Initial Algal Assay and Limiting Nutrient Sample Handling

1. SCOPE AND APPLICATION

1.1. This procedure 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) and limiting nutrient (LN) samples prior to inoculation. Tests covered by the procedure include: AA-AGP, AA-AGP-DCL, AA-AGP-SW, AA-AGP-pH, AA-AGP-SA, and AA-LIM-NUT.

2. SUMMARY OF THE METHOD

2.1. Samples are checked into the laboratory. Conductivity, salinity, temperature, and pH measurements are taken. Chlorine measurements are performed on effluent samples. After processing, chlorine is re-checked in samples having an initial TRC value of > 0.03 mg/L. Samples are dechlorinated as needed. Salinity-adjustments and pH-adjustments are made as needed. Samples are autoclaved and/or filtered based on salinity. Samples are stored between 0 ºC and 6 ºC until analyses are initiated. With the exception of *Everglades* samples, samples can be frozen for long term (> 30 day) storage.

3. INTERFERENCES

3.1. Interferences with the filtration process can include but are not limited to the following: highly turbid samples, contaminated glassware, contaminated filters and rinse water, plugged filter holders and torn or punctured filters.

4. SAMPLE COLLECTION AND PRESERVATION

4.1. See http://www.dep.state.fl.us/water/sas/sop/sops.htm and Laboratory Quality Manual, Section 5.7.

5. EQUIPMENT AND SUPPLIES

5.1. Initial handling of all samples

5.1.1. Specimen cup, 30 mL, one per sample

5.1.2. Mettler Toledo SevenMulti® meter S/N 1225117049, calibrated and used per TA-06.25

5.1.3. Accumet AR10 pH meter S/N AR93312105, calibrated and used per TA-06.23 or

5.1.4. Fisherbrand FE150 Benchtop pH meter, calibrated and used per TA-06.37

5.1.5. Label tape - red, blue, yellow, orange, and purple

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Note: Red is used to indicate sample has been autoclaved. Blue is used to indicate sample has been filtered. Yellow indicates sample may need pH adjustment. Orange indicates sample has a salinity of ≥ 1.0 parts per thousand (ppth) and may need salinity adjustment. Purple indicates sample may need chlorine adjustment. These colors can be changed as long as it's clear to all analysts what the color code scheme means.

5.1.6. Samples to be processed

Note: Sample should be in an approved Nalgene bottle. If not, get a clean bottle to transfer the sample to before autoclaving and/or filtering and create a non-conformance report (NCR) for the sample using the Laboratory/Administrative/Non-conformance Report module in LIMS.

5.2. Initial handling of samples < 1.0 ppth salinity

5.2.1. Tap water

5.2.2. Large bucket or ice chest

5.2.3. Autoclave – use per TA-06.18 or MB-03.2; should only be used by trained operators as improper use may result in personal injury or equipment damage

5.2.4. Autoclave gloves

5.2.5. Ice

5.3. Initial handling of samples 1.0-7.0 ppth salinity

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

5.3.2. Magnetic stir plate

5.3.3. Magnetic stir bar, 2.5-cm

5.3.4. Stir-bar retriever

5.3.5. Erlenmeyer flask, 500-mL for AGP samples or 2-L for AGL/LN samples

5.3.6. Graduated cylinder, 500-mL for AGP samples or 2-L for AGP/LN samples

5.3.7. Mettler Toledo PB 403-S/FACT balance S/N 1123052001, calibrated per TA-06.31

5.3.8. Weigh boat,

5.3.9. Metal spatula

5.4. Filtering

5.4.1. Fisher Durapore® membrane filters, 0.45 µm (or equivalent), prewashed per TA-06.16, at least 2 per sample

5.4.2. Fisher® 5.5 cm glass-fiber filters (or equivalent), prewashed per TA-06.16, at least 1 per sample

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5.4.3. Deionized (DI) water

5.4.4. Filter apparatus

Note: The filter apparatus usually remains set up, and it is only necessary to attach a filter flask, filter holder and filter funnel. If not set up, use materials 5.4.4.a-d to set up the filter apparatus according to Figure 1 below.

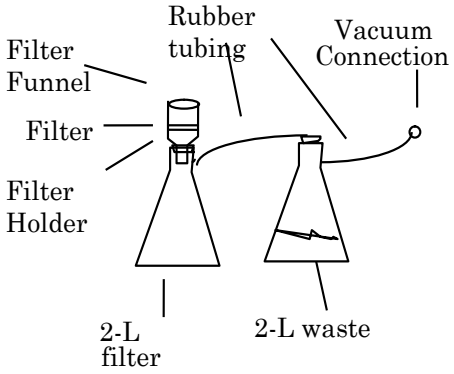
a. Filter flask for sample, 2 L
b. Filter flask for overflow waste, 2 L or 4 L
c. Whatman® or Millipore® 47 mm fritted-glass filter holder
d. No. 9 rubber stopper with hole

5.4.5. Permanent black marker

5.4.6. Black ink pen – Use only black ink pen to record log book entries. 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.

