Algal Growth Potential (AGP) and Limiting Nutrient (LN) Algal Assay Set-Up

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 determine the algal growth potential of fresh and saline waters and the bio-available nutrients in a water sample.

2. SUMMARY OF METHOD

2.1. Processed algal growth potential and limiting nutrient samples are poured into test flasks. Appropriate test algae are used to inoculate the test flasks. Various nutrients are added singularly or in combination to various test flasks for the limiting nutrient tests. Test flasks are placed in appropriate culture areas.

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. Erlenmeyer flasks with autoclaved foam plugs, 250 mL, autoclaved

3.2. Graduated cylinder, 100 mL

3.3. Kontes® repeating dispenser with 60-mL head and 2000 mL flask

3.4. Eppendorf Repeater and three 50 mL tips

3.5. Three sterile disposable plastic cups (labeled N, P, EDTA) for spikes

3.6. Limiting Nutrient (LN) spike chart

3.7. Sample *Set-up* Sheet, located in X:\Labs\Lab5\Biology\Bench Common\ToxCommon\AGP\AGP's Greatest Hits→Master Setup Sheets.xls

4. REAGENTS AND STANDARDS

4.1. AGP and AGP/LN samples, prepared and filtered, per SOP TA-08.01

4.2. Lab blanks, prepared and filtered, per SOP TA-08.08

4.3. Deionized (DI) water

4.4. Nitrogen, phosphorus, and EDTA nutrient spikes (prepared per TA-09.11)

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5. METHOD

5.1. Samples for AGP Tests

5.1.1. Place the autoclaved flasks on the algal assay set-up table in the Culture Lab (B246). Fill out the sample *Set-up* sheet by listing each freshwater sample (in LIMS Sample ID order) and assigning each sample a flask set. The flask set refers to the three flasks used with a particular sample for the AGP test. Starting with flask 1 of a new group of flasks, do the same for the saltwater samples. For both fresh and saltwater samples, assign one lab duplicate per every 10 samples or fraction thereof, and one lab blank per every 20 samples or fraction thereof, with a minimum of one duplicate and one lab blank per run. Randomly assign flask sets to the lab blanks and randomly place the sets among the samples.

5.1.1.1. A laboratory duplicate is created by setting up two flask sets from the same sample. This produces two separate results that are later compared. A minimum of 360 mL of sample is needed per laboratory duplicate. Do not create a laboratory duplicate of a field blank or a field duplicate.

5.1.2. Arrange the flasks in sets of 3, with one set per AGP sample according to the *Table Set-up Sheet*.

5.1.3. Invert the sample bottle to ensure the sample is mixed. **Verify** the flask set and sample combination from the *Table Set-up Sheet,* then use the graduated cylinder to pour 60 mL of the sample or laboratory blank into each of the assigned flasks. **Do not drop the foam plugs on the floor, the table or shaker.** Hold them in your hand, or place in original box or in a clean container.

5.1.4. **Verify** that each sample is matched to a set of flasks. Verify proper sample to proper flask set with each sample. Major problems arise from errors on this point.

5.1.5. Take the graduated cylinder to the sink and rinse thoroughly with DI water.

5.1.6. Place a small amount of the next sample into the cylinder and shake. Pour out when finished. This is to ensure that the sample will not be diluted.

5.1.7. Repeat Steps 5.1.3 through 5.1.6 until each sample has been poured into the proper flasks. Sample containers having leftover sample (should be >200 mL for all but containers having duplicate samples removed) are to be returned to the walk-in refrigerator. Refer to SOP TA-08.09 for sample disposal.

5.1.8. Ensure that all the flasks have plugs.

5.1.9. The samples are left on the algal assay set-up table overnight to acclimate and then they are inoculated with the appropriate alga. This usually occurs on Friday. Inoculate per SOP TA-08.03.

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5.1.9.1. Samples can be poured the morning of set-up, but they must be acclimated to room temperature before inoculation.

5.2. Samples for Limiting Nutrient Tests

5.2.1. 5.2.1 Place the autoclaved flasks on the algal assay set up table in the Culture Lab (B246). All LN tests have an AGP test performed with them.
NOTE: The AGP test does not require a LN test.

