Receiving and Maintaining Purchased Test Organisms

1. SCOPE AND APPLICATION

1.1. This procedure is used to ensure that required bioassay test organisms that are not cultured in-house are in the laboratory and ready for use in bioassay testing. Tests covered by this procedure are BSY-AS-FFS, BSY-AS-MMY, BSY-AS-MFS, BSY-CS-FFS, BSY-CS-MMY, BSY-CS-MFS, BSY-AD-FFS, BSY-AD-MMY, BSY-AD-MFS, BSY-CD-FFS, BSY-CD-MMY, and BSY-CD-MFS.

2. SUMMARY OF THE METHOD

The supplier is contacted and organisms (*Pimephales promelas*, *Cyprinella leedsi*, *Americamysis bahia* and *Menidia beryllina*) are ordered as needed in advance of scheduled bioassay tests. *Ceriodaphnia dubia* organisms are ordered once a year or when in-house culture health is poor for new culture tray preparation. Organisms are received in the laboratory, removed from the shipping packages and placed into appropriately labelled Carolina dishes. Parameters are measured on the "shipping" water and recorded in the *Organism Log-in Bench Sheets* logbook and LIMS. Holding water parameters are adjusted as needed to meet the bioassay test requirements. Organisms are fed and monitored (mortality and condition) prior to use in tests.

2.1. DEFINITIONS

2.1.1. Good condition: less than 5% mortality

2.1.2. Fair condition: between 5% and 10% mortality

2.1.3. Poor condition: >10% mortality

2.1.4. Conditionally Usable: mortality <10% and abnormal behavior returns to normal behavior after minimum of two hours acclimation in culture lab. Mortality >10% with no other observable issues.

2.1.5. Unusable: behavior that does not return to normal after acclimation period; organisms that appear to be physically damaged are unusable regardless of behavior.

3. EQUIPMENT AND SUPPLIES

3.1. Permanent marker (black, fine point)

3.2. Labeling tape

3.3. 200-ml specimen jars (1 per organism culture)

3.4. Large plastic Carolina dish (one per organism culture received)

3.5. Aeration device (air tubing and air stone or pipette) – 1 each per Carolina dish

3.6. *Artemia* nauplii, < 24 hours old (SOP TA-01.03 and TA-01.05)

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3.7. Wash bottle filled with deionized (DI) water

3.8. Scissors

3.9. *Organism Log-in Bench Sheets* logbook

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

3.11. Meters to measure physical-chemical parameters:

3.11.1. pH (Calibrate per SOP TA-06.43)

3.11.2. Dissolved Oxygen (DO) (Calibrate per SOP TA-06.44)

3.11.3. Digital Thermometer (Calibrate per SOP TA-06.13)

3.11.4. Salinity/Conductivity (Calibrate per SOP TA-06.36)

3.11.4.1. Salinity only for marine species

3.12. Hach kits to measure physical-chemical parameters:

3.12.1. Alkalinity and Hardness (Measure per SOP TA-06.08)

3.12.2. Ammonia (Measure per SOP TA-06.35)

3.13. Light table

3.14. 2.5 mL Plastic transfer pipettes

3.15. 2 L Beaker, two of each (for new *C. dubia* culture)

3.16. Large Glass Carolina dish (for new *C. dubia* culture)

3.17. 30-mL disposable plastic cups, 30 each (for new *C. dubia* culture)

3.18. 30-place Plexiglas trays (for new *C. dubia* culture)

3.19. 2.5 mL plastic transfer pipettes (for new *C. dubia* culture)

4. REAGENTS AND CHEMICALS

4.1. Synthetic Moderately Hard Water (MHW) (for *P. promelas* and *C. leedsi*) – prepared per SOP TA-03.15

4.2. Artificial Seawater (ASW) (for *A. bahia* and *M. beryllina*) – prepared per SOP TA-03.02

4.3. Synthetic Moderately Hard Water (MHWCD) (for *C. dubia*) – prepared per SOP TA-03.15

4.4. YCT (Yeast, Cereal Leaves, Trout Chow) (food for C. dubia) - prepared per SOP TA-01.01

4.5. Raphidocelissubcapitata (R. sub formerly Selenastrum capricornutum - algae for C. dubia) - prepared per SOP TA-01.04

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5. PREPARATION FOR TESTS

5.1. Use label tape and marker to label Carolina dishes (1 per received culture) with species name, test type (acute or chronic) and DEP batch number (the date received). (Label ex. "*P. promelas* acute 02.01.17").

Note: The DEP batch number is the date of receipt.

Note: All measurements and observations are to be recorded in the *Organism Log-in Bench Sheets* logbook. Organism age and supplier batch information will be on the paperwork supplied with the shipment. Ensure that the organisms are the correct age for the scheduled tests.

5.1.1. Acceptable organism age for scheduled tests:

Test	Acute	Chronic
C. dubia	<24 hours old	<24 hours old
P. promelas	1-14 days old	<48 hours old
C. leedsi	1-14 days old	<48 hours old
A. bahia	1-5 days old	7 days old
M. beryllina	9-14 days old	7-11 days old

5.2. Use label tape and marker to label the specimen jars as in 5.1.

5.3. Place a bag of organisms in the appropriately labeled Carolina dish.

5.4. Use the scissors to cut the top from each bag, leaving 3-4 inches of bag above the water level.

5.5. Pour approximately 150 mL of water from each bag into the corresponding specimen jar. Avoid pouring any organisms out during this process.

