Data Verification and LIMS Upload for Algal Growth Potential Tests

1. SCOPE AND APPLICATION

1.1. This method is used to verify data generated in AGP LN MANAGER for Algal Growth Potential (AGP) 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. Errors are corrected, non-conformance reports are generated (if necessary) and qualifiers are added to results (if necessary). Results are then uploaded into LIMS and all data is reviewed and authorized.

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 are both 3.0.

2.2.2.1. All samples with values below the MDL are reported as the MDL and U-qualified.

2.2.2.2. Duplicate samples below the MDL are not averaged. The first sample is reported as MDL U-qualified with a comment that the duplicate sample was less than the MDL.

3. EQUIPMENT AND SUPPLIES

3.1. Supplies

3.1.1. Verification/upload stamp

3.1.2. Outlier calculator – located in \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\AGP\AGP's Greatest Hits

3.1.3. AGP Sample Log-in book

3.1.4. Master Setup Sheet – located in \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\AGP\AGP's Greatest Hits

3.1.5. Black ink pen

3.2. Equipment

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3.2.1. Computer with access to LIMS and a printer

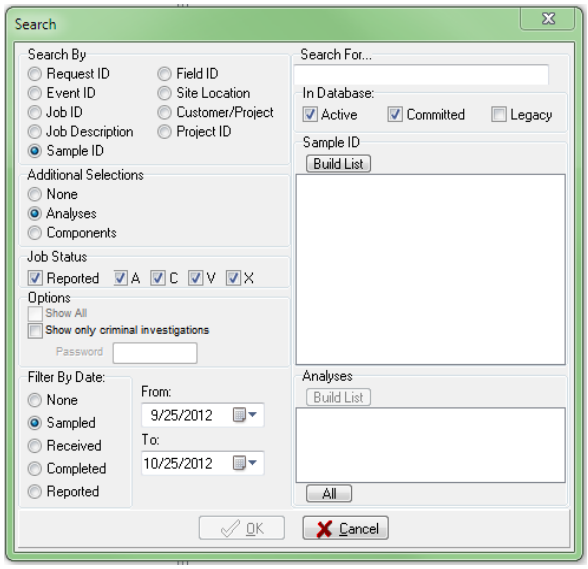
3.2.2. Calculator

4. DATA ENTRY

4.1. Using the AGP Sample Log-in book, complete the Prep Worksheet(s) created for the sample's run (this can also be completed after sample initiation).

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

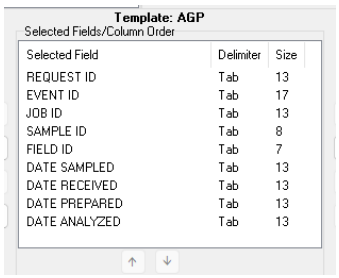
4.2. Log in to LIMS and select **General/LIMS Browser** to find all the samples in the run to be verified. Use the "Search By" and "Search For…" criteria displayed in Figure 1 below. Use the date range "From" as the date the first sample in the run was sampled (collected) and "To" as the date the last 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 (i.e. the AGP test codes). 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: Request ID (size 13), Event ID (17), Job ID (13), Sample ID (8), Field ID (7), Date Sampled (13), Date Received (13), Date Prepared (13), and Date Analyzed (1). Generate and print the report. (The manager can decide if they want the analyst to print this or if they want to print the report themselves.)

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Template: AGP
Selected Fields/Column Order

Selected Field	Delimiter	Size
REQUEST ID	Tab	13
EVENT ID	Tab	17
JOB ID	Tab	13
SAMPLE ID	Tab	8
FIELD ID	Tab	7
DATE SAMPLED	Tab	13
DATE RECEIVED	Tab	13
DATE PREPARED	Tab	13
DATE ANALYZED	Tab	13

4.3.1. 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.

4.3.2. The **Final Results** Tab in **AGP Manager** (step 4.20) needs to be completed prior to printing the report to populate all fields.

4.3.3. It is good practice to save a final copy of this report as a PDF in the AGP folder dedicated to the specific run, located here \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\AGP\AGP Counts [YY]

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 *Master Setup Sheet*, **verify** that the information in the “Background Data,” “Premix Data” and “Inoculation Check Data” is correct.

4.6. 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.

4.6.1. Counts will appear multiple times if a file was uploaded more than once. Sometimes it is necessary to compare Mean Cell Volumes 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, incorrect dilutions, and incorrectly uploaded counts.

4.8. Click on the **Flask Averages** tab. Compare the Mean Count of each flask set of 3 with the Outlier Calculator. When an outlier is found, make a note of which flask and day it belongs to (include this note with the data packet submitted to manager). High outliers will be the flask with the highest value of the 3 and low outliers will be the smallest value of the three flasks. Switch to the **Raw Counts Tab** and uncheck any

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flasks that are a High or Low Outlier, each flask noted as an outlier will have 3 flasks to uncheck. Make sure the Day and flask ID from the **Flask Average**s tab match the flasks that are being unchecked on the **Raw Counts** tab.

