Pseudokirchneriella subcapitata[*] Culture for *Ceriodaphnia dubia* Food ([*]Formerly *Selenastrum capricornutum*)

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 Edition. It can also be used for *Daphnia pulex* and *Daphnia magna* food.

2. SUMMARY OF METHOD

    2.1. Nutrients are added to sterilized-deionized water to prepare media in a flask. Algae is added to the media and allowed to grow for 7-8 days. The flask is moved to walk-in refrigerator for algae to settle. Supernatant is removed. Concentrated cells are counted. Cell concentration is adjusted by adding sterilized deionized water or further settling. Food is stored and used as needed.

    2.2. Interferences

        2.2.1. Unsterile glassware, media and reagents as well as airborne algae from other cultures create the potential for contamination of the *Pseudokirchneriella subcapitata* culture.

3. EQUIPMENT AND SUPPLIES

    3.1. Coulter® Counter Model Multisizer 4e (SN BA23044)

    3.2. Refrigerator, temperature range >0-≤6 ºC (temperature monitored per SOP TA-06.01)

    3.3. Erlenmeyer flask (quantity and volume can be adjusted to meet laboratory needs)

    3.4. 7-10-day old *Pseudokirchneriella subcapitata* stock culture, per SOP TA-04.08

    3.5. 2 mL Volumetric pipet for aeration

    3.6. 2, 5, or 10 mL sterile volumetric pipets for adding solutions

    3.7. Airline tubing, ~1-meter length

    3.8. Parafilm®

    3.9. “P. sub for C. dubia food Tracking log” excel sheet

    3.10. *Pseudokirchneriella subcapitata Food for* C. dubia *Log*

    3.11. Computer

4. REAGENTS AND STANDARDS

    4.1. Autoclaved deionized (DI) Water

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4.2. PAAP stock solutions (listed in SOP TA-03.03)

4.3. Alga-Gro®

4.4. 2 µg/mL working solution for sodium selenate, prepared per SOP TA-09.08

5. CULTURE PREPARATION

5.1. Obtain a clean, autoclaved Erlenmeyer flask. Label the flask "*Pseudokirchneriella subcapitata* for *C. dubia* food" along with the batch number, expiration date, and the analyst's initials.

5.1.1. The batch number is the date that the ingredients are mixed and poured into the flask. Format is mm/dd/yy.

5.1.2. Expiration date is 3 months after the preparation date.

5.2. Fill the flask halfway with autoclaved DI water. Add 1 mL per L of each of the eight PAAP stock solutions to the flask using sterile pipets.

5.2.1. Setting up a 4 L flask is the common practice.

5.3. Add 1 mL of sodium selenate working solution per liter of solution in the flask.

5.4. Add 5 mL of Alga-Gro® per liter of solution in the flask.

5.5. Inoculate the flask with 100 mL (adjust as needed) of concentrated *P. subcapitata* cells from the algal stock culture.

5.5.1. Refer to SOP TA-04.08. One flask per liter of ~100 mL of 7-10-day old *P. subcapitata* can be substituted for the concentrated *P. subcapitata*.

5.6. Fill the flask to the neck with autoclaved DI water.

5.7. Place the flask to the right of shaker rack C. This area has continuous lighting (cool-white, fluorescent bulbs) with an intensity of ~200-300 foot-candles for algae tests. (Although light intensity up to 400 foot-candles is appropriate.) Any culture tray with continuous lighting of similar intensity (within 10%) can be used.

5.7.1. Prior to placing the flask, check the light reading and record in "P. sub for C. dubia food Tracking log" excel sheet. This document is located ToxCommon within the folder "Dilution Water and Facility Sample Parameters for LIMS." If light readings drop below 100, or recent algae growth has been <200 mL, replace lights.

5.8. Connect the 2 mL pipet to the airline tubing. Place the pipet in the flask. Cover the flask with Parafilm®. Adjust the airflow to provide moderate aeration.

5.8.1. Fill in date and age of algae used in excel sheet.

5.9. After 7 (±1) days (does not include set-up day), remove the aeration pipet from the flask. Seal the flask with Parafilm® and place the flask in the walk-in refrigerator. A

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visual inspection of the flask should show a significant increase in turbidity (should be green) from the initial inoculation.

