Count Days for an AGP Test

1. SCOPE AND APPLICATION

1.1. This method is based on EPA 600/9-78-018, *The* Selenastrum capricornutum *Printz Algal Assay Bottle Test* and EPA 1974, *Marine Algal Assay Bottle Test*. It is used to determine growth of algal test species in freshwater and marine aqueous samples. Tests conducted by this procedure are AA-AGP, AA-AGP-DCL, AA-AGP-F, AA-AGP-PH, AA-AGP-SA, AA-AGP-SPK, and AA-AGP-SW.

2. SUMMARY OF METHOD

2.1. On days 10, 12, and 14 during the 14-day test period, aliquots of inoculated samples are collected, diluted and counted with an electronic particle counter. Counts are uploaded into a data reduction program. Test endpoint is growth. Results are entered into the Laboratory Information Management System (LIMS) per SOP TA-06.42.

2.2. Interferences: Toxic substances introduced by contaminants in rinse water or on hands, glassware, sample hardware, and testing equipment. Dissolved oxygen < 4.0 mg/L; pH < 6.0 SU or > 10.0 SU; salinity > 1 part per thousand for freshwater tests; salinity < 7 or > 35 parts per thousand for marine tests; chlorine, debris, indigenous organisms, etc.

3. EQUIPMENT AND SUPPLIES

3.1. 30 mL Fisher brand Dilu-Vials®

3.2. Coulter® Counter Model Multisizer 4e (S/N BA23044) (Calibrated and used per SOP TA-06.41.)

3.3. Fisherbrand Bottle top Dispenser (Volume range 1-10 mL) (Calibrated per SOP MB-03.3.)

3.4. 1L amber glass jar

3.5. Test tube racks (to hold the 30 mL vials)

3.6. Finnpipette II Pipettors (200-1000 µL adjustable volume and 1000 µL fixed volume) (Calibrated per SOP MB-03.3.)

3.7. Pipettor tips, to fit Finnpipette II Pipettors

3.8. Black ink pen

3.9. *Background Counts* Tab

3.10. *AGP Count Record* Tab

3.11. Computer with access to the Laboratory Information Management System (LIMS)

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4. REAGENTS AND STANDARDS

4.1. Autoclaved Deionized (DI) Water

4.2. Isoton®

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1. See SOP FS 1000 for sample collection and preservation.

6. SAMPLE PREPARATION

6.1. Refer to SOPs TA-08.01 and TA-08.03.

7. SAMPLE ANALYSIS

7.1. Fill a 1 L amber glass jar with Isoton® and attach the Fisherbrand Bottle top Dispenser to the top. Perform three background counts on a sample of Isoton® dispensed from this jar. Record the counts and the mean cell volumes (MCV) on the *Background* tab in the *Master Setup Sheet* and upload to LIMS, as per TA-06.41. The background count should be 400 cell counts or less.

7.1.1. Refer to SOP TA-06.41 to set up the Coulter® Counter and the LIMS AGP LN Manager.

7.2. For each sample flask that is to be counted, place one alpha-numerically numbered vial into the vial-holder rack. Use as many vials and racks as needed.

7.3. Use the 1000 µL pipettor to place 1 mL of sample from each test flask into separate 30 mL vials. Use a clean tip for each new sample. Record the flask and vial codes on the *AGP Count Record* tab found in the *Master Setup Sheet*.

7.4. Add 9.0 mL of Isoton® from the jar described in step 7.1 into each vial containing samples.

7.4.1. Tap to remove bubbles to prevent interference with the cell count.

7.5. Record the count start time and analyst initials on the *AGP Count Record Sheet*.

7.6. Perform three counts on the contents of each vial (this is automatic) and upload to LIMS, as per SOP TA-06.41.

7.6.1. As each set of three counts is uploaded to the AGP module in LIMS, use the 10% calculate found in \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\AGP\AGP's Greatest Hits to ensure the three counts are within 10% of each other. If they are not, another set of three counts should be done until you are able to pull three results within 10% of each other. Remove the counts you will not be using from the module by dragging them to the bottom of the list and right clicking and selecting delete. Only upload the three counts within 10% of each other to LIMS. If they do get uploaded by mistake, go into the raw counts and deselect them from the list by unchecking them.

