Culture of Algal-Assay Inoculation Stocks

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,* and culturing experience of laboratory personnel. It is used to ensure that *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*) and *Dunaliella tertiolecta* cultures are suitably maintained. Tests associated with this SOP are AA-AGP, AA-AGP-SA, and AA-AGP-SW.

2. SUMMARY OF METHOD

2.1. Cultures are transferred weekly to flasks containing fresh media and every other week to nutrient fortified agar plates. Health and purity 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.

2.3. Definitions

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

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

3. EQUIPMENT AND SUPPLIES

3.1. 250 mL Autoclaved Erlenmeyer flasks with foam stoppers

3.1.1. **Freshwater**: 3 flasks

3.1.2. **Saltwater**: 2 flasks

3.2. 10-mL pipets, sterile and individually wrapped

3.3. Pipettor

3.4. Parafilm

3.5. 100 µm-1000 µm adjustable pipet with sterile tips

3.6. Two 100-mL Graduated cylinders

3.7. Labeling tape (green and red)

3.8. Black, fine-tip, permanent marker

3.9. UV sterilizer (located in Microbiology or Taxonomy Lab)

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

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

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

3.12. *Algae Culture Record* logbook

3.13. **Freshwater monoculture**: *Raphidocelis subcapitata*

Saltwater monoculture: *Dunaliella tertiolecta*

3.13.1. One flask of each species containing the newest culture (7±1 days old) and the newest algal culture plate.

3.13.2. The morning of culture transfer, an algal taxonomist verifies the purity of the cultures in the flasks and initials the *Algae Culture Record* Logbook. If there is a problem with the health or purity of a culture, start a new culture from an agar plate (see Section 5.11).

4. REAGENTS AND STANDARDS

4.1. **Freshwater monoculture:**

4.1.1. PAAP medium, prepared in advance per SOP TA-03.03.

4.1.2. BG-11, prepared per SOP AB-12.

4.1.3. PAAP media agar plate, prepared in advance per SOP TA-03.13.

4.2. **Saltwater monoculture**:

4.2.1. ASW medium, prepared in advance per SOP TA-03.04.

4.2.2. ASW media agar plate, prepared in advance per SOP TA-03.13.

5. METHOD

5.1. Weekly, autoclave 5 foam-plug-stoppered flasks at 15 psi and 121 ºC for 15 minutes.

5.2. Once cooled, label flasks by writing the algae name, date, expiration date (one month from transfer date), medium and batch number, and analyst initials on label tape.

5.2.1. Label 3 flasks “R. sub” for *Raphidocelis subcapitata* using green tape.

5.2.2. Label 2 flasks “Dun” for *Dunaliella tertiolecta* using red tape.

5.3. Add 0.5 mL of BG-11 to each of the flasks labeled “R. sub”.

5.4. Add 90 mL of PAAP medium into each of the flasks labeled “R. sub”.

5.5. Add 90 mL of ASW media into each of the flasks labeled “Dun”.

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

5.6. 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. Sterilize each medium separately.

5.7. In the hood in the Tox lab, add 10 mL of the verified 6-8 day old "R. sub" to all the new "R. sub" flasks. "R. sub" should be completed first.

5.8. Repeat Steps 5.9 through 5.10, but using verified "Dun" in place of the R. sub.

5.9. Every other week, start a new algae culture on fortified nutrient agar.

5.9.1. Take one PAAP media agar plate and one ASW media agar plate from the walk-in refrigerator. Place in the hood to acclimate to room temperature.

5.9.2. Using black permanent marker, label the plates with the algal name, preparation date, media name, expiration date (30 days from prep date), and analyst initials.

5.9.3. Pipette 8 mL of verified freshwater algae onto the PAAP agar and seal with Parafilm.

5.9.4. Pipette 8 mL of verified saltwater algae onto the ASW agar and seal with Parafilm.

5.9.5. Culture the plates with the algae culture flasks. Place in a single layer for ample light penetration. Discard the oldest plate.

5.10. If a problem is noted in a culture flask, a new culture can start from an agar plate. Prepare flasks per steps 5.1 to 5.6. Use a sterile inoculation loop to gently scrape the surface of an algae plate culture that is less than 4 weeks old. Immerse the loop in the media and agitate the loop to dislodge the algae.

5.11. Remove the oldest "R. sub" and "Dun" culture flasks (≥14 days) and discard down the sink.

5.12. Place the newly inoculated "R. sub" flasks on shaker "B" in the Toxicology Lab or in an incubator at 25° C. Turn the shaker rack on to low speed (100 ± 10 rpm). Place the new "Dun" flasks in an incubator maintained at 20 ± 2 ºC.

5.12.1. Maintain 24-hour lighting over the algae cultures (360-440 foot-candles).

5.12.2. "Dun" flasks are hand swirled at least once each day.

5.13. Record the culture transfers and agar plate inoculation 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.

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

6.3. 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. QUALITY CONTROL

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

7.2. Using aseptic techniques to prevent cross-contamination, perform the freshwater transfers first. A new pipette is used for each culture transfer.

7.3. If the room temperature deviates from the preferred range, freshwater cultures are moved to an incubator with portable lighting.

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 FDEP Laboratory Quality Manual for sample disposal.

8.3. Refer to the Laboratory Health and Safety Plan.

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. DEP Laboratory Quality Manual

9.4. DEP Laboratory Health and Safety Plan

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

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 AB-12, *Preparation of BG-11 Medium Stock Solutions, Medium, and Agar Plates*

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

9.10. DEP SOP BG-05, *Biology Wash-party Procedures*

9.11. MSDS Information Sheets

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

APPENDIX OF CHANGES

09/25/2008 Added Scope and Application, Summary of the Method, Interferences, References and appendix of Changes.

01/29/2015 Formatted for 508 compliance.

03/06/2025 Combined SOPs TA-04.08, TA-04.18, & TA-04.19.

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