5.2.2. Arrange the flasks in sets of 21 if performing the typical nitrogen (N), phosphorus (P), and EDTA limiting nutrient test. Only 12 flasks are needed for a simple nitrogen and phosphorus addition test. A minimum sample volume of 1,300 mL of sample is required to run a typical LN analysis. If the sample volume is insufficient after preparation, eliminate the EDTA addition test (below) and then the P+EDTA addition test if needed.

5.2.2.1. Example of test set up:

Flasks *y*1, *y*2, and *y*3 are for the AGP test.
Flasks *y*4, *y*5, and *y*6 are for the N addition test.
Flasks *y*7, *y*8, and *y*9 are for the N + P addition test.
Flasks *y*10, *y*11, and *y*12 are for the EDTA addition test.
Flasks *y*13, *y*14, and *y*15 are for the P addition test.
Flasks *y*16, *y*17, and *y*18 are for the N + P + EDTA addition test.
Flasks *y*19, *y*20, and *y*21 are for the P + EDTA addition test.

5.2.3. On the sample *Set-up* sheet, list each LN sample and assign a 21-flask group per sample (or 12 flasks for a simple N and P addition test).

5.2.4. Select a sample (invert to mix) and pour it into the Kontes® pourer. **Verify** the flask set and sample combination from the sample *Set-up* sheet, then pour the sample from the pourer into each of the three flasks of its set. **Do not drop the foam plugs on the floor, the table or shaker.** Hold them in your hand, place in original box or a clean container. 60 mL of sample is placed into each flask. **Verify** that each sample is matched to a set of flasks. Verify proper sample to proper flask set with each sample. Major problems arise from errors on this point.

5.2.5. After each set of 21 is poured, rinse the pourer thoroughly with DI water.

5.2.6. Place a small amount of the next sample into the cylinder and shake. Pour out when finished. This is to ensure that the sample will not be diluted.

5.2.7. Repeat Steps 5.2.4 through 5.2.6 until each sample has been poured into the proper flasks. Sample containers having leftover sample are to be returned to the walk-in refrigerator. There will be insufficient sample remaining to set up a complete LN test. See supervisor if an additional test (based on results) needs to be set up. Refer to SOP TA-08.09 for sample disposal.

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5.2.8. Pour approximately 15 mL of **nitrogen spike** per limiting nutrient sample into plastic cup labeled N.

5.2.9. Use the Eppendorf repeater, one 50 mL tip, and nitrogen spike to place 1.0 mL of nitrogen nutrient solution into each flask numbered *y*4–*y*9 and *y*16-*y*18 in each set of the sample flasks.

5.2.9.1. Adding a nutrient solution to the sample is called spiking and the nutrient solution is referred to as a nutrient spike (nitrogen spike, phosphorus spike, EDTA spike). The nitrogen nutrient solution goes into 3 flasks for the N addition test, into 3 flasks in combination with phosphorus for the N + P addition tests, and into 3 flasks in combination with phosphorus and EDTA for the N + P + EDTA addition test.

5.2.9.2. Be sure to prime the pipettor well before beginning the nutrient addition to ensure that there are no bubbles in the tip. Bubbles result in adding less than 1.0 mL of spike to a flask. Use the limiting nutrient spike chart to determine which flasks receive which nutrient spikes.

5.2.10. Pour unused nitrogen spike down the sink and flush with tap water. Run water to help flush the sink out. **Never pour leftover spike back into the original container.** Dispose of the tip and cup used.

5.2.11. Pour approximately 15 ml of **phosphorus spike** per limiting nutrient sample into plastic cup labeled P.

5.2.12. Use the Eppendorf repeater, one 50 mL tip, and phosphorus spike to place 1.0 mL of the phosphorus nutrient solution into each flask numbered *y*7–*y*9 and *y*13-*y*21 in each set of the sample flasks. The phosphorus nutrient solution goes into 3 flasks for the P addition test, into 3 flasks for the N + P addition tests, into 3 flasks for the P + EDTA addition test, and into 3 flasks for the N + P + EDTA addition test.

5.2.12.1. Be sure to prime the pipettor well before beginning the nutrient addition to ensure that there are no bubbles in the tip. Bubbles result in adding less than 1.0 mL of spike to a flask.

5.2.13. Pour unused phosphorus spike down the sink and flush with tap water. Run water to help flush the sink out. **Never pour leftover spike back into the original container.** Dispose of the tip and cup used.