5.4.7. *AGP Sample Log-in Record* logbook – record any and all sample treatment and adjustments (i.e. pH, salinity, dechlorination), processing times, analyst initials and appropriate comments

Figure 1 – Filter Apparatus Set-up



6. PROCEDURE

6.1. All samples

6.1.1. Samples are retrieved from Receiving or the walk-in refrigerator and properly logged in and documented per SOP TA-08.09. Samples should be processed (filtered) within 72 hours of collection. Samples should be stored at 4 °C (on ice or in a refrigerator) with minimum exposure to light (e.g. in an opaque sample container) until processed.

6.1.2. If any of the conditions in Step 6.1.1 are not met, create an NCR in LIMS and qualify the sample result in LIMS accordingly when results are reported.

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6.1.3. If the sample is not in the appropriate opaque Nalgene bottle (usually pre-labeled for the appropriate test – 500 mL for AGP and 2 L for AGP/LN), transfer the sample to the appropriate sample container before autoclaving and/or filtering.

6.1.4. Pour 25-30 mL of sample into a 30-mL cup.

6.1.5. Measure the pH, temperature, conductivity and salinity of the sample **in the 30-mL cup** using the Accumet AR10 pH meter or Fisherband FE150 Benchtop pH meter and Mettler Toledo SevenMulti® meter. Rinse probes with DI water when going from one sample to another.

Caution: Do not measure parameters in the sample container. Measure all parameters in the cup.

Note: The salinity of the sample usually determines how the sample will be processed and which test alga will be used to inoculate the sample. An exception would be if the sample is known to be a sample of complex waste or organic compounds. **This situation is very rare.**

6.1.6. Record the measured values next to the log book label (generated per TA-08.09) or LIMS number for the appropriate sample in the *AGP Sample Log-in Record*.

6.1.7. If the pH is < 6.0 or > 10.0, label the sample bottle with a piece of yellow tape with the pH written on it. This sample will be pH adjusted prior to inoculation per SOP TA-08.06.

6.1.8. If the salinity is ≥ 1.0 ppth, label the sample bottle with a piece of orange tape with the salinity written on it. Salinity adjustment is described in Section 6.3.

6.1.9. If the sample is a complex waste or if the algal assay is specifically to study organic compounds (rare), do not autoclave the sample. Skip to Step 7.1 and filter the sample.

Note: "Autoclaving followed by filtration is the recommended pretreatment for nutrient limitation and heavy metal toxicity studies; however its use in studies of complex wastes and organic compounds may alter the chemical structure and bias the assay response. Presently, filtration (0.45 µm) is the only recommended pretreatment prior to the assay of organic compounds and complex wastes." (U.S. EPA-600/9-78-018)

6.1.10. 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 – one as freshwater and one as saltwater. Label sample container with orange tape.

Note: Check the facility summary or permit to determine if the receiving waters are fresh or marine. The intent is to determine potential effect of the effluent on the receiving waters, so the test type is not necessarily determined by the salinity of the effluent only.

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b. If an effluent has chlorine in it upon receipt, the chlorine must be measured again after the sample has been processed. If chlorine is still present (> 0.03 mg/L TRC), two AGP tests must be run – one chlorinated and one dechlorinated. Label the sample container with purple tape.

Note: Refer to TA-06.22 and TA-09.18 for chlorine measurement and dechlorination procedures.

6.1.11. 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, especially for sites at which previous freshwater samples have been analyzed.

6.2. Samples with salinity < 1.0 ppth

6.2.1. Loosen the lid of the sample container and place the sample container in the autoclave.

Note: At this point, the sample should be in the appropriate container, but make sure it is in the appropriate white, opaque Nalgene container before autoclaving.

6.2.2. Autoclave on the **slow liquid** setting 15 PSI @ 121 °C for 10 minutes per liter of sample in the container.

Note: Autoclave ≤ 1L for 10 minutes. For any amount over 1 L but less than 2 L, go to 20 minutes, etc.

6.2.3. 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. Place a piece of red tape on the sample container lid to indicate it has been autoclaved, and if the sample will not be filtered immediately after cooling, write the date and time of autoclaving on the tape.

CAUTION: Sample will be very hot at this point. Wear dry autoclave gloves to move the sample container. Failure to use caution can result in severe burns.

