Starting Algal Cultures in Flasks from Algal cultures on Agar Plates

(Based on Methods developed by Biology Program Staff)

1. SCOPE AND APPLICATION

1.1. This procedure is used to help maintain mono-cultures of test algae by starting new cultures from plated-cultures.

2. SUMMARY OF METHOD

2.1. Culture media is placed into culture flasks. Plated cultures are aseptically transferred to flasks. Flasks are placed in appropriate culture areas.

2.2. Interferences:

2.2.1. Cross-contamination can occur if aseptic techniques are not used during the culture transfers.

2.2.2. Incorrect incubation of the cultures can interfere with growth and viability of the cultures.

2.3. Definitions

2.3.1. ***Pseudokirchneriella subcapitata*:** freshwater algae; formerly known as *Selenastrum capricornutum* (labeled as P. sub)

2.3.2. ***Dunaliella tertiolecta*:** saltwater algae (labeled as Dun)

3. EQUIPMENT AND SUPPLIES

3.1 250 mL Erlenmeyer flasks with foam plugs, 2, sterilized per SOP TA-04.08

3.2 ¾" x 2" Labeling tape, 2 strips

3.3 100 mL Graduated cylinder, 2 (one for each species)

3.4 Shaker platforms, located in Tox lab

3.4.1 *P. subcapitata*—Shaker B, rotating 100±10 rpm

3.4.2 *D. tertiolecta*—Incubator #2, hand shaken daily (minimum 1X daily)

3.5 Disposable sterile inoculation loops, Fisherbrand® Steriloops™ 13-075-1

3.5.1 If single-use inoculation loops are not available, use a Bunsen burner and flint striker (or piezoelectric sparker) to flame sterilize a platinum-inoculation loop.

3.6 Milk dilution bottle

3.7 Black, fine-tip, permanent marker

4. REAGENTS AND STANDARDS

4.1. *P. subcapitata* and *D. tertiolecta*

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

4.1.1. *Pseudokirchneriella subcapitata* agar plate culture, 2-4 weeks old, prepared per SOP TA-04.18

4.1.2. *Dunaliella tertiolecta* agar plate culture, 2-4 weeks old, prepared per SOP TA-04.18

4.2. Culture media

4.2.1. *P. subcapitata*—PAAP, 250 mL, prepared in advance per SOP TA-03.03.

4.2.2. *D. tertiolecta*—ASW with NEPT, 500 mL of 30 ppth, prepared in advance per SOP TA-03.04.

CAUTION: Do not confuse with basal media which does not contain NEPT.

5. CULTURE PREPARATION

5.1. *Pseudokirchneriella subcapitata* and *Dunaliella tertiolecta*

5.1.1. When needed, gather the materials for the new algal cultures and place them in the laboratory hood. Usually, new cultures are started when problems are noted in the regular cultures by toxicology or algal taxonomy staff.

5.1.2. Using the black lab marker and the label tape (green for P. sub and red for Dun), label a 250 mL flask with the following:

a. Culture name
b. From plate batch #
c. Media (PAAP or 30 ppth ASW)
d. Transfer date
e. Analyst initials

5.1.3. Pour 90 mL ± 10 mL of media into the appropriately labeled 250 mL Erlenmeyer flask.

5.1.4. Place the flask containing media in the UV sterilizer (in the microbiology lab), and sterilize the flask for 2 minutes.

5.1.5. Remove the top from the agar plate.

5.1.6. Use a sterile disposable inoculation loop to gently scrape ½ of the surface of the agar covered by the algae, taking care not to go more than the diameter of the plastic forming the loop below the surface of the agar.

5.1.6.1. If a disposable inoculation loop is not available, prepare platinum wire-inoculation loop. **Use proper protective equipment and keep flammable materials away when using the Bunsen burner.**

5.1.7. Remove the foam plug from an appropriately labeled 250 mL flask. Immerse the loop in the media and agitate the loop to dislodge the algae. Remove the loop from the flask. Reinsert the foam plug in the flask, leaving half of the plug above the rim of the flask.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

5.1.8. Place the "Dun" flask in Tox lab Incubator #2. Place the "P. sub" flask on shaker platform "B" in the Tox lab. Turn the shaker off before placing the flask on it. Turn it back on when the flask is in place.

5.1.9. Record the flask set up 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 preparations 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.

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.

6.4. 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. A new, disposable sterile pipette is used for each transfer to prevent cross-contamination. If a platinum-inoculation loop is used, it is flame-sterilized before and after each use.

7.2. 2-4 week old agar plate cultures are used to ensure healthy, viable continuing cultures.

7.3. Cultures are examined for purity by the Biology Lab's Algal Taxonomic section.

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 the FDEP Laboratory Quality Manual for sample disposal.

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

8.4. Read MSDS information for reagent handling and disposal.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

9. REFERENCES

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

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

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

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

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

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

9.7. DEP SOP TA-09.09, *Preparation of PAAP-Medium Stock Solutions*.

9.8. DEP SOP TA-03.02, *Preparation of Artificial Seawater (ASW).*

9.9. DEP SOP MB-03.1, *Maintenance of Bacterial Cultures.*

9.10. MSDS information sheets

APPENDIX OF CHANGES

12/12/2007 Added Scope and Application; added Summary of the Method

07/26/2013 Reformatted SOP

06/12/2014 Corrected spelling error

01/29/2015 Formatted for 508 compliance.

06/01/2015 Removal of references to *Isochrysis galbana* and *Tetraselmis sp*.

06/08/2016 Updated links

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.