Culture of Algae on Agar Plates

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 maintain cultures of test algae.

2. SUMMARY OF METHOD

2.1. Algae cultures are transferred from culture flasks to agar plates, allowed to settle, then drained, sealed and placed in the appropriate culture area.

2.2. Interferences:

2.2.1. Cross-contamination of the cultures and age of the "old" cultures can interfere with the growth and purity of the "new" cultures. Failure to properly place the culture plates in the incubator can interfere with the amount of light available to the cultures and also creates the potential for cross-contamination.

2.3. Definitions:

2.3.1. ***Pseudokirchneriella subcapitata*:** freshwater algae; formerly known as *Selenastrum capricornutum* used for Algal Growth Potential (AGP) tests and food for *Ceriodaphnia dubia* (labeled as P. sub)

2.3.2. ***Dunaliella tertiolecta*:** saltwater algae, used for marine AGP tests (labeled as Dun)

3. EQUIPMENT AND SUPPLIES

3.1. 10 mL Serological disposable pipette, sterile and individually wrapped

3.1.1. The volume of each new lot is checked per SOP TA-06.32.

3.2. Pipette pump

3.3. Black, fine-tip, permanent marker

3.4. Parafilm®

3.5. Shelf or Incubator, maintained at 25 ± 3 °C with 24-hour fluorescent lighting (360-440 foot candles)

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

3.7. **Freshwater monoculture:** *Pseudokirchneriella subcapitata*

Saltwater monoculture: *Dunaliella tertiolecta*

3.7.1. One flask of each species containing the youngest or most recently dated culture (7 ± 1 days old).

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3.7.2. The morning of culture transfer, the Algal Taxonomy Group verifies the purity of the cultures in the flasks selected in 3.7.1. (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 media agar plate, prepared in advance per SOP TA-03.13

4.2. Saltwater monoculture:

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

5. METHOD

5.1. Every other week get the previously prepared agar-media plates from the walk-in refrigerator. Place plates bottom up in the hood.

5.1.1. There should be one PAAP media agar plate for the freshwater algal culture and one ASW media agar plate for the saltwater algal culture.

5.2. Remove the lid, shake off excess condensation, and replace the lid.

5.3. Using the black permanent marker, label the top of the agar plates with the following:

5.3.1. Algal name—*P. subcapitata* on PAAP plate, *D. tertiolecta* on ASW plate

5.3.2. Preparation date

5.3.3. Media name

5.3.4. Expiration date (30 days from the prep date)

5.3.5. Analyst initials

5.4. Obtain a verified flask of each culture and place the flasks in the lab hood near the agar plates. This helps prevent cross-contamination of the algal cultures.

5.5. Select the freshwater culture and the PAAP media plate. Pour (or pipette with sterile 10 mL pipette) 20-30 mL of the freshwater algae on to the PAAP agar. Do not allow the algae to overflow the agar plate, as this increases the chance of contamination. Replace the plate lid.

5.6. Repeat Step 5.5 for the saltwater algae, using the ASW agar.

5.7. Allow the agar plates with culture additions to sit undisturbed for two hours while the algae settle onto the surface of the agar.

5.8. After two hours carefully take each agar plate with culture to the sink and slowly pour off most of the liquid in the agar plate. Leave a thin layer of liquid on the agar. Replace the plate lid. Seal the inoculated agar plates with Parafilm®.

5.8.1. Be careful not to cross-contaminate the cultures.

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5.8.2. Leaving a thin layer of liquid on the agar ensures the presence of the algae. Settled algae should be visible as a green colored residue.

5.9. **Alternatively**: 8 mL of algae may be pipetted on to the appropriate agar plate and sealed with Parafilm®.

5.10. Place the inoculated freshwater agar plate on a paper towel next to Shaker B in the Tox lab. The temperature should be maintained at 25 ± 3 °C with 24-hour fluorescent lighting maintained at 360-440 foot-candles.

5.11. Place the inoculated saltwater agar plate in an incubator maintained at 20 ± 2 °C and with 24-hour fluorescent lighting maintained at 360-440 foot-candles.

5.12. Do not stack the newly inoculated plates on the old plates, or vice versa. This increases the chance of cross-contamination and also creates less than optimum growing conditions by blocking light from the lower agar plates.

5.13. Record the 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 culture 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, date and initial 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.

6.3.2.1. 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.).

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. Aseptic techniques are used to prevent cross-contamination of the cultures. A new, sterile pipette is used for each culture transfer.

7.3. Complete the steps for transferring the freshwater species before starting to transfer the saltwater species.

7.4. Young cultures (7±1 days old) are used to create the new culture plates. Older cultures are more likely to be less viable and/or have a greater potential of being contaminated.

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7.5. Plates are placed in the incubator in a single layer to prevent cross-contamination and to permit the light to penetrate the plates equally.

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.

Degrees Celsius (ºC) = (ºF – 32) / 1.8

Degrees Fahrenheit (ºF) = (1.8)(ºC) + 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:

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).

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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 Manual and Hazardous Waste Management. 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 SOP TA-03.13, Preparation of Plate-Count Agar (PCA).

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

9.5. Laboratory Health and Safety Manual https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

9.6. MSDS safety sheets.

APPENDIX OF CHANGES

12/21/2007	Added Scope and Application; added Summary of Method; corrected SOP reference TA-03.15 to TA-03.13.
07/24/2013	Reformatted.
01/29/2015	Formatted for 508 compliance.
07/07/2015	Updated links.
07/11/2016	Updated links.
09/13/2017	Added to the Equipment and Supplies list; added temperature monitoring to Quality Control section.

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