Preparation of Artificial Seawater (ASW)

1. SCOPE AND APPLICATION

1.1. This method is based on EPA 821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Ed. October 2002. It can be used with well water, deionized water, or a suitable surface freshwater.

2. SUMMARY OF METHOD

2.1. Artificial sea-salts are dissolved in well water. The salinity of the solution is measured and adjusted as needed. The batch information is recorded in the Laboratory Information Management System (LIMS).

2.2. Interferences:

2.2.1. Contaminants in the salts or water. Water containing substances at or near saturation can interfere with the salts dissolving in the solution.

2.2.2. Contaminated containers can cause bacterial, fungal, or algal slime growth in the solution and render it useless for bioassay testing.

3. EQUIPMENT AND SUPPLIES

3.1. Scrub brush

3.2. 1 L Wide-mouth glass, jar

3.3. 250 mL Beaker

3.4. 12 L Nalgene bucket

3.5. Sterile glass pipet for aeration

3.6. 4.76 mm ID air-line tubing

3.7. Labeling tape

3.8. Black water-proof marker

3.9. 113 L Nalgene drum/carboy with lid

3.10. Fisherbrand Accumet AB315 pH/mV Meter, calibrated per SOP TA-06.43

3.11. Fisherbrand Traceable Bench Conductivity meter, calibrated per SOP TA-06.36

3.12. Total residual chlorine (TRC) Hach Pocket Colorimeter, per SOP TA-06.22

3.13. Ammonia Hach Pocket Colorimeter, per SOP TA-06.35

3.14. Alkalinity Hach Kit, per SOP TA-06.08

3.15. Computer with access to the Laboratory Information Management System (LIMS)

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4. REAGENTS AND STANDARDS

4.1. Deionized (DI) Water

4.2. Chlorine bleach

4.3. 10% HCl solution

4.4. Sodium thiosulfate ($Na_2S_2O_3$), 0.025 N

4.5. Well water

4.6. Commercial artificial sea-salt mix

4.6.1. This lab uses Crystal Sea™ Marine Mix (Bioassay Laboratory Grade)

5. PREPARATION

5.1. Cleaning the Nalgene 100 L carboy

5.1.1. If the carboy is new: wash with soap and water, acid rinse with 10% HCl and rinse with copious amounts of well water.

5.1.2. If the carboy is being reused: Empty remaining ASW from the Nalgene carboy and clean with soap, bleach, sodium thiosulfate, and DI water (see 5.1.4. – 5.1.7.).

5.1.3. ASW can be emptied down the drain.

5.1.4. Pour 20-30 mL chlorine bleach in the bottom of the carboy and add approximately 1 L of DI water. Use the scrub brush to remove algae from the sides and bottom of the carboy. Wear a lab coat, gloves, and respirator. Turn the carboy on its side to reach the bottom. After the carboy has been cleaned, empty the bleach water down the drain.

5.1.5. Rinse the carboy several times with DI water to remove the debris and most of the bleach. Empty the DI water down the drain.

5.1.6. Add 100 mL of 0.025 N sodium thiosulfate to the carboy. The sodium thiosulfate solution should cover all interior surfaces of the carboy to neutralize any remaining chlorine bleach.

5.1.6.1. A pipette or beaker can aid in covering the entire interior surface of the carboy with sodium thiosulfate. Pour the sodium thiosulfate from the beaker while tilting the carboy.

5.1.7. Rinse the carboy with copious amounts of DI water and dispose of water down the drain.

5.2. MAKING THE ASW

5.2.1. Place approximately three 1 L jars full of Crystal Sea® marine mix bioassay grade artificial sea salt into the bottom of the carboy. Add well water to the

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100 L mark (approximately 10.2 cm from the top of the carboy; this allows the addition of well water if needed to adjust salinity).

5.2.1.1. In lieu of using the 1 L jars, weigh out approximately 2100 g of sea salts using the Mettler Toledo PB403-S/FACT balance. Add these salts to the carboy.

