Data Verification and LIMS Upload for Algal Growth Potential and Limiting Nutrient Tests

1. SCOPE AND APPLICATION

1.1. This method is used to verify data generated in Algal Growth Potential (AGP) and Limiting Nutrient (LN) tests prior to uploading results to the Laboratory Information Management System (LIMS) for reporting to clients.

2. SUMMARY OF THE METHOD

2.1. A review of a record of preparation is performed to ensure that samples were processed within holding time and that the appropriate test was performed. A non-conformance report is generated for samples that were not processed within hold-time. Inoculation data, count data, and result data are reviewed simultaneously by two people. Errors are corrected, non-conformance reports are generated (if necessary) and qualifiers are added to results. Reviewed results are then uploaded into LIMS. Dated and initialed data sheets are filed.

2.2. Method Detection Limits (MDL) and Practical Quantification Limits (PQL):

2.2.1. Freshwater AGP MDL and PQL are 0.3 and 0.9, respectively.

2.2.2. Saltwater AGP MDL and PQL is 3.0 for both.

3. 3. EQUIPMENT AND SUPPLIES

3.1. Supplies

3.1.1. Ruler or straight-edge

3.1.2. Verification/upload stamp

3.1.3. Outlier calculator – located in X:\Labs\Lab5\Biology\Bench Common\ToxCommon\AGP\AGP's Greatest Hits

3.1.4. AGP/LN Sample Log-in book

3.1.5. Run Set-Up Sheet

3.1.6. Inoculum Preparation Worksheet

3.1.7. Daily Counts Sheets

3.1.8. Black ink pen – black ink should be used for all logbook and benchsheet entries. To correct raw data entries, place a single line through the incorrect or illegible entry (do not overwrite), write the corrected entry near the error and initial the correction.

3.2. Equipment

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3.2.1. Computer with printer access and access to LIMS

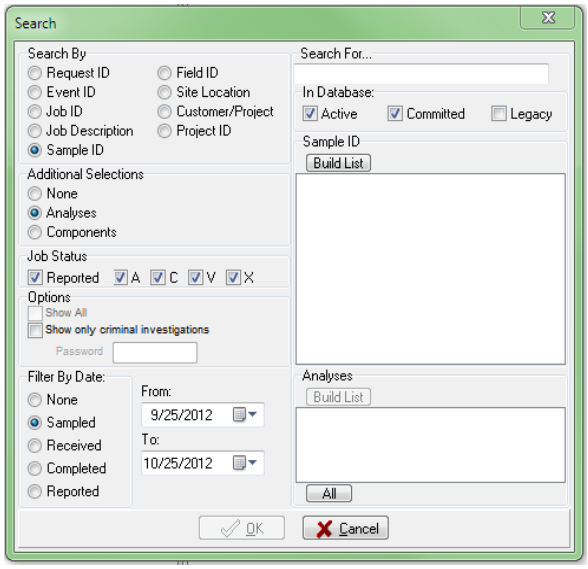
3.2.2. Calculator

4. DATA ENTRY

4.1. Using the AGP/LN Sample Log-in book, complete the Prep Worksheet(s) created for the samples run.

4.1.1. Prep Worksheets are created and completed per SOP TA-08.09.

4.2. Log in to LIMS and select **General/LIMS Browser** to find all the samples in the run to be verified. Use the "Search By" and "Search For…" criteria displayed in Figure 1 below. Use the date range "From" as the date first sample in the run was sampled (collected) and "To" as the date last sample in the run was sampled. Select "Build List." Choose all samples ID's displayed and choose the tests ("Analyses") applicable to the run being verified. Click the [**OK**] button to generate a list of events.



4.3. Once the query completes, a list of LIMS events will display. Click on the 

button at the top of the screen. Use the **Reports/File Formats** utility to generate a tab-delimited report with the following information for the samples: Event ID (field size 22), Sample ID (8), Field ID (18), Priority (7), Date Sampled (19), Date Received (19), Date Prepared (19), and Date Analyzed (19). Generate and print the report. Stamp with the verification/upload stamp.

