Preparation of 20% Dilute Mineral Water (DMW)

1. SCOPE AND APPLICATION

1.1. This method is based on information found in EPA 821-R-02-012 and practical laboratory experience. 20 % Dilute Mineral Water (DMW) is used to prepare culture media for *Ceriodaphnia dubia*. It is also used as control-water and dilution water for *Ceriodaphnia dubia* bioassay tests.

2. SUMMARY OF THE METHOD

2.1. 16 liters of distilled water and 4 liters of plain Perrier® water are placed into a clean 20-L carboy. 20-mL each of 2 µg/mL selenium solution and 2 µg/mL B12 are added to the carboy. The solution is aerated for approximately 24 hours. Physical-chemical parameters are measured on the solution prior to use. Batch number and expiration date are placed on the container. Preparation is recorded in *Tox Record of Preparation* logbook and the FDEP Laboratory Information Management System (LIMS).

3. EQUIPMENT AND SUPPLIES

3.1. Nalgene carboy, 20 L

3.2. Graduated cylinder, 250 mL

3.3. Pipet with cotton plug removed, 1 mL

3.4. Airline tubing, 1.52 m long, 4.76 mm ID

3.5. Parafilm ™, 10x15 cm strips, 2 each

4. REAGENTS AND STANDARDS

4.1. Distilled water

4.1.1. Purchased through a local grocery store in 3.78 L containers

4.2. Deionized water

4.3. B12 working solution

4.3.1. Prepare in advance per SOP TA-09.08

4.4. Selenium working solution

4.4.1. Prepare in advance per SOP TA-09.08

4.5. Plain Perrier ® water. 4 liters for each 20 L batch of 20% DMW

4.5.1. Five full 750-ml bottles bottles and 250 mL from a sixth bottle, or four 1-L bottles are needed.

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5. METHODS

5.1. Clean carboy with a scrub brush, detergent and water. Rinse copiously with tap water. Acid rinse with 10% HCl and thoroughly rinse with DI water. Cover inner surface with 10% bleach solution. Rinse with tap water. Cover inner surface with a 10% sodium thiosulfate solution. Triple rinse with DI water.

5.2. Add 16 L of distilled water to the carboy.

5.3. Add 4 L of Perrier® water to the carboy.

5.3.1. If using 750-mL bottles of Perrier®, add to the carboy five full bottles and 250 mL from the sixth bottle. Recap the sixth bottle and save the excess for the next batch.

5.3.2. If using 1-L bottles of Perrier®, add 4 full bottles to the carboy.

5.4. Add 20 mL of selenium working solution and 20 mL of B12 working solution to the carboy.

5.4.1. Selenium and B12 are necessary nutrients for growth and reproduction. The 20 mL of working solutions yields 2 µg/L Se and 2 µg/L of B12.

5.5. Connect the airline tubing to the pipet and to the compressed-air outlet located to the right of the fume hood in the Culture room.

5.6. Place the pipet into the carboy so that the tip will be near the bottom of the carboy.

5.7. Cover the mouth of the carboy with the strips of Parafilm™ and aerate vigorously for 24 hours. After 24 hours, remove the pipet from the carboy and close the air outlet. Seal the mouth of the carboy with Parafilm™.

5.7.1. Parafilm™ removes carbonation and stabilizes the medium.

5.8. Measure the pH, hardness, and alkalinity of an aliquot of the aerated 20% DMW. Record the results in the *Tox Record of Preparation* logbook.

5.8.1. Value ranges normally obtained for moderately hard water are:

5.8.1.1. pH: 7.8-8.3

5.8.1.2. Hardness: 80-100

5.8.1.3. Alkalinity: 60-70

5.8.2. Adjust as necessary by either diluting with distilled water or adding more Perrier® water. Add additional selenium (1 mL working solution per liter of additional dilution) as needed.

5.8.2.1. Adding distilled water decreases alkalinity and hardness; adding Perrier® water increases alkalinity and hardness. After additional aeration, the pH can be adjusted with hydrochloric acid or sodium hydroxide. Thus far, adjustments have not been necessary.

5.9. Using the labeling tape and the lab marker, label the carboy "20% DMW", "Batch #", "Date prepared", "Se added", “B12 added”, and the preparer’s initials. Add an

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expiration date of 14 days from the preparation date to the label. Store the 20% DMW on the counter top to the immediate right of the fume hood.

5.9.1. Discard leftover 20% DMW 14 days after preparation. The 20% DMW can be safely poured down the sink.

5.9.2. NOTE: If necessary, 20% DMW can be used after the 14-day expiration date if no visual evidence of contamination exists (fungal/bacterial growth), and if the 20% DMW has been stored in a sealed container.

5.10. Log the Prepared 20% DMW into the FDEP LIMS database using the Standard Preparation Tracker. Print a label with a legible LIMS Serial Number and attach the label to the carboy near the hand-written label from step 5.9

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 and 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.

6.5. Preparation is recorded in FDEP Laboratory Information Management System (LIMS)

6.6. Record batch number in the *Tox Record of Preparation* logbook and on bioassay bench sheets.

7. QUALITY CONTROL

7.1. Refer to Laboratory Quality Manual for corrective action and handling out-of-control data.

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 the 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.

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9. REFERENCES

9.1. EPA-821-R-02-012 Method for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Available here.

9.2. Environment Canada Report EPS 1/RM/21: Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*. Available here.

9.3. Laboratory Quality Manual. Available here.

9.4. Laboratory Health and Safety Manual and Hazardous Waste Management. Available here.

9.5. DEP SOP LB-002. Non-Conformance Reporting System. Available here.

9.6. DEP SOP TA-09.08. Preparation of Selenium Solution for use in 20% DMW. Available here.

APPENDIX OF CHANGES:

03/03/2008	Added *Scope and Application*, *Summary of the Method*, and *Appendix of Significant Changes.* Added reference to SOP LB-002 to Documentation and Corrective Action *Step 2* comment.
03/18/2009	Added *References* Section Added extended use note to Methods STEP 8 Comment. Deleted specifically from *Scope and Application* section. Added "Plain" to item 7 in Materials list.
12/13/2013	Reformatted SOP.
02/09/2015	Formatted for 508 compliance.
11/04/2015	Updated links.
11/30/2016	Reformatted to expand sections.
10/12/2017	Added B12 addition and new reference.
11/21/2017	Updated hyperlinks for all references. Added step 5.9 to the "Methods" section to include creating a LIMS label and logging the media into LIMS.

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