Preparation of Algal Assay Nutrient Spike-Verification Solutions

1. SCOPE AND APPLICATION

1.1. This method is based on Miller, W. E., T. E. Maloney, and J. C. Greene. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA-600/9-78-018. U. S. EPA, Cincinnati, Ohio. 126 p. These solutions are used in growth reference tests. Solutions without nitrogen and/or phosphorus should not produce growth. For example, the PAAP-P solution should not produce growth until P (phosphorus) is added. The solutions with nutrient spikes added provide data of actual growth to expected growth per unit of nutrient.

2. SUMMARY OF METHOD

2.1. Chemicals are weighed and added singularly to deionized water to make nutrient solutions of known concentration. Solutions are stored and used as needed in limiting nutrient algal assays.

2.2. Interferences include contaminated glassware, improperly prepared and contaminated nutrient solutions, dust and airborne microscopic organisms.

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. Autoclave

3.2. 2 L Volumetric flasks with stoppers, 6

3.3. 2 mL Disposable glass pipets

3.4. Parafilm^{TM} (stopper substitute)

4. REAGENTS AND STANDARDS

4.1. Nutrient spike-verification solutions based on PAAP medium. All solutions should be prepared in advance per SOP TA-09.09.

4.1.1. PAAP stock solution $NaNO_3$

4.1.2. PAAP stock solution KH_2PO_4

4.1.3. PAAP stock solution EDTA

4.1.4. PAAP stock solution Trace plus Micro

4.1.5. PAAP stock solution $MgCl_2$

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4.1.6. PAAP stock solution $CaCl_2 \cdot 2H_2O$

4.1.7. PAAP stock solution $MgSO_4 \cdot 7H_2O$

4.1.8. PAAP stock solution $NaHCO_3$

4.1.9. Autoclaved deionized (DI) water

4.2. Nutrient spike-verification solutions based on ASW medium. All except ASW basal stock solution are prepared in advance per SOP TA-09.09. ASW basal stock solution is prepared in advance per SOP TA-09.10.

4.2.1. ASW basal stock solution

4.2.2. PAAP stock solution $NaNO_3$

4.2.3. PAAP stock solution KH_2PO_4

4.2.4. PAAP stock solution EDTA

4.2.5. PAAP stock solution Trace plus Micro

4.2.6. Autoclaved DI water

5. METHOD

5.1. PAAP-medium-based solutions: Nitrogen spike-verification solution

5.1.1. Label one of the flasks with “PAAP minus Nitrogen,” the batch number, an expiration date of one year from the preparation date (batch number), and the analyst’s initials. The nutrient spike-verification solutions are PAAP medium or ASW medium without the nutrient of the spike. This acts as a check on the strength of the nutrient spike as the nutrient is expected to stimulate a known amount of algal growth.

5.1.2. Fill the labeled flask halfway with autoclaved DI water.

5.1.3. Add 2 mL of each of the **listed** PAAP stock solutions (**do not add $NaNO_3$ or EDTA**) to the flask and fill the flask to the 2L mark with autoclaved DI water. Stopper the flask.

5.1.4. Add 1 mL of the following PAAP nutrient stock solutions to the flask for each liter of "PAAP minus Nitrogen -EDTA":

5.1.4.1. $MgCl_2 \cdot 6H_2O$

5.1.4.2. $CaCl_2 \cdot 2H_2O$

5.1.4.3. $MgSO_4 \cdot 7H_2O$

5.1.4.4. KH_2PO_4

5.1.4.5. $NaHCO_3$

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5.1.4.6. Trace plus Micro

5.2. PAAP-medium-based solutions: Phosphorus spike-verification solution

5.2.1. Label one of the flasks with “PAAP minus Phosphorus,” the batch number, an expiration date of one year from the preparation date (batch number), and the analyst’s initials.

