Culture of Algal-Assay Inoculation Stocks

1. SCOPE AND APPLICATION

1.1. This method is based on and modified from EPA-600/9-78-018, *The* Selenastrum capricornutum *Printz Algal Assay Bottle Test* and EPA, 1974, *Marine Algal Assay Bottle Test* and the test organism culturing experience of laboratory personnel. It is used to ensure that *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) and *Dunaliella tertiolecta* bioassay test organism cultures are maintained so as to provide suitable test organisms. Tests associated with this SOP are AA-AGP, AA-AGP-LN, AA-AGP-SA, BSY-CS-FSC, and BSY-CD-FSC.

2. SUMMARY OF METHOD

2.1. Culture flasks are prepared by washing and autoclaving. Cultures are transferred weekly to flasks containing fresh media and every other week to nutrient fortified agar plates. Health of the cultures and contamination of the cultures is monitored by the Algal Taxonomic Group and recorded in the *Algae Culture Record* logbook.

2.2. Interferences:

2.2.1. Contaminated culture flasks and culture media and the failure to use aseptic techniques when transferring culture stocks can be detrimental to maintaining algal cultures.

3. EQUIPMENT AND SUPPLIES

3.1. 250 mL Autoclaved Erlenmeyer flasks with foam stoppers. Number may vary depending on laboratory requirements.

3.1.1. **Freshwater**: 6 flasks

3.1.2. **Saltwater**: 4 flasks

3.2. 2 10 mL Serological pipets, sterile and individually wrapped

3.3. 100 µm-1000 µm adjustable pipet with sterile tips

3.4. 2 100 mL Graduated cylinders

3.5. ¾ -inch wide labeling tape (green, red, and teal)

3.6. Black, fine-tip, permanent marker

3.7. UV sterilizer (located in Microbiology Lab); operate per instruction manual

3.8. Shaker rack, maintained at 25 ± 3 ºC with 24-hour fluorescent lighting (360-440 foot-candles)

3.9. Incubator, maintained at 20 ± 2 ºC with 24-hour fluorescent lighting (360-440 foot-candles)

3.10. **Freshwater monoculture**: *Pseudokirchneriella subcapitata*

Saltwater monoculture: *Dunaliella teriolecta*

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3.10.1. One flask of each species containing the youngest or most recently dated culture (7±1 days old) in addition to the most recently dated algal culture plate.

3.10.2. The morning of culture transfer, the Algal Taxonomy Group verifies the purity of the cultures in the flasks selected in 3.9.1. (See Section 7--Quality Control). The taxonomist should initial the *Algae Culture Record* Logbook and indicate whether the culture is suitable for use.

4. REAGENTS AND STANDARDS

4.1. **Freshwater monoculture:**

4.1.1. PAAP medium, at least 600 mL, prepared in advance per SOP TA-03.03.

4.1.2. BG-11, <10 mL, prepared by the Algal Taxonomy Group per SOP AB-12

4.2. **Saltwater monoculture:** ASW medium with NEPT, at least 400 mL, prepared in advance per SOP TA-03.04.

4.3. Nutrient fortified agar plates, prepared in advance per SOP TA-03.13

4.3.1. 2 plates per algae species (1 serves as backup)

4.3.2. Only needed every other week (refer to SOP TA-04.18)

5. METHOD

Note: Precautions must be taken to avoid cross-contamination of the *P. subcapitata* and *D. tertiolecta*. Refer to the Quality Control Section of this SOP.

5.1. Each Thursday, autoclave 10 foam-plug-stoppered flasks at 15 psi and 121 ºC for 15 minutes (day can be changed due to holidays, weather events, etc.).

5.1.1. This can be done one day prior to culture transfer. Take care to keep flasks sterile until use.

5.2. Remove the flasks from the autoclave and let cool to room temperature.

5.3. Label flasks by writing the algal name, date, expiration date (one month from transfer date), medium, and your initials on label tape. Stick one tape label around each flask.

