Raphidocelis 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 autoclaved 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 autoclaved 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 *Raphidocelis subcapitata* culture.

3. EQUIPMENT AND SUPPLIES

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

    3.2. Refrigerator, temperature range >0 to ≤6 ^{o}C

    3.3. 4L Erlenmeyer flask (quantity and volume can be adjusted to meet laboratory needs)

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

    3.5. 2 mL sterile glass pipette for aeration

    3.6. Sterile glass volumetric pipettes for adding solutions (usually 5 and 10 mL)

    3.7. Airline tubing

    3.8. Parafilm®

    3.9. *Raphidocelis subcapitata Food for C. dubia* Logbook (formerly *Pseudokirchneriella subcapitata Food for C. dubia Logbook*)

    3.10. Computer

4. REAGENTS AND STANDARDS

    4.1. Autoclaved deionized (DI) Water

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

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4.3. Alga-Gro®

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

5. CULTURE PREPARATION

5.1. Obtain a clean, autoclaved Erlenmeyer flask. Label the flask "*Raphidocelis subcapitata* for *C. dubia* food" along with the batch date, expiration date (3 months from batch date), LIMS ID, and the analyst's initials.

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

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

5.3. Add 1 mL per L of sodium selenate working solution to the flask.

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

5.5. Inoculate the flask with 100 mL (for 4L volume, adjust as needed for other volumes) of concentrated *R. subR. subcapitata* cells from the algal stock culture.

5.5.1. Refer to SOP TA-04.08. One flask per 4 liters of ~100 mL of 7-10-day old *R. subR. subcapitata* can be substituted for the concentrated *R. subR. subcapitata*.

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

5.7. Place the flask on the box between Incubator #9 and the shaker rack. This area has continuous lighting (cool-white, fluorescent or LED bulbs) with an intensity of ~200-300 foot-candles for algae tests. (Although light intensity up to 400 foot-candles is appropriate.) Any area with continuous lighting of similar intensity (within 10%) and access to aeration can be used.

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

5.9. Enter the new *R. sub* batch into LIMS Standard Preparation Tracker. The *R. sub* concentrate and the Autoclaved DI water do not have LIMS numbers, they are noted in the "Additional Information" area.

5.10. After 7 (±1) days (does not include set-up day), remove the aeration pipette from the flask. Seal the flask with Parafilm® and place the flask in the walk-in refrigerator. A visual inspection of the flask should show a significant increase in turbidity (should be green) from the initial inoculation.

5.10.1. 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, or to increase low growth yields.

5.11. When the algae has settled, and a distinct clear layer has formed above a distinct green layer, siphon or pour off the clear supernatant. Separation should occur within

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2-3 days but if sufficient time is available, best results occur when the algae is left to settle for a week or more.

5.11.1. Setup an additional 2 L flask labeled "*R. sub* SUPERNATANT" with a matching batch number to the setup algae with an "S" at the end. Ex: ##.##.##S. First pour off ~500 mL of supernatant down the drain. The rest of the supernatant is poured into the SUPERNATANT flask until a dark stream of green algae can be seen.

5.11.1.1. The supernatant algae is primarily used as a backup to the regular algae and is not essential. If the algae is allowed to settle for a week, there will be little algae in the supernatant. This is fine if you always have two bottles of algae ready for use in case of spillage or other loss of the algae.

5.11.2. Pour offs should be performed at a moderate and continuous speed and stopped right before the dark green stream of algae enters the flask. Do not return the flask to its upright position during pour off as this will homogenize the separated liquid. It usually takes 2 or 3 total pour offs to reach counting levels (1 to 2 if the algae has settled for a week). The algae will appear dark green, and the main batch will likely be 300 to 500 mL from a 4 L flask.

5.12. Count culture density using the Coulter® Counter Multisizer 4e. 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).

5.12.1. *R. sub* counts are performed with 0.1 mL of the sample and 9.9 mL of Isoton. Set to "*R. sub* counts2025" SOP before starting. Take the average "Number" from the run and multiply by 200 to get the density.

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

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

5.13. The R. sub can last 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.

5.13.1. In an emergency situation, a batch of *R. sub* can be used longer than 90 days if there is no suitable batch available. A fresh batch should be made ready as soon as possible.

5.14. Record batch preparation and count information in the *Raphidocelis (Pseudokirchneriella) subcapitata Food for C. dubia Logbook*.

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6. DATA ARCHIVAL

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

6.2. Record preparation in the *Raphidocelis (Pseudokirchneriella) subcapitata Food for C. dubia Logbook*.

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.

6.3.3. 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. 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. Cultures 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 Assurance Manual for sample disposal.

8.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management Plan.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

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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*

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-09.08, *Preparation of B12 and Sodium Selenate for use in 20% DMW, MHWCD, and Pseudokirchneriella subcapitata Culture Media*

9.6. FDEP Laboratory Quality Assurance Manual

9.7. FDEP Laboratory Health and Safety Manual

9.8. 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.

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