# Termination of Algal Assay Tests

## 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, AA-AGP-SW, AA-LIM-NUT and AA-LN-NT-F.

## 2. SUMMARY OF METHOD

2.1. On various days 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), 40 slots, as needed

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 Sheet*

3.10. *AGP/LN Count Record Sheet*

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-08.02 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 Sheet*.

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

7.2.1. For the limiting-nutrient test, use your “shaker map” (SOP TA-08.03) to locate each flask to be drawn up.

7.3. Use the 1000 µL Finnpipette II pipettor to place 1 mL of sample from each test flask into separate 30 mL vials. Use a clean tip for each set of three flasks. Record the flask and vial codes on the *AGP/LN Count Record 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. Add the Isoton® to the vials one rack at a time to minimize settling of the algal cells. After one rack of vials has been counted, the Isoton® can be added to the next rack.

7.4.2. Wait for any bubbles that appear to disperse before counting the algal cells. If it is not dispersing gently tap the vial on the table to get rid of any lingering bubbles. This will prevent them from being included in the cell counts.

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

7.6. Perform three counts on the contents of each vial, as per SOP TA-06.41.



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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 on dry paper towels to dry. Discard the vials after three to six months of usage or 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, empty the contents down the sink, and rinse the flasks with tap 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, verified, uploaded, verified again, 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 bench sheet: *Background Counts Sheet* or *AGP/LN Count Record Sheet*.

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 | Florida Department of Environmental Protection 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).



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9.2.3. Section administrator is notified.

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

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

9.3. Refer to https://floridadep.gov/dear/quality-assurance/content/dep-sops 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. The timer activates at 0800 and 1500. Always make sure the on/off switch is in the “off” position when placing flasks on and removing flasks from the shaker rack.

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 Manual for sample disposal.

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

10.5.1. https://www.floridadep.net/resources/health-safety-manual

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 Bottle Test*

11.3. DEP SOP TA-08.02 *Algal Growth Potential AGP (AGP) and Limiting Nutrient (LN) Algal Assay Set-Up*

11.4. DEP SOP TA-08.03 *Inoculation of Algal Assay Tests*

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

11.6. DEP SOP TA-08.07 *Data Verification and LIMS Upload for Algal Growth Potential and Limiting Nutrient Tests*

11.7. DEP Laboratory Quality Assurance Manual and SOPs | Florida Department of Environmental Protection



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11.8. Laboratory Health and Safety Plan.

11.9. MSDS Information Sheets

**APPENDIX OF SIGNIFICANT CHANGES**

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| 08/28/2008 | Added Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes. |
| 07/13/2009 | Updated Quality Manual reference. |
| 05/18/2010 | Added “CAUTION Individuals allergic to latex should not use latex gloves. A suitable substitute should be found and used,” and deleted “Vinyl gloves can be substituted for individuals allergic to latex” in Safety Section comments. Updated the reference to the Biology Quality Manual. |
| 04/26/2011 | Changed Biology Quality Manual reference to Bureau Quality Manual. Replaced “manipulate” with “reduce” and added “raw” to Step 9 of Counting an Algal Assay Sample. |
| 02/06/2015 | Formatted for 508 compliance. |
| 03/15/2016 | Updated references to FDEP Laboratory Quality Manual and FDEP Laboratory Health and Safety Plan |
| 03/28/2017 | Updated links. |
| 02/28/2018 | Updated links. |
| 02/24/2021 | Updated Coulter Counter. Updated with universal language. |
| 02/15/2022 | Updated links and checked for grammar. |
| 02/27/2023 | Changed some sentence structure for clarify. Fixed grammar mistakes. |
| 02/26/2024 | Added SOP TA-06.42. Updated links. Added how to disperse bubbles in vials. Added “Autoclave” to the DI water since that is what we use to clean vials. |



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