Initial Algal Assay Sample Handling

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10. REFERENCES

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1. SCOPE AND APPLICATION

1.1. This method is based on EPA-600/9-78-018 *The* Selenastrum Capricornutum *Printz Algal Assay Bottle Test.* It is used to process algal growth potential (AGP) samples prior to inoculation. Tests covered by the procedure include AA-AGP, AA-AGP-DCL, AA-AGP-SW, AA-AGP-pH, and AA-AGP-SA.

2. SUMMARY OF THE METHOD

2.1. Samples are checked into the laboratory. Conductivity, salinity, temperature, chlorine and pH measurements are taken. Samples are dechlorinated as needed. Salinity and pH adjustments are made as needed. Samples are autoclaved and/or filtered based on salinity. Samples are stored between 0°C and 6°C until analyses are initiated. Samples can be frozen for long term (>30 day) storage.

3. INTERFERENCES

3.1. Interferences with the filtration process can include highly turbid samples, contaminated glassware, contaminated filters and rinse water, plugged filter bases and torn or punctured filters.

4. EQUIPMENT AND SUPPLIES

4.1. Initial handling of all samples

4.1.1. 30 mL disposable plastic cup, one per sample

4.1.2. Traceable Bench Conductivity Meter, S/N 170814827, calibrated and used per TA-06.36.

4.1.3. Fisherbrand Accumet AB315 pH/mV Meter, calibrated and used per TA-06.43

4.1.4. Autoclave tape

4.2. Initial handling of samples < 1.0 ppth salinity

4.2.1. Large bucket or ice chest

4.2.2. Autoclave – use per BG-08

4.2.3. Ice

4.3. Initial handling of samples 1.0-7.0 ppth salinity

4.3.1. Marine Mix artificial sea salts – lab uses Crystal Sea Marine Mix. Artificial sea salts used should be bioassay laboratory formula and should not contain EDTA, sodium thiosulfate, nitrates, or phosphates.

4.3.2. Magnetic stir plate

4.3.3. Magnetic stir bar, 2.5-cm

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4.3.4. Stir-bar retriever

4.3.5. 500 mL Erlenmeyer flask

4.3.6. 500 mL Graduated cylinder

4.3.7. Mettler Toledo PB 403TS/00 balance S/N B815673069, calibrated per TA-06.34

4.3.8. Weigh boat

4.3.9. Metal spatula

4.4. Filtering

4.4.1. Prewashed 0.45 µm Fisher Durapore® membrane filters (or equivalent), at least 2 per sample

4.4.2. Prewashed 1.2 µm Fisher® glass-fiber filters (or equivalent), at least 1 per sample

4.4.3. 8-inch Carolina dish lined with Kimwipes

4.4.4. Small forceps, flat tipped

4.4.5. Drying oven

4.4.6. Autoclaved Deionized (DI) water

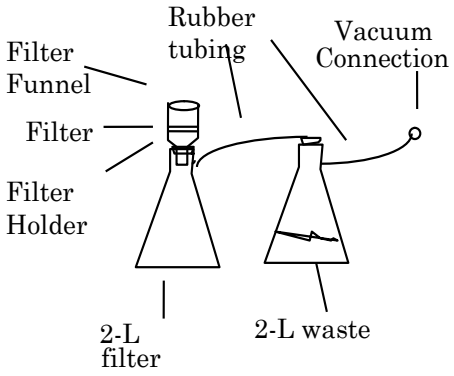
4.4.7. Filter apparatus

a. 2 L Filter flask for sample
b. 2 L Filter flask for overflow waste, or 4 L
c. Filter clamp
d. Plastic sidearm and bushing
e. 47 mm Whatman® or Millipore® fritted-glass filter base and stopper
f. Whatman® or Millipore® fritted-glass filter funnel
g. Airline hose
h. No. 9 rubber stopper with hole
i. Small forceps, flat tipped

4.4.8. *AGP Sample Log-in Record* logbook – record all sample treatment and adjustments, processing times, analyst initials and appropriate comments.

