Preparation of Plate-Count Agar (PCA)

(Based on Difco® medium instructions)

1. SCOPE AND APPLICATION

1.1. This method is based on Difco® medium instructions for preparing agar. Prepared in appropriate media, it is used for long term maintenance of algal cultures.

2. SUMMARY OF METHOD

2.1. Agar is added to appropriate liquid medium, melted, poured into Petri dishes, covered and allowed to cool. They are then stored until used for culturing algae.

2.2. Interferences:

2.2.1. Aseptic techniques must be used to ensure that there is no contamination of the culture via the media, reagents, supplies or the analyst.

2.2.2. Use Class-A glassware to ensure accurate volume measurements.

2.2.3. Avoid burning or boil-over of media. Overheating can cause nutrient degradation.

3. EQUIPMENT AND SUPPLIES

3.1. 500 mL Erlenmeyer flask, 2

3.2. 250 mL Graduated cylinder, 2

3.3. Weigh boats, 2

3.4. Mettler PB403-S/FACT balance, calibrated per SOP TA-06.31

3.5. Hot-plate with stirrer (Corning® Model 320)

3.6. 1” Magnetic stir-bars, 2

3.7. 100 x 15 mm Sterile disposable Petri dishes, 20 (Falcon® lab ware, Falcon® 1029 from Fisher Scientific)

3.8. 2” Labeling tape, 4 pieces

3.9. Permanent black marker

3.10. Clean spatula

3.11. Parafilm®

4. REAGENTS AND STANDARDS

4.1. PAAP freshwater alga media, prepared in advance per SOP TA-03.03.

4.2. Artificial Seawater (ASW) saltwater alga media, prepared in advance per SOP TA-03.04.

4.3. Bacto-Agar, dehydrated. (Difco® Bacto-Agar from Fisher Scientific. Located in the dessicator in the Microbiology Lab.)

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5. MEDIA PREPARATION

5.1. Using the label tape and black marker, label one 500 mL flask and one 250 mL cylinder "PAAP." Label the other 500 mL flask and 250 mL cylinder "ASW Media."

5.2. Separate the Petri dishes into two groups of 10. Using labeling tape and a black marker, label the top of each dish of one set with the date and "PAAP Agar." Label the other set with the date and "ASW Media Agar." Add an expiration date of one year from the preparation date to each Petri dish. Label each dish with a common batch number for each media type.

5.2.1. Keep the Petri dishes "bottom up" when not in use.

5.2.2. The batch number is the date that the solution was made. If more than one batch of the same solution is made on that date, add a letter. (Example: 04-06-15A and 04-06-15B.)

5.3. Using the graduated cylinder labeled "PAAP," measure and pour 250 mL of PAAP media into the 500 mL flask labeled "PAAP."

5.4. Using the graduated cylinder labeled "ASW media," measure and pour 250 mL of ASW media into the 500 mL flask labeled "ASW Media."

5.5. Using the balance, a weigh boat, and the spatula, weigh out 3.5 g of Bacto-Agar and add it to the "PAAP" flask.

5.5.1. Weighing QC requires that two "S" weights that bracket the desired weight (weigh boat tared) be weighed and recorded. Example: 3 g and 5 g "S" weights can be used to bracket the desired weight of 3.5 g.

5.6. Repeat step 5.5, only put the agar into the "ASW Media" flask.

5.7. Put a magnetic stir-bar into each of the 500 mL flasks.

5.8. Place the "PAAP" flask on the hot-plate stirrer. Adjust the temperature control to "high." Adjust the stirrer speed as needed so as to cause a small vortex to form at the surface of the liquid.

5.9. Monitor the liquid at the bottom of the flask for air bubble formation and monitor the surface of the liquid for the formation of foam. These are indicators that the liquid is about to boil.

5.10. **WARNING! HOT LIQUID!!** As soon as the foam starts to rise toward the mouth of the flask, use the autoclave gloves to remove the flask from the hot-plate stirrer. Place the flask on the counter top.

The liquid will rapidly foam out of the flask if it is not removed from the hot plate. **Do not look away!**

5.11. Set the hot-plate stirrer controls to the "off" position.

5.12. Use the gloves to carry the "PAAP" media flask to the "PAAP Agar" Petri dishes. **WARNING! HOT!!** Remove the top from a "PAAP Agar" Petri dish and carefully pour the "PAAP" liquid into the bottom half of the Petri dish, filling the dish

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approximately one-half full (~25 mL). Place the top back on the Petri dish. Repeat for the remaining nine appropriately labeled dishes.

5.13. Repeat Steps 5.8-5.12 using the flask labeled "ASW Media" and Petri dishes labeled "ASW Media Agar."

5.14. Take both flasks to a sink, use copious amounts of hot water and rinse any remaining agar down the drain. This saves later clean up time, since the agar should still be liquid.

5.15. Allow the Petri dishes containing the agar to cool for approximately 1 hour or until the agar has solidified.

5.16. After the agar has solidified, wrap with Parafilm®, then invert the dishes (bottom up and top down).

5.17. Put the Petri dishes in the walk-in refrigerator. Place them on the second shelf from the top of the second set of shelves to the left as you enter the refrigerator.

5.18. Record batch information in the *AGP Record of Preparation* logbook.

6. DATA ARCHIVAL

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

6.2. Record preparation in the *AGP Record of Preparation* 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. Autoclaved, Class-A glassware is used to minimize the potential for contamination and to ensure accurate volume measurements.

7.2. Two "S" weights that bracket the desired weights should be weighed and recorded before reagents are weighed. This is done before the first use of the balance on a given day, or if it has been greater than 2 hours since the previous use of the balance on the same day. If the amount of reagent needed is outside of the previously weighed bracket weights, then a new set of bracket weights should be weighed and recorded to include the desired weight. (See SOP TA-06.31.)

7.3. Media is monitored during heating to make sure it doesn't burn or boil over.

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7.4. Petri dishes are stored upside down to prevent condensation pooling on the agar.

7.5. Media is not used after the expiration date.

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. Expired media is taken to the microbiology lab and placed in the "to be autoclaved" bag for disposal. (Refer to DEP Laboratory Quality Manual for media disposal.

8.3. Refer to the DEP Laboratory Health and Safety Manual.

8.4. Read MSDS information sheets for reagent handling and disposal.

9. REFERENCES

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

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

9.3. Difco® medium instructions for preparing agar.

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

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

9.6. DEP SOP TA-06.31, Calibration and Use of Mettler PB403-S/FACT Balance.

APPENDIX OF CHANGES

12/11/2007	Added Scope an Application and Summary of Method.
11/07/2008	Added References.
06/05/2013	Reformatted SOP.
02/10/2015	Formatted for 508 compliance.
04/30/2015	Removed saving excess agar from process.
03/30/2016	Updated links and references.
03/30/2017	Updated links.

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