5.6. Measure the temperature, pH, conductivity, salinity, (marine species only), and dissolved oxygen of the water in the specimen jars and record in the *Organism Log-in Bench Sheets* logbook. Set the jars aside for later measurements of alkalinity, hardness (freshwater species only), and ammonia. All parameters are recorded in LIMS in addition to the *Organism Log-in Bench Sheets* logbook. Ammonia is recorded in LIMS and the *Stand-up Parameters Bench Sheet*.

5.7. Return to the Carolina dish, carefully lift the bag (containing the organisms) from one side and gently transfer the organisms and water into the Carolina dish. Use DI water to rinse any organisms remaining in the bag into the dish.

5.8. Take the dishes containing the organisms to the light table. Observe the organisms, noting the general condition. Count and remove any dead organisms (immediately before loading tests, check organisms again and remove any additional dead organisms). Organisms received that are to be used the following day are to be checked and dead organisms removed upon receipt. Repeat again immediately prior to being used in tests. Record observations (number dead and date/time checked) in the *Organism Log-in Bench Sheets* logbook.

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5.9. Place the dishes containing the organisms on Water Bath shelf B-4. Feed the organisms from 1 to 2 mL of *Artemia* nauplii (adjust quantity as needed). Organisms should be ready to use approximately 2 hours after feeding. Organisms should not be used < 2 hours after initial feeding without supervisor approval.

5.10. Place an aeration device (pipette) into each dish and gently aerate.

5.11. Scan the supplier's receipt page and save the file to X:\Labs\Lab5\Biology\FYI_Materials_&_Reports\FYI3_Reports\Organism_Receipts

Note: The file name should be supplier name and date of receipt. Example: "MBL 2.2.21" or "Aquatic Indicators 7.20.21"

6. PREPARATION FOR NEW *C. DUBIA* CULTURES

6.1. Use label tape and marker to label a 2 L beaker with *C. dubia* (name of supplier) and DEP batch number (the date received).

Note: The DEP batch number is the date of receipt (label ex. "*C. dubia* MBL 02.01.24").

Note: All measurements and observations are to be recorded in the *Organism Log-in Bench Sheets* logbook. Organism age and supplier batch information will be on the paperwork supplied with the shipment. Ensure that the organisms are the correct age for the scheduled tests.

6.1.1. To start a new culture, about 100 *C. dubia* neonates around 2 days of age when received are required.

6.1.2. Newly established *C. dubia* cultures must be observed for 3 weeks after tray setup before they can be used for testing purposes.

6.2. Use label tape and marker to label the specimen jar as in 6.1.

6.3. Hold the bag of organisms over the beaker and specimen jar. You may place the specimen jar and/or beaker in a Carolina dish if there are concerns of spilling.

6.4. Use the scissors to cut the top from each bag, leaving 3-4 inches of bag above the water level.

6.5. Pour approximately 150 mL of water from each bag into the specimen jar for parameters. Try to avoid pouring out organisms during this process. Inevitably, some *C. dubia* will be poured into the jar. Do not pipette *C. dubia* back into the bag.

6.6. Measure the temperature, pH, conductivity, and dissolved oxygen of the water in the specimen jars and record in the *Organism Log-in Bench Sheets* logbook. Set the jar aside for later measurements of alkalinity, hardness, and ammonia. All parameters are recorded in LIMS in addition to the *Organism Log-in Bench Sheets* logbook. Ammonia is recorded in LIMS and the *Stand-up Parameters Bench Sheet*.

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6.7. Carefully lift the bag (containing the organisms) from one side and gently transfer the organisms and water into the beaker. Use DI water to rinse any organisms remaining in the bag into the beaker.

6.8. Take the beaker containing the organisms to the light table. Observe the organisms, noting the general condition. If the majority of *C. dubia* are floating and moving, mark the condition as "good". Dead organisms are not recorded. Record observations (date/time checked) in the *Organism Log-in Bench Sheets* logbook.

6.9. Place the beaker on the Water Bath shelf B-4 until ready to transfer starting at step 6.11.

6.9.1. Transfers may occur in the afternoon but must occur the same day of organism arrival.

6.10. Scan the supplier's receipt page and save the file to X:\Labs\Lab5\Biology\FYI_Materials_&_Reports\FYI3_Reports\Organism_Receipts

Note: The file name should be supplier name and date of receipt. (Example: "MBL 2.2.21" or "Aquatic Indicators 7.20.21")

6.11. Label a 2 L beaker *C. dubia Mass Culture D*. Write the current date on the label and the current MHWCD batch #. Fill with at least 1 L of MHWCD. Feed 2 mL of a mixture of 50% YCT and 50% Raphidocelis subcapitata per SOP TA-02.00.