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Figure 1 – Filter Apparatus Set-up



5. PROCEDURE

5.1. All samples

5.1.1. Samples are retrieved from Receiving or the walk-in refrigerator, logged in and documented per SOP TA-08.09. Samples should be processed (filtered) within 72 hours of collection. Samples should be stored on ice or in a refrigerator with minimum exposure to light until processed. If these conditions are not met, create an NCR and qualify the sample result as needed.

5.1.2. If the sample is not in the appropriate opaque Nalgene bottle, transfer the sample to the appropriate sample container before processing.

5.1.3. Pour 25-30 mL of sample into a 30 mL cup. Measure the pH, temperature, conductivity, and salinity of the sample in the 30 mL cup using the Fisherband Accumet AB315 pH/mV pH meter and Traceable Bench Conductivity meter. Rinse probes with DI water between samples.

5.1.4. Record the measured values next to the logbook label or LIMS number for the appropriate sample in the *AGP Sample Log-in Record*.

5.1.5. If the pH is < 6.0 or > 10.0, the sample must be pH adjusted prior to inoculation per SOP TA-08.06.

5.1.6. If the sample is a complex waste or if the algal assay was collected specifically to study organic compounds (rare), do not autoclave the sample. Skip to Step 6.1 and filter the sample.

5.1.7. If the sample is an effluent sample from a facility for which an FYI3 or FYI5 event has been performed, apply the following:

a. If a freshwater sample (salinity ≤ 1.0 ppth) is salinity adjusted for a toxicity test, or if freshwater effluent discharges into marine waters, then two AGP tests must be run, freshwater and salinity adjusted.

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b. If an effluent has detectable chlorine upon receipt, measure the chlorine again after sample processing (i.e. filtering). If chlorine is still present (> 0.03 mg/L TRC), two AGP tests must be run, chlorinated and dechlorinated. Refer to TA-06.22 and TA-09.18 for chlorine measurement and dechlorination procedures.

5.1.8. If the sample salinity is > 1.0 ppth but is suspected to originate from a source other than seawater, then both freshwater and saltwater tests must be run.

5.2. Samples with salinity < 1.0 ppth

5.2.1. Loosen the lid of the sample container and place the sample container in the autoclave. Place a piece of autoclave tape on every lid.

5.2.2. Autoclave on the slow liquid setting 15 PSI @ 121 °C for 10 minutes per liter of sample in the container (minimum 10 minutes).

5.2.3. After autoclaving, remove the sample container from the autoclave and place it upright in a large bucket or ice chest. Since the cap is still loose, use care not to allow the sample container to tip and spill. If the sample will not be filtered immediately after cooling, write the date and time of autoclaving on the tape.

5.2.4. If not being filtered the same day, tighten the sample lid and store in the walk-in refrigerator. If filtering immediately after autoclaving, surround the sample container with ice and pour tap water over the ice, taking care not to completely immerse the sample container.

5.2.5. Complete the preparation section of the *AGP Sample Log-in Record*. In the "Prep" column, write "A" indicating the sample has been autoclaved.

5.2.6. Once the sample has cooled, proceed to Section 6.1 and filter the sample.

5.3. Samples with salinity ≥ 1.0 and < 7.0 ppth

5.3.1. Using a graduated cylinder, measure the suggested volume of sample for the test being performed. (While ~200 mL is typically needed, it is highly recommended to make 500 mL of salinity adjusted sample in case of spilling or necessary duplicates.) Pour the sample into an Erlenmeyer flask. Save any excess sample in the sample container.

5.3.2. Use the Mettler balance and a weigh boat to weigh out the artificial sea salts needed to increase sample salinity to 7 ± 0.2 ppth for samples with known seawater composition; and the sea salts needed to increase the sample salinity by 7 ± 0.2 ppth (original 'salinity' + 7 ppth) for samples in which the basis for the original sample salinity is unknown. Conversion note: 1 g of artificial sea salt ≈ 1 ppth/L.