5.2.14. Pour approximately 15 mL of **EDTA spike** per limiting nutrient sample into plastic cup labeled EDTA.

5.2.15. Use the Eppendorf repeater, one 50 mL tip, and EDTA spike to place 1.0 mL of the EDTA nutrient solution into each flask numbered *y*10-*y*12 and *y*16-*y*21 in each set of the sample flasks. The EDTA nutrient solution goes into 3

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flasks for the EDTA addition test, into 3 flasks for the P + EDTA addition test, and into 3 flasks for the N + P + EDTA addition test.

5.2.15.1. Be sure to prime the pipettor well before beginning the nutrient addition to ensure that there are no bubbles in the tip. Bubbles result in adding less than 1.0 mL of spike to a flask.

5.2.16. Pour unused EDTA spike down the sink and flush with tap water. Run water to help flush the sink out. **Never pour leftover spike back into the original container**. Discard the used 50-mL tip and cup.

5.2.17. Ensure that all the flasks have plugs.

5.2.18. The samples are left on the algal assay set-up table overnight to acclimate, and then they are inoculated with the appropriate alga. This usually occurs on Friday. Inoculate per SOP TA-08.03.

5.2.19. Return the nutrient solution bottles to the walk-in refrigerator.

6. DATA ARCHIVAL

6.1. 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.

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

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

6.2. 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.).

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

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

7. QUALITY CONTROL

7.1. REMINDER: Growth reference tests are generally performed quarterly with both test alga species. The growth reference test is used to verify nutrient strength of growth media and to confirm absence/presence of nutrients in spike solutions. Chemical nutrient analyses can serve the same function as long as algal cultures are maintained and monitored per SOP TA-04.19.

7.2. AGP and AGP/LN samples are prepared, filtered, and stored in the walk-in refrigerator per SOP TA-08.01. If a sample's initial pH was <6.0 or >10.0, recheck the pH and adjust the sample prior to set-up per SOP TA-08.06.

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7.3. Lab blanks are prepared per SOP TA-08.08. Run one freshwater laboratory blank with any samples of <1.0 % salinity. Likewise, run one saltwater laboratory blank with any samples of ≥7.0 % salinity. At this point, no sample should have a salinity between ≥1 % and <7 % salinity.

7.4. AGP tests require 3 flasks per sample, laboratory blank, and laboratory duplicate. LN tests require 3 flasks per singular nutrient addition and 3 flasks per combined-nutrient additions. A typical LN test (with nitrogen, phosphorus, and EDTA) requires 21 flasks per sample. When only a reduced sample volume is available, a smaller N and P LN test (with the primary nutrients: nitrogen and phosphorus) requires 12 flasks per sample (3 flasks AGP, 3 flasks N-spike, 3 flasks P-spike, and 3 flasks N+P spikes).

7.5. Foam plugs should be replaced semi-annually or whenever the white color changes to a medium yellow-brown. Plugs should also be replaced immediately if any crumbling is noticed.

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.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. The *Selenastrum capricornutum Printz* Algal Assay Bottle Test, EPA-600/9-78-018.

9.2. Marine Algal Assay Bottle Test, EPA 1974.

9.3. DEP SOP TA-04.19, Starting Alga Cultures in Flasks From Alga Cultures on Agar Plates.

9.4. DEP SOP TA-08.01, Initial Algal Assay Sample Handling.

9.5. DEP SOP TA-08.03, Inoculation of Algal Assay Tests.

9.6. DEP SOP TA-08.04, Filtering Algal Growth Potential and Limiting Nutrient Samples.

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

9.8. DEP SOP TA-08.09, Sample Custody, Log Book Labels and Prep Worksheet Manager for Algal Growth Potential (AGP) Samples.

9.9. DEP SOP TA-09.01, Preparation of Nutrient Solutions for Limiting Nutrient Algal Assays.

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

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

APPENDIX OF CHANGES

12/12/2007	Scope and Application added; added Summary of the Method
11/17/2008	Added Interference and Reference sections.
08/12/2013	Reformatted SOP.
01/28/2015	Formatted for 508 compliance.
07/14/2015	Updated links.
07/13/2016	Updated links.
09/14/2017	Added *Set-up* sheet to Equipment and Supplies; minor reformatting in AGP Method section; in the Quality Control section, AGP/LN preparation, filtering, and storage edited to reflect they are now in one SOP
08/27/2018	Minor reformatting: removed double-spaces after periods, changed font to Calibri

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