4.8.1. The outlier calculator uses the following formula to detect outliers. Data is rank ordered such that $X1 \leq X2 \leq X3$. Where X1 is lowest dry weight; X2 is the second lowest/ second highest dry weight and X3 is the greatest dry weight value. The sample's critical value (c) is determined as follows: If X3 is an outlier, $c = (X3 - X(3-1)) / (X3 - X1)$ If X1 is an outlier, $c = (X2 - X1) / (X3 - X1)$ If the value of c exceeds the critical value (0.941 for N = 3)) at $\alpha = 0.05$, that dry weight is discarded, and calculations are performed using the remaining two values.

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 \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\AGP\AGP Counts [YY]. 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**. Do the same for the first column. (This gives a blank row and column on the outside edge of the table.)

4.12. If there is more than one sample, highlight all the data, including the headings, and select **Data/Sort**. Sort by Sample ID, ascending. Click **OK**.

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

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

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** is less than 10, use Day 10.

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

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

4.16. For the duplicate sample, highlight each sample's data based on the above and then write the average of the two next to the line. This will be the result, A-qualified, of the sample.

4.16.1. If the result is less than the MDL, report the first sample as MDL U-qualified and add the following comment: "Laboratory duplicate result was [MDL] U."

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4.16.2. If the result is between the MDL and PQL (freshwater only), report the first sample as [result] I-qualified and the following comment: "Laboratory duplicate was [result] I."

4.17. Ensure the blank sample is below the MDL.

4.17.1. If the blank is above the MDL (failed), look at the sample results:

4.17.1.1. If the sample results are greater than 10 times the blank value, add a comment that says, "The laboratory blank failed but the result is > 10 times the blank result."

4.17.1.2. If the sample results are less than 10 times the blank result, V-qualify the data.

4.18. 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 "% Change". Press [Enter] and type the Run ID. Click **OK** twice and **Print**.

Ex. % Change
AGP-20##-##-##-##

4.19. Save the file and close Excel.

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

4.20. 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. (see 4.16)

4.20.1. For each duplicate sample, if either result is below MDL or between MDL and PQL, do not calculate the average. If the result is below the MDL, report the MDL with a U qualifier and make a comment stating that the laboratory duplicate was "MDL U." If the result is between MDL and PQL, select the higher of the two results for uploading and make a comment stating the result of the laboratory duplicate. Refer to Section 2 for MDL and PQL limits.

4.21. Stamp the printout with the verification/upload stamp and affix the note with the removed outliers.

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

4.22.1. **The Prep Worksheet must be completed before the Final Results tab can be completed.**

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

4.22.3. 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.

4.22.4. 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

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not select a day. Enter the average in the empty field using 3 significant figures. Click **OK**.

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

4.22.5.1. If the result is an average, enter an "**A"**.

4.22.5.2. If the result is less than MDL, enter a "**U"** (and enter the result as the MDL value).

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

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

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

4.25. On 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.)

4.26. Make sure the following tabs from the *Master Setup Sheet are filled out*: Setup, CtRcrd10, CtRcrd12, CtRcrd14, Bkgd, and FWInoc and/or SWInoc (depending on the type of tests run).

4.27. Place all printouts in a manilla folder. (Currently, only the table printout with the stamp and outliers note is required, but this can change per the manager's preference.)

5. VERIFICATION

5.1. Completed by manager or as assigned.

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

5.3. Using the AGP Sample Log-in book, verify that the Prep Worksheets were completed correctly (right now, only manager has access to completed prep worksheets).

5.4. Using the LIMS printout, verify hold times.

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

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

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

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

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

5.7.1. Unchecked raw data will not show on the **Flask Averages** tab. To check the outliers, the data will need to be selected and then once done un-checked again if they were outliers.

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5.7.2. If different outliers are unchecked from the ones originally provided, the final results will be different. A new % change table must be made and new results must be entered.

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

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

5.10. Initial and Date the **Verified** lines on the Excel printout.

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

6. AUTHORIZING

6.1. Completed by manager.

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

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

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

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

6.6. When the test results come up, check once more for any errors.

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

6.8. Data can then be authorized by going to **LIMS→Laboratory→Administrative→Date Review/Authorize** (results can also be checked in data review instead of results editor).

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

7. DATA ARCHIVAL

7.1. Use a black, waterproof pen for all bench sheets and logbooks.

7.2. Record information in appropriate logbooks (AGP Sample Log-in book).

7.3. To correct raw data entries, place a single line through the incorrect or illegible entry, write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

7.3.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

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7.3.2. If the correction requires an explanation, write a comment, and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

7.3.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

7.4. FDEP Laboratory Information Management System (LIMS).

8. REFERENCES

8.1. DEP Laboratory Quality Assurance Manual

8.2. DEP Health and Safety Manual

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

8.4. EPA 1974, Marine Algal Assay Procedure Bottle Test.

8.5. DEP SOP TA-08.09, Sample Custody, Logbook Labels, and Prep Worksheet Manager for Algal Growth Potential (AGP) Samples.

APPENDIX OF CHANGES

01/04/2008 Added Scope and Application, Summary of Method and Appendix of Changes

01/28/2015 Formatted for 508 compliance.

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.

09/02/2020 Added data archival section.

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