Note: Choosing the field sizes listed above in parentheses will enable all the information to fit on one page. This information can be saved as a template so that it does not have to be set up each time a report is generated. For more information on using the LIMS Browser and/or Report utility, contact the Laboratory Support Section or someone with the Biology Section who is familiar with the program.

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4.4. In LIMS, select **Laboratory/Biology/AGP LN Manager**. Find and double-click the correct Run ID.

4.4.1. To minimize the number of runs to search through, check the "use date filter?" box.

4.5. In the **Run Setup** screen, using the *Inoculum Preparation* Worksheet verify that the information in the "Background Data," "Premix Data" and "Inoculation Check Data" is correct.

4.6. Using criteria in SOP TA-06.19, uncheck the boxes that are to the left of undesired (incorrect) data or that represent duplicate counts. Unchecked data will not be used in calculations.

Note: Counts will appear multiple times if a file was uploaded more than once. Sometimes it is necessary to compare Mean Cell Volumes in order to choose the correct count.

4.7. Click on the **Raw Counts** tab. Using the Daily Counts Sheets, verify that all Flask IDs, Vial IDs, counts and dilutions are correct. In the Raw Counts tab, uncheck any invalid counts.

4.7.1. Invalid counts include counts in excess of three counts per flask/vial/count day, incorrect flasks and vials, and incorrectly uploaded counts.

4.8. Click on the **Flask Averages** tab. Compare the Mean Count of each flask set with the Outlier Calculator. Uncheck any flasks that are a High or Low Outlier.

Note: This requires experience and professional judgment. Not every flask set must be checked with the calculator, but it is better to err on the side of checking too often.

4.9. Click on the **% Change** tab. Highlight samples and select **View NCR**. Note any Non-Conformance Reports (NCRs) related to the samples in the LIMS upload comments. Click on the **Export** button and save the file to X:\Labs\Lab5\Biology\Bench Common\ToxCommon\AGP\AGP Counts [Current Year]. Use the Run ID as the file name. The default file-type is .txt.

4.10. Open Microsoft Excel and open the text file generated in Step 4.9. In the dialogue box that appears, select **Finish**. **Save as** an Excel Workbook (.xlsx).

4.11. Highlight the header row of the exported data. Right-click and choose **Insert**. Repeat until four new rows have been inserted.

4.12. Highlight all the data, including the headings, and select **Data/Sort**. Sort by Sample ID, ascending. Click **OK**.

4.12.1. For each LN sample, the AGP should be listed first and then the flask treatments in the order they were set up for easy reference.

4.13. For any sample lab duplicate (LD) move the row so that it lies directly beneath the original sample.

4.14. While the data are highlighted, **Format** as a basic table.

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4.15. For each sample, determine which day's count will be uploaded. Highlight each of those counts.

4.15.1. If **% Change 10-12** less than 10, use Day 10.

4.15.2. If **% Change 10-12** greater than 10 and **% Change 12-14** less than 10, use Day 12.

4.15.3. If **% Change 10-12** greater than 10 and **% Change 12-14** greater than 10, use Day 14.

4.16. In **Print Preview** choose the landscape orientation for printing. Go to **Page Setup** and select the **Header/Footer** tab. Add a **Custom Header** and in the "Center Section" type in the document title ("LN % Change" for limiting nutrients and "AGP % Change" for AGP). Hit [Enter] and type the Setup Date in mm-dd-yy format. Press [Enter] and type the Run ID. Click **OK** twice and **Print**.

4.17. Save the file and close Excel.

4.17.1. If the file was not previously saved as an Excel Workbook (.xlsx), do so at this point.

4.18. For each duplicate sample, use the Excel printout to calculate the average of the two results. Write the averages to the side of or below the table.

4.18.1. For each duplicate sample, if either result is below MDL or between MDL and PQL, do not calculate the average. Select the higher of the two results for uploading. Refer to Section 2 for MDL and PQL limits.

