter-analyst variability) will be examined.

4.6.7.2 If similarities are >70%, the QC exercise has passed.

5. QUALITY CONTROL AND DOCUMENTATION

5.1 Use a pen with black ink for all logbook and bench sheet entries.

5.2 DEP Laboratory Information Management System (LIMS)

5.3 QC spreadsheets

5.4 QC benchsheets

5.5 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.

5.6 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.

5.7 See the DEP Laboratory Quality Manual for further QC and documentation requirements.

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6. REFERENCES

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

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

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

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

6.5 SOP AB-05, *Phytoplankton Non-Diatom Taxonomic Analysis*

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

6.7 SOP AB15, *Biological Community Comparisons using PRIMER_E Software*

6.8 SOP AB18, *Analysis of Algal Bloom and Algal Identification Samples*

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

Appendix of Significant Changes:

03/17/2008 Combined the analysts' portions of AB09, AB09_01, AB09_02, and AB09_03 to create AB-14-1.0. Updated file names and location references for bench sheets and ongoing taxa lists. Added requirement for documenting ongoing proficiency. Added requirement to include date as part of filename for QC data files. Removed the requirement to keep a spreadsheet of results from the Monthly QC (formerly called Monthly Round Robin). Added Scope and Application, Summary, and Appendix of Significant Changes.

01/25/2011 Re-organized layout to reduce redundancy.

03/25/2015 Formatted for 508 compliance. Updated links.

09/20/2017 Added the initial demonstration of proficiency requirements for BLOOM-ID and ALGAL-ID samples; Added referenced SOPs to the References section and standardized SOP naming format.

10/23/2019 Changed "Quarterly QC" to "Annual QC" throughout the document. Changed "Monthly QC" to "Group QC" throughout and modified the language in that section to allow flexibility during times of high sample load, too few analysts, and lack of appropriate samples to QC. Added a new section for BLOOM-ID/ALGAL-ID QC. Reformatted

01/04/2021 Modify note in 4.5.1.

12/03/2021 Added note in 4.5.2. pertaining to microscope sterilization during Group QC.

11/15/2022 Added note 4.2.3.1 (date of reidentification) for excel spreadsheet.

11/20/2023 Modified Group QC to Quarterly Group QC; removed links to network folders and replaced with file paths; updated link for Lab QA Manual; Sect 2.2: Ongoing QC for BLOOM-ID/ALGAL-ID is performed monthly, not weekly; corrected the referenced SOPs in 4.2.2 (should be AB18, not AB03 &AB05); added SOP AB18 to the References section.

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11/13/2024 Clarified in Section 2.1 that IDOP is passed after 10 samples for phytoplankton (PKD/DTY) and periphyton (PRN/DTM) analysis. Deleted SIMPER analysis instructions as PRIMER 7 does not support this analysis. Deleted sections on PRIMER analysis for BLOOM-ID/ALGAL-ID QC as this is not performed. Updated names of QC folders.

05/19/2025 More definitive tone, fewer “if able” options throughout the SOP; re-organized steps to help with the flow of procedures. Added SOP AB-02.1 to the Reference section. For Individual Ongoing QC, minimum one QC per quarter was added. Section 4.2.3, included the logic behind the qualitative IDOP. Created electronic version of Phytoplankton and Periphyton Algal Identification QC Log; Section 3 adjusted, accordingly.

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