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 Disposable pipette

3.2. 10 mL Re-pipettor

3.3. Black, fine-tip, permanent marker

3.4. Parafilm®

4. REAGENTS AND STANDARDS

4.1. Freshwater monoculture:

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

4.1.2. *Pseudokirchneriella subcapitata* culture

4.1.2.1. Use the youngest or most recently dated culture, which should be 7±1 days old. A slide prepared from the culture should be examined under a microscope to verify that the culture is not contaminated.

4.2. Saltwater monoculture:

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

4.2.2. *Dunaliella tertiolecta* culture

4.2.2.1. Use the youngest or most recently dated culture, which should be 7±1 days old. A slide prepared from the culture should be examined under a microscope to verify that the culture is not contaminated.

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 each freshwater algal culture and one ASW media agar plate for each 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 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 an algal culture and a corresponding media plate. Pour (or pipette with sterile 10 mL pipette) 20-30 mL of the appropriate algae on to the appropriate 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 other culture.

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.

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.

5.8.3. Alternatively: approximately 10 mL of the appropriate algae may be poured on to the appropriate agar plate and sealed with Parafilm®.

5.9. Place the inoculated freshwater agar plate on a paper towel next to Shaker B in the Tox lab. Place the inoculated saltwater agar plate in Incubator #2.

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5.9.1. 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.

5.10. 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. Record culture preparation in the *Algae Culture Record* logbook.

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

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

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

6.2.2.1. 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. Culture purity is checked by making a slide of the “old” culture and looking for contamination. If it is not contaminated, then “new” cultures can be made.

7.2. Aseptic techniques are used to prevent cross-contamination of the cultures. A new, sterile pipette is used for each culture transfer. Also, the saltwater culture and the freshwater culture are kept separate from each other.

7.3. 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.

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

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 Bureau Quality Manual for sample disposal
http://www.dep.state.fl.us/labs/library/lab_sops.htm

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8.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

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

9.4. Bureau 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.

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