Initial Algal Assay and Limiting Nutrient Sample Handling

DEPARTMENT OF ENVIRONMENTAL PROTECTION LABORATORY
BIOLOGY SECTION
TALLAHASSEE, FLORIDA

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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) 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: highly turbid samples, contaminated glassware, contaminated filters and rinse water, plugged filter bases and torn or punctured filters.

4. SAMPLE COLLECTION AND PRESERVATION

4.1. See https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops and Laboratory Quality Manual, Section 5.7.

5. EQUIPMENT AND SUPPLIES

5.1. Initial handling of all samples

5.1.1. 30 mL Disposable plastic cup, one per sample

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

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

5.1.4. Autoclave tape

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

Note: Autoclave tape 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 scheme means.

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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 BG-08; 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. 500 mL Erlenmeyer flask, for AGP samples or 2 L for AGL/LN samples

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

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

5.3.8. Weigh boat

5.3.9. Metal spatula

5.4. Filtering

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

5.4.2. 1.2 µm Fisher® glass-fiber filters (or equivalent), prewashed per TA-06.16, at least 1 per sample

5.4.3. Autoclaved 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 base and filter funnel. If not set up, use materials 5.4.4.a-i to set up the filter apparatus according to Figure 1 below. Two filter stations with vacuum capability: Artemia station and Taxonomy lab by sinks.

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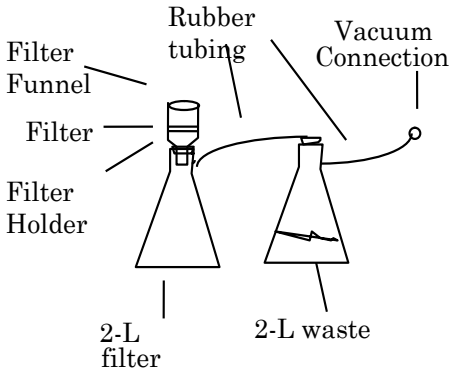
a. 2 L Filter flask for sample
b. 2 L Filter flask for overflow waste, or 4 L
c. Filter clamp
d. Plastic sidearm and bushing
e. 47 mm Whatman® or Millipore® fritted-glass filter base and stopper
f. Whatman® or Millipore® fritted-glass filter funnel
g. Airline hose
h. No. 9 rubber stopper with hole
i.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

5.4.5. Permanent black marker, for tape marking

5.4.6. Black, waterproof ink pen, for logbook 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.

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),

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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 Fisherband Accumet AB315 pH/mV pH meter and Traceable Bench Conductivity meter. Rinse probes with DI water when going from one sample to another.

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 logbook 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.

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

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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. Place a piece of fresh autoclave tape on every lid.

Note: Make sure the sample is in the 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. The tape should now have a black stripe visible to indicate it has been autoclaved successfully, 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.

6.3.1.1. Highly recommended to make 500 mL of Salinity Adjusted sample in case of spilling or duplicates needed.

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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 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 (examples are papermills located inland away from obvious marine water input and RO water treatment plants using ground water as the source water). **Conversion note: 1 g of artificial sea salt ≈ 1 ppth.**

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. 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

Example: 500 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 500 mL sample is needed, multiply 2 ppth by 0.5 L. Result: Add 1 gram of sea salt to the 500 mL sample to obtain 7 ppth sample.

Example: 500 mL of sample is needed for an AGP test. A sample of 2 ppth salinity (unknown seawater basis) would require that 5 grams of sea salts (7-2=5) be added to one liter of sample to increase the salinity to 7 ppth. Since only 500 mL sample is needed, multiply 5 ppth by 0.5 L. Result: Add 2.5 grams of sea salts to the 500 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-06.36, 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 base into the mouth of the sample filter flask. Seat the stopper firmly to ensure a tight seal.

Note: If the filter base 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 or the valve handle.

7.1.3. Place the appropriate filter on the filter base using the small forceps. 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 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.

7.1.5.2. Seat the filter base 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 base. Place the filter funnel back on top of the filter base 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 autoclaved DI water. Shake out/off the excess water.

7.1.7. 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 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 the filter base at least with autoclaved DI. Filtering all but the vacuum tubes and waste as well is optional.

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. You may also write an "F" on the tape for clarity. 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 autoclaved 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.

7.1.13.1. Note: Always 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.

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.

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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

8.4. Latex or nitrile gloves

8.5. Safety glasses

9. DATA ARCHIVAL

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

9.2. 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.

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

9.2.2. 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.

9.2.3. 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.).

9.3. Record information in appropriate logbooks.

10. QUALITY CONTROL

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

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

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

10.3.1. 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.

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11. REFERENCES

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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 comment of **Methods Step 3**. Replaced ≥ with > in comment of **Methods Step 7**.

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.

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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.

11/25/2019 Updated equipment names and links.

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

11/12/2021 Update links. Balance update. Added Table of Contents

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11/8/2022	Check links and grammar. Updated pH meter. Added mention of autoclave tape. Expanding filtering details. Changed some filter language. Updated salinity conversion instructions.

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