5.3.2.1. To obtain the amount of sea salts needed per liter of sample, subtract the measured salinity of the sample (known seawater

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composition) from 7. To calculate the amount of sea salts needed for the actual sample:

a. First calculate grams per liter: 7 ppth - (*ppth of sample*) = *a*
b. Convert volume of sample needed into liters
c. Multiply the result (of *a*) by the volume to get grams of salt needed

5.3.3. Place the flask containing the sample on the magnetic stir plate, place the stir bar in the flask, and turn the stirrer on. Add sea salts to the flask and allow to stir until salts are dissolved.

5.3.4. Measure the salinity of the sample per TA-06.36, and if needed, add more sea salts to obtain the desired salinity. Add sea salts slowly to allow time for salts to dissolve.

5.3.5. If the desired salinity is exceeded, use the sample reserved in Step 5.3.1 to dilute the salinity-adjusted sample.

5.3.6. Once the desired salinity is reached, remove the flask from the stir plate and turn the stirrer off. Rinse the stir bar retriever with DI water and then use the retriever to remove the stir bar from the flask.

5.3.7. In the "Comments" section of the *AGP Sample Log-in Record,* record the sample's final salinity, the date, the analyst's initials, and the final amount of marine salts added to the sample.

5.3.8. Proceed to Section 6.1 and filter the sample.

5.4. Samples with salinity ≥ 7.0 ppth

5.4.1. Proceed to Section 6.1 and filter the sample.

6. SAMPLE FILTRATION

6.1. Filtering Samples

6.1.1. Place the stopper and filter base into the mouth of the sample filter flask. Seat the stopper firmly to ensure a tight seal. If needed, wet both pieces with DI water for easier insertion.

6.1.2. Connect the sample filter flask to the waste flask using the rubber tubing and turn the vacuum on at the valve in the wall. Turn the vacuum on and off during filtration using the small stopcocks in the rubber tubing assembly or the valve handle.

6.1.3. Place a clean filter on the filter base using the small forceps. For clear AGP samples start with a 0.45 µm filter. For turbid AGP samples start with a 1.2 µm filter.

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6.1.3.1. To clean filters, filter 100 mL of DI water. Use forceps to place filter in a Carolina dish lined with Kimwipes. Dry the filters for one hour at 70-100°C in the drying oven. Filters can be stored in a covered Carolina dish.

6.1.4. Slowly pour the sample into the filter funnel.

6.1.5. If a filter becomes clogged, change the filter.

6.1.5.1. If sample is still in the filter funnel (reservoir), use the stopcock or valve to turn off the vacuum and gently remove the **clamped** filter funnel and filter base from the neck of the filter flask. Use caution not to pull on the filter funnel, thus breaking the seal between it and the filter base. Pour the unfiltered sample from the filter funnel back into the original sample container.

6.1.5.2. Set the filter base back on the filter flask. Release the clamp, remove the filter funnel, remove the clogged filter, and place a new filter on the filter base. Place the filter funnel back on top of the filter base and clamp it in place. Resume filtering.

6.1.6. While the last of the sample is filtering, rinse the sample container and lid thoroughly with autoclaved DI water. Shake off the excess water.

6.1.7. Remove the filter base from the filter flask and pour the sample into the rinsed sample container. If the sample has been only pre-filtered through a 1.2 µm filter, proceed to Step 6.1.8. If the sample has been filtered through a 0.45 µm filter, skip to Step 6.1.10.

6.1.8. Disassemble the filter apparatus and thoroughly rinse the filter base with autoclaved DI.

6.1.9. Reassemble the filter apparatus with a 0.45 µm filter and filter the sample again as in Steps 6.1.4-6.1.7.

6.1.10. Record the date and time that the sample was filtered and the initials of the analyst in the *AGP Sample Log-in Record* logbook. In the “Prep” column of the logbook, write “F” indicating the sample has been filtered.

6.1.11. Make sure the sample container lid is secure. If the sample is to be set up within 1-30 days, place the container in the walk-in refrigerator. If the sample is to be set up after 30 days (rare occurrence), place the sample in the walk-in freezer.