6.12. Prepare *C. dubia* culture Tray D as per SOP TA-04.01. Soaked or triple rinsed disposable plastic cups should be filled with 20 mL of MHWCD. Feed 0.2 mL of a mixture of 50% YCT and 50% Raphidocelis subcapitata per SOP TA-02.00. Feed 0.4mL of mixture into the reserve cup.

6.13. Bring the beaker of received organisms over from the water bath to the light tables. Grab a clean glass Carolina dish and empty some of the water from the beaker into this dish.

6.13.1. Culture tray: Using a clean plastic pipette, transfer a neonate from the glass dish into each cup as per SOP TA-04.01. Place 5-10 neonates in the reserve cup.

6.13.2. Beaker: Using a clean plastic pipette, transfer the remaining *C. dubia* from the glass dish into the mass culture beaker. All *C. dubia* from the original receiving beaker should be transferred over to the clean beaker.

6.14. Place the newly prepped mass culture beaker over on the Water Bath shelf B-4 area and tray D in Incubator #4 in room B246.

6.14.1. Any time after the 3-week observation, the weakest of the four *C. dubia* culture trays may be removed. Tray D will become whichever tray is removed.

6.14.2. After 3 weeks, neonates from culture D may be used as reserve replacements for weaker trays.

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7. DATA ARCHIVAL

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

7.2. Record information in the *Organism Log-in Bench Sheets* logbook.

7.3. 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.

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

7.5. 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.

7.6. 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.).

7.7. FDEP Laboratory Information Management System (LIMS).

8. QUALITY CONTROL

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

8.2. The method recommends tests and test cultures be maintained at 25±1°C (A. bahia tests are recommended to be maintained at 26±1°C). The method requires test temperatures not deviate more than 3° during the test period (max-min).

8.2.1. Because of the imprecise nature and thermal cycling of laboratory incubators, the Tox lab strives to maintain tests and cultures at 25±1°C but follow the 3° requirement as a test acceptability criterion. The incubators are set to alert if the temperature exceeds the range by a degree on either side for an hour. Temperature fluctuations below this threshold are minor enough to not impact the test/culture water temperatures significantly.

8.2.2. To ensure accuracy of test and culture temperatures within the incubators, the Tox lab has set correction factors to the continuous monitoring temperature probes within the incubators to ensure the temperature they are recording is as close to the actual test/culture water temperature as possible. The correction factors were established by taking an average of the test/culture temperatures on the shelf the monitor is on with an NIST-traceable thermometer and correcting against the temperature the continuous monitoring probe was recording.

8.2.3. In the case that a test exceeds the three-degree test acceptability criterion, a comment must be added to the data as follows:

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8.2.3.1. Test temperature >3°, no toxicity seen: “Temperature of test solutions deviated from method specifications.”

8.2.3.2. Incubator temperature >3°, no toxicity seen: “Continuous temperature monitoring in surrogate test vessels deviated from method specifications.”

8.2.3.3. Test temperature >3°, with toxicity seen: [“J” qualify] “Estimated value. Deviation of test solution temperatures from method specifications may be contributing to toxicity.”

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1. Refer to the FDEP Laboratory Health and Safety Plan.

9.2. Read MSDS information for reagent handling and disposal.

9.3. Use proper safety equipment including the lab hood or chemical ventilation system, a lab coat, safety glasses, a respirator and latex gloves (or a suitable substitute if allergic to latex) when working with chemicals and unprocessed samples.

10. REFERENCES

10.1. DEP Health and Safety Manual

10.2. DEP Laboratory Quality Assurance Manual

10.3. DEP SOP TA-01.01, *YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) Preparation*

10.4. DEP SOP TA-01.03, *Artemia Nauplii Culture*

10.5. DEP SOP TA-01.04, *Raphidocelis subcapitata* Culture for Ceriodaphnia dubia Food (*Formerly Selenastrum capricornutum)*

10.6. DEP SOP TA-01.05, *Decapsulation of Artemia cysts*

10.7. DEP SOP TA-02.00, *Feeding Test-Organism Cultures*

10.8. DEP SOP TA-03.02, *Preparation of Artificial Seawater (ASW)*

10.9. DEP SOP TA-03.15, *Preparation of Synthetic Moderately Hard Freshwater*

10.10. DEP SOP TA-04.01, *Maintenance of Ceriodaphnia dubia Mass and Chronic-Bioassay Cultures*

10.11. DEP SOP TA-06.08, *Measuring Total Alkalinity and Hardness*

10.12. DEP SOP TA-06.13, *Thermometer Accuracy Check and Use*

10.13. DEP SOP TA-06.35, *Measuring Total Ammonia using HACH Ammonia Nitrogen Pocket Colorimeter II Test Kit*

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

10.15. DEP SOP TA-06.43, *Calibration and Use of Fisherbrand accumet AB315 pH/mV Meter*

10.16. DEP SOP TA-06.44, *YSI MultiLab 4010-1 Meter Calibration and YSI IDS ProOBOD DO Probe Maintenance*

10.17. MSDS Information Sheets

APPENDIX OF CHANGES:

07/25/2008 Added Scope and Application, Summary of the Method, References and Appendix of Changes

02/06/2015 Formatted for 508 compliance.

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