5.2.2. Fill the labeled flask halfway with autoclaved DI water.

5.2.3. Add 2 mL of each of the **listed** PAAP stock solutions (**do not add K_2HPO_4**) to the flask and finish filling the flask to the 2L mark with autoclaved DI water. Stopper the flask.

5.2.4. Add 1 mL of the following PAAP nutrient stock solutions to the flask for each liter of "PAAP minus Phosphorus":

5.2.4.1. $MgCl_2 \cdot 6H_2O$

5.2.4.2. $CaCl_2 \cdot 2H_2O$

5.2.4.3. $MgSO_4 \cdot 7H_2O$

5.2.4.4. $NaNO_3$

5.2.4.5. $NaHCO_3$

5.2.4.6. EDTA

5.2.4.7. Trace plus Micro

5.3. PAAP-medium-based solutions: EDTA spike-verification solution

5.3.1. Label one of the flasks with “PAAP minus EDTA,” the batch number, an expiration date of one year from the preparation date (batch number), and the analyst’s initials.

5.3.2. Fill the labeled flask halfway with autoclaved DI water.

5.3.3. Add 2 mL of each of the **listed** PAAP stock solutions (**do not add EDTA**) to the flask and finish filling the flask to the 2L mark with autoclaved DI water. Stopper the flask.

5.3.4. Add 1 mL of the following PAAP nutrient stock solutions to the flask for each liter of "PAAP minus EDTA":

5.3.4.1. $MgCl_2 \cdot 6H_2O$

5.3.4.2. $CaCl_2 \cdot 2H_2O$

5.3.4.3. $MgSO_4 \cdot 7H_2O$

5.3.4.4. $NaNO_3$

5.3.4.5. $NaHCO_3$

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5.3.4.6. KH_2PO_4

5.3.4.7. Trace plus Micro

5.4. ASW-medium-based solutions: Nitrogen spike-verification solution

5.4.1. Label one of the flasks with “ASW minus Nitrogen,” the batch number, an expiration date of one year from the preparation date (batch number), and the analyst’s initials. The nutrient stock solutions used in preparation of the ASW-based solution*s* are the same as those used for the various PAAP solutions. These stock solutions are also labeled "PAAP." The “batch #” is the date that the solution is prepared. Format is mm/dd/yy, ex: 01/11/10. Add an expiration date to the label of one year after preparation date (batch number), ex: 01/11/11 for the 01/11/10 batch.

5.4.2. Fill the labeled flask halfway with autoclaved DI water.

5.4.3. Add 2 mL of each of the **listed** PAAP stock solutions (**do not add $NaNO_3$ or EDTA**) to the flask and finish filling the flask to the 2L mark with ASW basal solution. Stopper the flask.

5.4.4. Add 1 mL of the following PAAP nutrient stock solutions to the flask for each liter of "ASW minus Nitrogen":

5.4.4.1. KH_2PO_4

5.4.4.2. Trace plus Micro

5.5. ASW-medium-based solutions: Phosphorus spike-verification solution

5.5.1. Label one of the flasks with “ASW minus Phosphorus,” the batch number, an expiration date of one year from the preparation date (batch number), and the analyst’s initials. The “batch #” is the date that the solution is prepared. Format is mm/dd/yy, ex: 01/11/10. Add an expiration date to the label of one year after preparation date (batch number), ex: 01/11/11 for the 01/11/10 batch.

5.5.2. Fill the labeled flask halfway with autoclaved DI water.

5.5.3. Add 2 mL of each of the **listed** PAAP stock solutions (**do not add KH_2PO_4**) to the flask and finish filling the flask to the 2L mark with ASW basal solution. Stopper the flask.