6.2.4. Surround the sample container with ice and then pour tap water over the ice, taking care not to completely immerse the sample container (i.e. flood the loose cap and contaminate the sample).

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

6.2.6. Once the sample has cooled, proceed to Section 7.1 and filter the sample.

6.3. Samples with salinity ≥ 1.0 and < 7.0 ppth

6.3.1. Using a graduated cylinder, measure the suggested volume of sample for the test being performed (~200 mL for AGP or ~1,200 mL for AGP/LN) and pour the sample into the appropriate size Erlenmeyer flask. Save any excess sample in the sample container in case it is needed for further manipulation or sample dilution.

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6.3.2. 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 seasalts 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 (examples are papermills located inland away from obvious marine water input and RO water treatment plants using ground water as the source water).

6.3.2.1. To obtain the amount of sea salts needed per liter of sample, subtract the measured salinity of the sample (known seawater composition) from 7. To calculate the amount of sea salts needed for the actual sample:

a. Divide the sample volume by 1 liter (1,000 mL)
b. Multiply the result (of *a*) by 100 to convert to percent
c. Multiply amount of sea salts needed for one liter by this percent

Example: 200 mL of sample is needed for an AGP test. A sample of 5 ppth salinity (known seawater basis) would require that 2 grams of sea salts (7-5=2) be added to one liter of sample to bring the salinity to 7 ppth. Since only 200 mL sample is needed, 20% ((200 mL/1,000 mL) x 100) of the salts required for one liter of sample need to be added to the 200 mL sample. Add 0.4 gram (2 g x 20%) of sea salts to the 200 mL sample to obtain 7 ppth sample.

Example: 200 mL of sample is needed for an AGP test. A sample of 2 ppth salinity (unknown seawater basis) would require that 7 grams of sea salts be added to the one liter of sample to increase the salinity by 7 ppth. Since only 200 mL sample is needed, 20% ((200 mL/1,000 mL) x 100) of the salts required for one liter of sample need to be added to the 200 mL sample. Add 1.4 grams (7 g x 20%) of sea salts to the 200 mL sample to increase the salinity by 7 ppth.

6.3.3. Place the flask containing the sample on the magnetic stir plate, place the stir bar in the flask, and turn the stirrer on.

6.3.4. Add sea salts to the flask and allow to stir until salts are dissolved.

6.3.5. Measure the salinity of the sample per TA-06.25, and if needed, carefully add more sea salts to obtain the desired salinity. Be patient and add sea salts carefully to allow time for salts to dissolve.

Note: The sea salt addition is deliberately underestimated in Step 6.3.2 and more will probably need to be added. The underestimation is a result of not oven drying the sea salts prior to weighing. It is better to have to add more salt than to run out of sample while trying to dilute a sample with too high a salinity.

6.3.6. If the desired salinity is exceeded, use the sample reserved in Step 6.3.1 to dilute the salinity-adjusted sample.

6.3.7. Once the desired salinity is reached, remove the flask from the stir plate and turn the stirrer off. Use the stir bar retriever to remove the stir bar from the flask.

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6.3.8. In the "Comments" section of the *AGP Sample Log-in Record* record the sample's final salinity, the date and the analyst's initials.

6.3.9. Proceed to Section 7.1 and filter the sample.

Note: "Autoclaving is not recommended for marine or estuarine samples at this time." U.S. EPA, 1974, Marine Algal Assay Procedure Bottle Test

6.4. Samples with salinity ≥ 7.0 ppth

6.4.1. Proceed to Section 7.1 and filter the sample.

Note: "Autoclaving is not recommended for marine or estuarine samples at this time." U.S. EPA, 1974, Marine Algal Assay Procedure Bottle Test

7. SAMPLE FILTRATION

7.1. Filtering Samples – refer to Equipment and Supplies Step 5.4.4 and Figure 1

7.1.1. Place the stopper and filter holder into the mouth of the sample filter flask. Seat the stopper firmly to ensure a tight seal.

Note: If the filter holder is not already seated in a stopper, wet (with DI) both pieces to make it easier to insert the stem into the stopper.

7.1.2. Connect the sample filter flask to the waste flask using the rubber tubing and turn the vacuum on at the valve in the wall. Leave the vacuum on at the wall and turn it on and off during filter using the small stopcocks in the rubber tubing assembly.

7.1.3. Place the appropriate filter on the filter holder. For clear AGP samples start with a 0.45 µm filter. For turbid AGP samples or LN samples start with a 1.2 µm filter.

Note: All samples can be pre-filtered with the larger 1.2 µm filter if desired, but often 200 mL of a clear sample will go through the 0.45 µm filter before the filter clogs. For more turbid samples or for the larger volume of sample required for an LN test, pre-filter the sample with the 1.2 µm filter to remove debris that will quickly clog the 0.45 µm filter. Otherwise, a turbid sample or larger sample volume could take hours to filter through the 0.45 µm filter without pre-filtering.