5.3.1. Label 6 flasks “P. sub” for *Pseudokirchneriella subcapitata* using green tape.

5.3.2. Label 4 flasks “Dun” for *Dunaliella tertiolecta* using red tape.

5.3.3. Additionally, label one flask from each culture “From Plate” using the teal tape.

5.4. Use an adjustable pipet to add 0.5 mL of BG-11 to each of the flasks labeled “P. sub.”

5.5. Use a graduated cylinder to pour 90±1 mL of PAAP medium into each of the flasks labeled “P. sub.”

5.6. Use a second graduated cylinder to pour 90±1 mL of ASW media into each of the flasks labeled “Dun.”

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5.7. Place the flasks containing media in the UV sterilizer in the Microbiology Lab, up to three at a time, and sterilize each set of flasks for two minutes.

5.7.1. Do not place flasks containing PAAP medium and flasks containing ASW medium in the UV sterilizer at the same time! Sterilize each medium separately. (Refer to the Quality Control Section of this SOP.)

5.8. Place the "P. sub" and "Dun" flasks in the hood in the Toxicity room.

5.9. Inoculate all but the "from plate" new "P. sub" flasks with 10 mL of the verified 6-8 day old "P. sub" using a sterile serological pipet.

5.10. In the remaining flask, pour "P. sub" from the most recently dated algal culture plate until the media appears to have a hint of green color.

5.11. Repeat Steps 5.8-5.9 adding 10 mL of the verified "Dun" to all but the "from plate" flask using a new sterile serological pipet and utilizing the most recent dated "Dun" plate to pour into the remaining flask.

5.12. Every other week, follow SOP TA-04.18 to start a new algae culture on fortified nutrient agar.

5.13. Remove the oldest "P. sub" and "Dun" culture flasks (≥14 days) from their respective maintenance areas to make room for the newly inoculated culture flasks.

5.13.1. You will need the appropriate number of empty slots on the shaker rack and places in the incubator.

5.13.2. The contents of the old culture flasks can be discarded down the sink.

5.14. Place the newly inoculated "P. sub" flasks on shaker "B" in the Toxicity room. Turn the shaker rack on to low speed (100±10 rpm). Place the new "Dun" flasks in an incubator maintained at 20 ± 2 ºC .

5.14.1. Specific shaker racks and incubators may vary.

5.14.2. Maintain 24-hour lighting over the algae cultures (light intensity should be 360-440 foot-candles).

5.14.3. Shaker racks are controlled by a wall-mounted electronic timer. The switch on the shaker rack should be in the on position.

5.14.4. "Dun" flasks are hand shaken once daily Monday-Friday and once daily on Saturday or Sunday when temperatures are taken.

5.15. Record the culture transfers in the *Algae Culture Record* logbook.

6. DATA ARCHIVAL

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

6.2. Record preparation in the *Algae Culture Record* logbook.

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

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6.3.1. The corrected entry is written as near its appropriate space as possible or else in the comment box on the appropriate bench sheet.

6.3.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

6.3.3. 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. QUALITY CONTROL

7.1. 6-8 day old cultures are generally in a rapid-growth phase (log phase) and the algae that are transferred to fresh media are viable and stay in the log phase. Cultures that are 2-3 weeks old have usually depleted most of the nutrients from the culture media and are dying off. Algae in depleted media are generally more susceptible to physiological changes and may form a type that differs from the original culture (Personal communication circa 1988 to Marshall Faircloth FDEP, from D. Schultz (now retired), U.S. EPA, Athens, GA.).

7.2. Slides are prepared from the cultures and are examined under a microscope by the Algal Taxonomy Group to verify the culture is not contaminated.

7.3. The daily steps for one species should be done before doing the daily steps for the second species.

7.4. Perform the freshwater transfers first. Discard the pipets used for one species before using a new pipet for the other species.

7.5. Flasks should be washed in the biology section's dishwasher and then placed in the drying oven until dry. If the flasks are put through the standard Biology Section glassware washing procedure (see SOP BG-05), phosphate detergents are avoided because of the potential of skewed test results from phosphate-contaminated glassware. Use only non-phosphate detergent.