5.5.4. Add 1 mL of the following PAAP nutrient stock solutions to the flask for each liter of "ASW minus Phosphorus":

5.5.4.1. $NaNO_3$

5.5.4.2. EDTA

5.5.4.3. Trace plus Micro

5.6. ASW-medium-based solutions: EDTA spike-verification solution

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5.6.1. Label one of the flasks with "ASW minus EDTA," the batch number, an expiration date of one year from the preparation date (batch number), and the analyst's initials. The "batch #" is the date that the solution is prepared. Format is mm/dd/yy, ex: 01/11/10. Add an expiration date to the label of one year after preparation date (batch number), ex: 01/11/11 for the 01/11/10 batch.

5.6.2. Half fill the labeled flask with ASW basal solution.

5.6.3. Add 2 mL of each of the **listed** PAAP stock solutions (**do not add EDTA**) to the flask and finish filling the flask to the 2L mark with ASW basal solution. Stopper the flask.

5.6.4. Add 1 mL of the following PAAP nutrient stock solutions to the flask for each liter of "ASW minus EDTA":

5.6.4.1. $NaNO_3$

5.6.4.2. KH_2PO_4

5.6.4.3. Trace plus Micro

5.7. For **All** Prepared Nutrient Verification Solutions

5.7.1. Check the pH of the media and pH adjust as necessary per SOP TA-08.06. The end pH of the PAAP-based solutions should be 7.5 ± 0.1. The end pH of the ASW-based solutions should be 8.0 ± 0.11.

5.7.2. Filter solutions through prewashed 0.45 µm membrane filter per SOP TA-08.01 Step 7. Filters are washed per SOP TA-06.16. **Caution:** carefully rinse filtration glassware to avoid nutrient contamination.

5.7.3. Record the nutrient-verification solution batch information in the LIMS Standard Preparation Tracker.

5.7.4. Store the nutrient-verification solutions in the walk-in refrigerator. Nutrient verification solutions may be kept for 12 months at > 0 to ≤ 6°C.

6. DATA ARCHIVAL

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

6.2. 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.

6.2.1. The corrected entry is written as near as possible to the error or 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. Do not attempt to put all information (correction and comments) into a small space.

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6.2.3. 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. If a problem occurs during sample processing, a nonconformance report (NCR) is submitted describing the problem and the corrective action.

7.2. All stock solutions must be less than 1-yr old (check expiration date on flask labels).

7.3. Newly prepared solutions (minimum of annually) are submitted to the FDEP chemistry laboratory for QA/QC analyses. Contact QA/QC officer.

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 Laboratory Quality Manual for sample disposal.

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

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. Miller, W. E., T. E. Maloney, and J. C. Greene. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA-600/9-78-018. U. S. EPA, Cincinnati, Ohio. 126 p.

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

9.3. DEP SOP TA-09.10, Artificial Seawater Basal Stock Solution for Culturing Marine Algae.

9.4. DEP SOP TA-08.06, pH adjustment of Samples: Algal Assay and Toxicity Bioassay

9.5. DEP SOP TA-08.01, Initial Algal Assay and Limiting Nutrient Sample Handling

9.6. DEP TA-06.16, Washing Filters for Algal Assay to Remove Trace Contaminants

9.7. Laboratory Quality Manual DEP Laboratory Quality Assurance Manual and SOPs | Florida Department of Environmental Protection

9.8. Laboratory Health and Safety Manual https://www.floridadep.net/resources/health-safety-manual

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APPENDIX OF CHANGES

12/21/2007 Added Scope and Application; Summary of Method and Appendix of Changes. Transferred comment from Materials to Scope and Application.

11/18/2008 Added Interference and Reference sections.

08/14/2013 Reformatted SOP.

02/11/2015 Formatted for 508 compliance.

07/14/2015 Updated links.

07/18/2016 Updated links.

09/14/2017 Updated links.

08/29/2019 Updated links.

09/03/2020 Updated data archival section with universal language.

05/07/2021 Updated grammar and links.

09/01/2022 Updated links. Added SOP mentions. Edited equipment section.

09/05/2023 Fixed display list of PAAP nutrients within Method. Checked links.

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