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7.7. When finished with all the counts, perform the shut-down procedures on the Coulter® Counter equipment, as per SOP TA-06.41.

7.8. After use, rinse the vials thoroughly with autoclaved DI water. Shake to remove excess water and invert to dry. Discard the vials whenever they appear dirty or cracked, or if any contamination is noted.

7.9. On count day 14, after counting has been completed, remove the appropriate flasks from the shaker racks or incubators, empty the contents down the sink, and rinse the flasks with DI water. Place the flasks on the dirty glassware cart(s).

7.10. Analyze the raw data per SOP TA-08.07. Data is then corrected, uploaded, verified, and authorized in LIMS.

8. DATA ARCHIVAL

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

8.2. Record counts in the appropriate tabs from the Excel document *Master Setup Sheet*: *Background* tab or *AGP/LN Count Record* tab.

8.3. 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.

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

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

8.3.2.1. 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.).

8.4. Enter the reduced raw data into the DEP Laboratory Information Management System (LIMS).

9. QUALITY CONTROL

9.1. Refer to DEP Laboratory Quality Assurance Manual and SOPs for corrective action and handling out-of-control data.

9.2. If a problem occurs during the sample processing or testing, the following steps are performed:

9.2.1. Lab supervisor is notified.

9.2.2. Client is notified (client makes decision for additional testing).

9.2.3. Section administrator is notified.

9.2.4. A non-conformance report (NCR) is submitted describing the problem and the corrective action.

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9.2.5. To create an NCR: open **LIMS** and go to **Laboratory/Administrative/Non-Conformance Report.**

9.3. Refer to FS1000 for sample collection and preservation, and the Laboratory Quality Manual for corrective action and handling out-of-control data.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves, and safety glasses, is required when working with chemicals and unprocessed samples.

10.2. The shaker rack can snare loose clothing, especially lab coats, and can cause serious bodily injury. BE CAREFUL! The shaker racks are operated by a timer system that activates the shakers twice daily for a 60-minute period. Always make sure the on/off switch is in the "off" position when placing flasks on and removing flasks from the shaker rack. The shaker rack in Inc #9 will automatically shut off and remain off while the door is open.

10.3. When replacing fluorescent lamps, make sure the shaker rack and the light switch are both turned off.

10.4. Refer to the DEP Laboratory Quality Assurance Manual for sample disposal.

10.5. Refer to the DEP Laboratory Health and Safety Plan.

10.6. Read MSDS information for reagent handling and disposal.

11. REFERENCES

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

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

11.3. FS1000, General Sampling.

11.4. DEP SOP MB-03.3, Calibration and Use of mechanical and Disposable Pipettes.

11.5. DEP SOP TA-06.41, Calibration and Use of the Coulter Counter Model Multisizer 4e.

11.6. DEP SOP TA-06.42, LIMS Entry for Laboratory Parameters.

11.7. DEP SOP TA-08.01, Initial Algal Assay Sample Handling.

11.8. DEP SOP TA-08.03, Set-Up and Inoculation of Algal Assay Tests

11.9. DEP SOP TA-08.07, Data Verification and LIMS Upload for Algal Growth Potential Tests.

11.10. DEP Laboratory Quality Assurance Manual.

11.11. DEP Laboratory Health and Safety Manual.

11.12. MSDS Information Sheets.

APPENDIX OF SIGNIFICANT CHANGES

08/28/2008 Added Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes.

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02/06/2015	Formatted for 508 compliance.
11/07/2024	The Title of this SOP was changed from "*Termination of Algal Assay Tests*" to "*Count Days for an AGP Test*".

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