7.6. Temperatures are monitored daily.

7.6.1. If the **Room B246** temperature deviates more than ±2 ºC from the preferred range of 25 ± 3 ºC for any sustained length of time (meaning more than 2 hours at one time or 4 total hours during a 24-hour period):

7.6.1.1. Check the thermostat and record the room temperature and setting from the thermostat (include the date and time of observation). The thermostat units will need to be converted from degrees Fahrenheit to degrees Celsius. Go to 7.6.5 for procedure to report Room B246 temperature issue.

$$\text{Degrees Celsius } (^{o}C) = (^{o}F - 32) / 1.8$$

$$\text{Degrees Fahrenheit } (^{o}F) = (1.8)(^{o}C) + 32$$

7.6.2. If the "Dun" incubator has a sustained temperature deviation of ±2 ºC from the preferred temperature range of 20 ± 2 ºC:

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7.6.2.1. Check and record the incubator setting. Include the date and time of observation.

7.6.2.2. Notify supervisor(s) that the temperature is out of range and give them your observations.

7.6.2.3. Adjust the incubator setting and monitor the temperature for one hour, upon supervisor's approval.

7.6.2.3.1. Refer to the incubator manual for instructions on adjusting the temperature.

7.6.2.4. If the temperature does not improve, the supervisor should submit a purchase request for repair. "Dun" cultures can be moved to another incubator (lighting and temperature adjusted as needed).

7.6.3. If the temperature of room **B246** continues to fall out of range or does not improve, contact **Nhon Vo** (FDEP Scientific Support Services—245-8137 Lab Building A 416E) via the infrastructure problem reporting system in the Lab Support Help Desk: https://fldeploc.dep.state.fl.us/hd/helpdesk.asp and report the problem. Do **NOT** report it to him verbally. All issues must be tracked in the Lab Support Help Desk.

7.6.3.1. Until temperature is consistently within range, "P. sub" cultures can be moved into an incubator if necessary (lighting and temperature adjusted as needed).

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

8.2. Refer to Laboratory Quality Manual for sample disposal.

8.3. Refer to the Laboratory Health and Safety Plan and Hazardous Waste Management.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

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

9.2. EPA, 1974, *Marine Algal Assay Bottle Test*.

9.3. FDEP Laboratory Quality Manual
http://www.dep.state.fl.us/labs/library/lab_sops.htm

9.4. FDEP Laboratory Health and Safety Plan
http://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

9.5. DEP SOP TA-03.03, *Preparation of Preliminary Algal Assay Procedure (PAAP) Medium for Freshwater Algae Cultures*.

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9.6. DEP SOP TA-03.04, *Preparation of Artificial Seawater (ASW) medium for Culturing Dunaliella tertiolecta*.

9.7. DEP SOP TA-03.13, *Preparation of Plate-Count Agar (PCA)*

9.8. DEP SOP TA-04.18, *Culture of Algae on Agar Plates*

9.9. DEP SOP AB-12, *Preparation of BG-11 Medium Stock Solutions, Medium, and Agar Plates*

9.10. DEP SOP TA-08.03, *Inoculation of Algal Assay Test*.

9.11. DEP SOP BG-05, *Biology Washparty Procedures*.

9.12. MSDS Information Sheets.

APPENDIX OF CHANGES

09/25/2008	Added Scope and Application, Summary of the Method, Interferences, References and appendix of Changes. Added date and organization to comment section of Methods Step 10.
06/06/2013	Reformatted SOP.05/28/2014 Revised Method section.
01/29/2015	Formatted for 508 compliance.
06/13/2016	Updated links.
07/03/2017	Added shaker rack and incubator to Equipment and Supplies; Incubator #2 no longer in use, removed it from the document. Included BG-11 in the method. Added references to DEP SOPs AB-12, TA-03.13, and TA-04.18.

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