5.2.2. Use the designated large plastic pipe to mix the salt into the water thoroughly.

5.2.3. Place a sterile glass pipette attached to an airline into the carboy and adjust to a moderate level of aeration. Place the lid on the carboy. The aeration serves as a mixing aid.

5.2.4. Use tape to make a label: “ASW,” “batch number (preparation date)”, “expiration date (14 days from prep date)”, “LIMS ID number”, and “preparer’s initials.” Place the label on the lid. Apply a “Do Not Use” label to the carboy lid. Also place a red “parameters” label to the lid. Let water aerate for 24 hours.

5.2.4.1. ASW can be recertified in LIMS after the initial 14 days for an additional 14 days for use in *artemia* culturing absent any algal growth and/or other visible changes and as long as the *artemia* cultures remain stable.

5.2.5. Record preparation in LIMS Standard Prep Tracker. Print the LIMS serial number and place on the lid next to the label.

5.2.6. After 24 hours, measure the salinity of the water in the carboy. If necessary, adjust the salinity to the required salinity of 20 ± 0.5 parts per thousand (ppth).

5.2.6.1. Measure salinity per SOP TA-06.36.

5.2.6.1.1. If the salinity is less than 19.5 ppth, add sea salts at the rate of approximately 100 g per desired 1 ppth increase in salinity.

If sea salts are added, mix (15-30 min) before measuring salinity again.

When desired salinity is met, allow the ASW to sit for an additional 1-2 hours before use.

5.2.6.1.2. If the salinity is greater than 20.5 ppth, add well water at the rate of approximately 4 liters per desired 1 ppth decrease in salinity.

If well water is added, mix (4-5 minutes) before measuring salinity again.

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When desired salinity is met, the ASW can be used.

5.2.6.1.3. Continue aeration during salinity adjustments.

5.2.7. When the desired salinity is reached, prior to first use, measure the pH, conductivity, alkalinity, total residual chlorine, and ammonia of the ASW, and record in the appropriate parameter sections of LIMS logbook.

5.2.8. Filter the ASW into a smaller carboy using a 60 µm filter and a pump. This removes the coating that is on the salt which could negatively affect the organisms. Place a "filtered" label on the smaller carboy along with a label that has the batch number, expiration date, LIMS number.

5.2.9. Label the ASW drum with the final measured salinity. Salinity should also be checked prior to use of ASW.

6. DATA ARCHIVAL

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

6.2. Record preparation in LIMS Standard Prep Tracker.

6.3. To correct raw data entries, place a single line through the incorrect or illegible entry. Write the corrected entry near the error, initial and date the correction. Do not overwrite entries.

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

6.3.2. 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.

6.3.3. 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. Thoroughly clean and rinse the drum before making new ASW to prevent bacterial, fungal, and algal slime contamination.

7.2. When adjusting the salinity with additional sea-salt or well water, mix the solution before re-checking the salinity.

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.

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8.2. Refer toDEP Laboratory Quality Assurance Manual for sample disposal.

8.3. Refer to the FDEP Laboratory Health and Safety Manual.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. DEP Laboratory Quality Assurance Manual

9.2. DEP Health and Safety Manual

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

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

9.5. SOP TA-06.22, Measuring Total Residual Chloring Using the Hach Pocket Colorimeter and Colorimeter II Test Kits

9.6. SOP TA-06.35, Measuring Total Ammonia Using the Hach Annobia Nitrogen Pocket Colorimeter II Test Kit

9.7. SOP TA-06.08, Measuring Total Alkalinity and Hardness

9.8. EPA 821-R-02-012 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Ed. October 2002

9.9. MSDS Information Sheets

APPENDIX OF CHANGES

09/24/2008 Added Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes

02/09/2015 Formatted for 508 compliance.

03/04/2026 Changed expiration period from 30 days to 14 days with the allowance for recertification of ASW for an addition 14 days for use as *artemia* culture water only.

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