5.9.1. Flask can be left for longer than seven days prior to placing in the refrigerator, if visual observation suggests continued growth (greening) in the flask.

5.9.2. Record in excel sheet the aeration time.

5.10. When the algae have settled, and a distinct clear layer has formed above a distinct green layer (usually within 2-3 days), siphon or pour off the clear supernatant.

5.10.1. Setup an additional 2 L flask labeled "P. sub SUPERNATE" with a matching batch number to the setup algae, but an "s" at the end. Ex: ##.##.##s. First pour off, ~500 mL of supernate can go down the drain. The rest of the supernate is poured into the SUPERNATE flask hence forth.

5.10.2. Pour offs should be performed at a moderate speed and stopped right before the dark green patches of algae enter the flask. It usually takes 2 or 3 total pour offs to reach counting levels. The algae will appear dark green and the main batch will likely be below 500 mL from a 4 L flask.

5.11. Count culture density using the Coulter® Counter. Refer to SOP TA-06.41 for instructions on use of the counter. A density of 3.9 - 4.6 x 10^7 cells/mL is required to provide a sufficient concentration of cells in the *C. dubia* vials (containing 20 mL of media).

5.11.1. P. sub counts are performed with 0.1 mL of the sample and 9.9 mL of Isoton. Set to "P.sub counts2022" SOP before starting. Take the average "Number" from the run and multiply by 200 to get the density.

5.11.2. If the cell density is less than required, concentrate by allowing additional settling.

5.11.3. If the cell density is greater than required, dilute as needed with autoclaved DI water.

5.12. Store between 0º and 6 ºC in the walk-in refrigerator. The batch can be stored for up to six months in the refrigerator; however, as a food source, it is preferable to use a batch within 90 days. Batches of algae less than 90 days old can be combined, especially if there is only a little of the "old" and a lot of the "new."

5.13. Record batch preparation and count information in the *Pseudokirchneriella subcapitata Food for* C. dubia *Log.* Record in "P. sub for C. dubia food Tracking log" excel sheet the final count, ~mL Yield and any significant comments.

6. DATA ARCHIVAL

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

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6.2. Record preparation in the *Pseudokirchneriella subcapitata Food for* C. dubia *Log* and "P. sub for C. dubia food Tracking log" excel sheet.

6.3. 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.

6.3.1. The corrected entry is written as near as possible to the error or 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.

6.4. 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. Sterile, autoclaved glassware and sterile media and reagents are used to prevent contamination of the culture.

7.2. The culture is grown under ~200-400 foot-candles ±10% of continuous, cool-white lighting.

7.3. Culture can but should not be used for food beyond 90 days from the date of preparation.

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.

9. REFERENCES

9.1. EPA-821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition*, Test Method 2002.0.

9.2. DEP SOP TA-03.03, Preparation of Preliminary Algal Assay Procedure (PAPP) Medium for Freshwater Algae Cultures.

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9.3. DEP SOP TA-04.08, Culture of Algal-Assay Inoculation Stocks.

9.4. DEP SOP TA-06.41, Calibration and Use of the Coulter® Counter Model Multisizer 4e.

9.5. DEP SOP TA-06.19, Temperature Monitoring of Walk-in Refrigerator, Freezer, and Toxicity Laboratory Incubators.

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

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

9.8. Laboratory Health and Safety Manual Health and Safety Manual

9.9. MSDS Information Sheets.

APPENDIX OF SIGNIFICANT CHANGES

12/12/2007	Added Scope an Application; added Summary of the Method.
06/24/2013	Reformatted SOP.
02/09/2015	Formatted for 508 compliance.
07/11/2016	Updated links.
09/13/2017	Added acceptable algae age range; corrected *Tox Record of Preparation* to Pseudokirchneriella subcapitata *Food for* C. dubia *Log*
08/22/2018	Updated links and changed font to Calibri.
07/02/2019	Updated references.
08/26/2020	Updated order and added sterile pipets to equipment list.
07/02/2021	Edited for clarity and universal language in data archival section. Updated Coulter Counter SOP.
07/21/2023	Added logbook, excel sheet and computer to materials. Added excel sheet instructions. Expanded pour off instructions. Expanded multisizer directions.

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