4.19. Stamp the printout with the verification/upload stamp.

4.20. In AGP Manager, navigate back to the current run and select the **Final Results** tab.

4.20.1. The Prep Worksheet must be completed before the final results page can be completed.

4.20.2. In the Final Results tab, the **Analyst** and **Analysis Date** must be entered first.

4.20.3. In the **Comments** section, always include day(s) used and test species. Add any other appropriate comments (refer to AGP/LN Sample Log-in book), including any NCRs associated with the samples.

4.20.4. For each sample, click the **Result** header. In the new window, select the result (i.e. Day 10, 12, or 14) to be uploaded. If the result is an average, do not select a day. Enter the average in the empty field. Click **OK**.

4.20.5. Add any appropriate qualifiers by clicking on the **Remarks** heading.

4.20.5.1. If the result is an average, enter an **A**.

4.20.5.2. If the result is less than MDL, enter a **U**.

4.20.5.3. If the result is between MDL and PQL, enter an **I**.

4.21. The status should automatically change to "C."

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4.22. Close out of the run and out of AGP Manager.

4.23. On the LIMS printout and the Excel printout, initial and date the **Data Entry** line when all data had been checked in LIMS AGP Manager. Initial and date the **Uploaded** line when the sample status changes to "C." (Data Entry and Uploaded dates are often the same.)

5. VERIFICATION

5.1. The person who verifies should not be the same person who uploaded. As each step is being checked, note anything that may need correcting.

5.2. Using the AGP Sample Log-in book, verify that the Prep Worksheets were completed correctly.

5.3. Using the LIMS printout, verify hold times.

5.3.1. Collection Time to Prep Time should be less than 72 hours.

5.3.2. Collection Time to Analysis Time should be less than 30 days.

5.4. Verify that the correct Background counts, Premix counts, and Inoculation counts were selected in the **Run Setup** tab.

5.5. Verify that the dilutions are correct in the **Raw Counts** tab.

5.6. Check for outliers in the **Flask Average** tab. If there are outliers, make sure they were un-checked in the Raw Counts tab.

5.7. Using the Excel printout, verify that the correct results have been selected for uploading and that the calculated averages are correct.

5.8. Verify that the **Final Results** tab was completed correctly.

5.9. Initial and Date the **Verified** lines on the LIMS and Excel printouts. Return the packet to the person who uploaded.

5.10. If any corrections were made after the initial verification, the packet should be re-verified.

6. UPLOADING

6.1. After all corrections have been made and verified, open **LIMS→Laboratory→Technical→Results Editor**.

6.2. Under **Sort by…**, select **Sample ID**.

6.3. Under **Analysis Group…** and **Test Status…**, check the box next to "C" and select the appropriate analysis groups.

6.4. In the **Select test…** and **Select sample ID…** boxes, select the appropriate tests and sample IDs.

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6.5. When the test results come up, check once more for any errors. If everything is correct, select the **Authorize Tests in Grid with Status "C"** icon (**A**) at the top of the page.

6.6. Click **OK** and exit out of Results Editor.

6.7. On the verification/upload stamp, initial and date the **Uploaded LIMS** line.

7. REFERENCES

7.1. EPA 821-R-02-013 Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Ed. October 2002.

7.2. The *Selenastrum capricornutum Printz* Algal Assay Bottle Test, EPA-600/9-78-018.

7.3. Marine Algal Assay Bottle Test, EPA 1974.

APPENDIX OF CHANGES

01/04/2008	Added Scope and Application, Summary of Method and Appendix of Changes
12/30/2008	Added Reference Section. Deleted Step 8 'Balance capable of weighing to +/- 0.00001 grams' and associated comment 'OHAUS® 250 D located in the Microbiology Lab (Room B212)' from Materials.
10/27/2011	Replaced "easily readable" with "legible" in the Comment for Step 4.
10/23/2012	Reformatted SOP.
01/28/2015	Formatted for 508 compliance.
09/11/2015	Updated links.
09/15/2016	Updated links.
09/15/2017	Added MDLs and PQLs to Summary of Method section; included Prep Worksheet completion in Data Entry section; completed the Data Entry section and added a Verifying section and an Uploading section.
09/05/2018	Minor clarifications throughout; corrected % Dry Weight to Mean Count in Section 4.8; changed font to Calibri.

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