7.1.4. Slowly pour the sample into the filter funnel but do not overflow the funnel.

7.1.5. If a filter becomes clogged (either 0.45 or 1.2 µm), change the filter.

7.1.5.1. If sample is still in the filter funnel (reservoir), use the stopcock to turn off the vacuum and gently remove the **clamped** filter funnel and filter holder 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 holder. Pour the unfiltered sample from the filter funnel back into the original sample container.

7.1.5.2. Seat the filter holder back on the filter flask. Release the clamp, remove the filter funnel, remove the clogged filter, and place a new

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filter on the filter holder. Place the filter funnel back on top of the filter holder and clamp it in place. Resume filtering.

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

7.1.7. Remove the filter holder 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 7.1.8. If the sample has been filtered through a 0.45 µm filter, skip to Step 7.1.10.

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

7.1.9. Reassemble the filter apparatus with a 0.45 µm filter, and filter the sample again as in Steps 7.1.4-7.1.7.

7.1.10. Record the date and time that the sample was filtered and the initials of the analyst doing the filtering on a piece of blue tape and affix to the sample container. Record the same information in the *AGP Sample Log-in Record* logbook. In the "Prep" column of the logbook, write "F" indicating the sample has been filtered.

7.1.11. 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.

Note: The turn-around time for entering AGP results in LIMS (complete or 'C' status) is 35 days, so it will be rare that a sample is not set up within 30 days. This should be reserved for situations where holding the sample longer is unavoidable.

A sample may be stored for up to 30 days at 4 °C. If frozen, it may be stored for up to 4 months. **Exception: Everglades samples are never to be frozen.**

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

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

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

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Refer to the Laboratory Health and Safety Plan

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

8.3. Lab coat

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8.4. Latex gloves (**Caution: Individuals allergic to latex should not use latex gloves. A suitable substitute such as nitrile gloves should be used instead.**

8.5. Safety glasses

9. REFERENCES

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

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

9.3. FDEP Standard Operating Procedures http://www.dep.state.fl.us/water/sas/sop/sops.htm

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

9.5. DEP SOP TA-06.25, Calibration and Use of Mettler Toledo SevenMulti® Meter for Measuring Conductivity, Salinity and TDS

9.6. DEP SOP TA-06.23, Calibration and Use of Accumet Model AR10 pH Meter

9.7. DEP SOP TA-06.18, AMSCO Renaissance Series 3021 Autoclave Calibration and Use

9.8. DEP SOP TA-06.31, Calibration and Use of Mettler Toledo PB 403-S/FACT Balance

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

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

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

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

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

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

APPENDIX OF CHANGES

Changes to SOP TA-08.01

03/31/2008 Added Scope and Application, Summary of Method and Appendix of Changes. Under **Materials** All Samples inserted Accumet AR10 pH meter and inserted comment Calibrate per SOP TA-06.23.

04/01/2008 Deleted pH and SOP #TA-06.24 from comment of **All Samples Step 1.** Replaced 24 with 23 in coment of **Methods Step 3**. Replaced ≥ with > in comment of **Methods Step 7**.

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03/19/2009 Added References section. Changed TA-06.06 to TA-06.25 in Step 5 comment section of If sample salinity is ≥1.0 and .0 ppth.

02/26/2010 Replaced 'Vinyl gloves can be substituted for individuals allergic to latex." with 'CAUTION Individuals allergic to latex should not use latex gloves. A suitable substitute should be found and used.' in Safety Section. Updated reference to Biology Quality Manual.

02/25/2011 Updated reference to Bureau Quality Manual.

02/02/2012 Updated reference to Bureau Quality Manual. Replaced Mettler PC 440 balance with Mettler Toledo PB 403-S/FACT balance

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.

12/17/2009 Deleted 'Vinyl gloves can be substituted for individuals allergic to latex' and added 'CAUTION Individuals allergic to latex should not use latex gloves. A suitable substitute should be found and used.' Updated reference to Biology Quality Manual.

11/16/2010 Updated reference to Biology Quality Manual.

10/27/2011 Updated reference to Biology Quality Manual. Added "do not overwrite entries" to Step 1 of Documentation and Correction Action section.

09/11/2015 Updated links.

Changes to Combined SOPs as TA-08.01

09/15/2016 Updated references and links.

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.

04/17/2017 Clarified salinity adjustment process.

11/30/2017 Aligned example in Step 6.3.2.

11/29/2018 Changed font to Calibri. Added Fisherbrand FE150 Benchtop pH meter.

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