6.1.12. Disassemble the filter apparatus and thoroughly rinse all parts with autoclaved DI water except for the vacuum tubes and waste flask.

6.1.13. Reassemble the filter apparatus and repeat the procedure for the next sample.

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6.1.13.1. Carefully rinse between sample sets and ensure sample #'s match when filtering multiple bottles at a time. If filtering both freshwater and saltwater samples, start with freshwater. The same equipment may be used for both with careful rinsing, or a separate flask and filter set may be used for saltwater samples afterward.

6.1.14. When filtering is complete, place used glassware on the dirty glassware cart in B246. Used filters should be discarded in the wastebasket.

7. SAFETY/HAZARDOUS WASTE MANAGEMENT

7.1. Refer to the Laboratory Health and Safety Plan

7.2. Lab hood or chemical ventilation system – proper laboratory protection is required when working with chemicals and unprocessed samples

7.3. Lab coat

7.4. Latex or nitrile gloves

7.5. Safety glasses

8. DATA ARCHIVAL

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

8.2. 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.

8.2.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

8.2.2. If the correction requires an explanation, write a comment and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

8.2.3. Use sequential alphabetical annotation to identify errors and corresponding comments.

8.3. Record information in appropriate logbooks.

9. QUALITY CONTROL

9.1. Glassware is washed, autoclaved, and stored in accordance with DEP SOP BG-05 to minimize the potential for contamination of the stock solution.

9.2. Aseptic techniques are used to help prevent the contamination of samples and cultures.

9.3. If a problem occurs during the sample processing or testing, the following steps are performed: 1) Lab supervisor is notified. 2) Client is notified (client makes decision

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for additional testing). 3) Environmental administrator is notified. 4) A non-conformance report (NCR) is submitted describing the problem and the corrective action.

9.3.1. To create NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to https://floridadep.gov/dear/quality-assurance/content/dep-sops for sample collection and preservation, and the Laboratory Quality Manual for corrective action and handling out-of-control data.

10. REFERENCES

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

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

10.3. FDEP Standard Operating Procedures https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

10.4. Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

10.5. DEP SOP TA-06.36, Calibration and Use of the Fisherbrand Traceable Bench Conductivity Meter for Conductivity and Salinity.

10.6. DEP SOP TA-06.43, Calibration and Use of Fisherbrand Accumet AB315 pH/mV Meter

10.7. DEP SOP TA-06.34, Calibration and Use of Mettler Toledo PB 403TS/00 Balance

10.8. DEP SOP TA-06.16, Washing filters for Algal Assay to Remove Trace Contaminants

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

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

10.11. DEP SOP TA-06.22, Measuring Total Residual Chlorine using the Hach Pocket Colorimeter Test Kit

10.12. DEP SOP TA-09.18, Dechlorination Procedure using Sodium Thiosulfate

10.13. U.S. EPA, 1974, Marine Algal Assay Procedure Bottle Test

10.14. DEP SOP BG-05, Biology Wash Party Procedures

10.15. DEP SOP BG-08, STERIS AMSCO Series 250LS Autoclave Calibration and Use

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APPENDIX OF CHANGES

Changes to SOP TA-08.01

03/31/2008 Added Scope and Application, Summary of Method and Appendix of Changes.

03/19/2009 Added References section.

01/28/2015 Formatted for 508 compliance.

Changes to SOP TA-08.04

02/05/2008 Added Scope and Application, Summary of Method and Appendix of Changes.

01/13/2008 Added Interferences and References Sections.

Changes to Combined SOPs as TA-08.01

Major Revision

10/24/2012 Combined SOP's TA-08.01 and TA-08.04 and reformatted the combined SOP.

10/01/2014 Added Safety/Hazardous Waste Section to SOP.

11/12/2020 Added data archival and quality control section.

11/12/2021 Added Table of Contents

08/06/2024 Combined TA-06.16 with TA-08.01

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