Decapsulation of *Artemia* cysts

1. SCOPE AND APPLICATION

    1.1. This procedure serves to enhance hatch rate, reduce effort required to separate hatched *Artemia* from cysts, and disinfect the *Artemia*.

2. SUMMARY OF THE METHOD

    2.1. *Artemia* cysts are placed in chlorine bleach, stirred for several minutes, placed on a screen, rinsed to remove chlorine, and then set up per TA-01.03.

3. EQUIPMENT AND SUPPLIES

    3.1. *Artemia* cysts

    3.2. Air-line and pipette(s)

    3.3. 30 mL disposable plastic cup

    3.4. *Artemia Culture and Feeding* logbook

    3.5. 300 mL Beaker

    3.6. Magnetic stirrer

    3.7. Magnetic stir bar (~1 cm)

    3.8. *Artemia* screen (100-120µm Nitex® screen)

    3.9. 1 2 L separatory funnel (filled with 15-20 ppth salinity ASW to within 6 cm of the top)

    3.10. Wash bottle containing bleach (unscented, regular strength)

    3.11. Wash bottle containing ASW

4. REAGEANTS AND CHEMICALS

    4.1. Artificial seawater (ASW), prepared per SOP TA-03.02

    4.2. Deionized (DI) water

    4.3. Bleach (unscented, regular)

5. PREPARATION

    5.1. Pour ~ 200 mL of ASW into the 300 mL beaker.

    5.2. Place 2 level teaspoons of *Artemia* cysts into the beaker.

    5.3. Aerate moderately for 1 to 2 hours.

    5.4. Remove the aeration device from the beaker. Use deionized (DI) water to rinse the cysts from the beaker onto the screen. Rinse the beaker with DI water.

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5.5. Use the wash bottle (containing bleach) to rinse the cysts back into the beaker. Place the stir bar in the beaker and place the beaker on the magnetic stirrer. Stir at moderate speed (slight vortex).

Note: ~ 50 -100 mL of bleach are used in this process. DO NOT PROCESS LONGER THAN 3 minutes after bleach contacts the cysts.

5.6. Constantly watch the beaker for the formation of a layer of foam.

Note: Formation of foam takes ~ 30 seconds to 2 minutes. Brownish-white bubbles should appear, then spread throughout the solution, then form a layer on the surface.

5.7. After the foam layer forms, watch for a color change in the solution from brown to grayish orange. When the intensity of the grayish orange color appears stable (2-3 minutes), take the beaker and the Artemia screen to the sink (B246). Pour the solution through the screen. Use the DI water (hose) to thoroughly rinse the decapsulated *Artemia* that are on the screen. A bleach odor should not remain.

Note: If in doubt about a bleach odor coming from the *Artemia*, the *Artemia* can be returned to the beaker (with about 100 mL of DI water), treated with 2-4 mL of 0.025N sodium thiosulfate, and rescreened and thoroughly rinsed as above after about 30 seconds.

5.8. Use the DI water hose to rinse the *Artemia* from the screen into the plastic cup. Use the wash bottle containing ASW to rinse the *Artemia* into the separatory funnel.

5.9. Place the funnel in the holder. Provide moderate aeration. Record the "date", "*Artemia* lot #", "*Artemia* batch #" and additional information as required in the *Artemia Culture and Feeding* logbook.

5.10. 18-24 hours after Step 5.9, process the *Artemia* following Step 6 - Step 12 of SOP TA-01.03.

6. DATA ARCHIVAL

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

6.2. 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.

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

6.2.2. 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.

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6.2.3. 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.).

6.3. Record information in appropriate logbooks.

7. QUALITY CONTROL

7.1. Artemia are cultured as needed to feed test organisms. Cysts are set up approximately 24 hours before Artemia are needed for food. In-house tests suggest that hatching doesn't begin or is insignificant prior to twelve hours after setup. Thus, setup time from 0600 – 1800 the day before use should provide nauplii < 24 hours old for use in feeding test organisms.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Refer to the FDEP Laboratory Health and Safety Plan

8.2. Read MSDS information for reagent handling and disposal.

8.3. 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.

9. REFERENCES

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

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

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

APPENDIX OF CHANGES:

07/25/2008	Added Scope and Application, Summary of the Method, References and Appendix of Changes
03/18/2013	Reformatted the SOP.
03/09/2015	Formatted for 508 compliance.
02/03/2016	Updated link and moved it to the reference section. Re-organized sections per SOP LB-001
11/29/2018	Changed font to Calibri
11/10/2020	Updated data archival and quality control section. Updated links.

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