Culture of *Microcystis aeruginosa* Stocks

(Based on and modified from EPA-600/9-78-018,
The *Selenastrum capricornutum* Printz Algal Assay Bottle Test)

1. SCOPE AND APPLICATION

1.1 This SOP describes the maintenance of pure cultures of *Microcystis aeruginosa* under laboratory conditions. The laboratory's *Microcystis aeruginosa* cultures were originally collected from blooms or stock that was confirmed to produce the hepatotoxin, microcystin, a toxin able to damage the liver. Cultures are checked periodically to assess toxin production. It is good practice to use precautionary measures when handling and avoid direct contact with the cultures.

2. SUMMARY OF METHOD

2.1 Cultures are maintained on a rotary shaker at 24 ± 3 °C under 24-hour light conditions.

2.2 Flasks of fresh BG-11 medium are inoculated (2 flasks/culture) with seven-day old culture each week and the three-week old culture is discarded.

2.3 Plates of fresh BG-11 agar are inoculated with seven-day old culture once a month for reserve stock.

3. APPARATUS AND EQUIPMENT

3.1 Erlenmeyer flasks with foam stoppers (2 per culture, 250 mL, autoclaved)
3.2 Disposable pipettes (10mL and 1mL)
3.3 Pipettor (10 mL and 1 mL)
3.4 Graduated cylinder (100 mL)
3.5 Labeling tape, 3/4-in wide or waterproof label sheet for computer printing
3.6 Black, fine-tip, permanent marker
3.7 UV sterilizer -- Located in Microbiology Lab (B212). Operate per instruction manual.
3.8 Microcystis Culture Logbook

4. REAGENTS AND CHEMICALS

4.1 BG-11 media, approximately 200 mL per culture (prepared in advance per SOP AB-12)
4.2 BG-11 media agar plates (prepared in advance per SOP AB-12)
4.3 *Microcystis aeruginosa* cultures from different sources

5. SAMPLE COLLECTION AND PRESERVATION

5.1 Samples used for isolating a *Microcystis aeruginosa* culture are collected from blooms where it has been confirmed that the algal toxin, microcystin, is being produced.

5.2 Samples should be carried directly to the laboratory or shipped overnight without preservation. Even ice preservation has the potential to damage the algal cells and halt toxin production. Living cells or colonies are needed for lab isolation of new cultures lab.

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6. CULTURE MAINTENANCE PROCEDURES

The culture flasks are maintained on a shaker rack in room B246. Algae from the youngest or most recently dated culture from each source, which should be 7±1 days old, is transferred to fresh growth media each week. Every three months, a slide prepared from each culture should be examined under a microscope to verify that the culture is not contaminated before transferring.

6.1 Flask Culture Maintenance Methods

Precautions must be taken to avoid cross-contamination of the cultures. Use a new pipette for each flask. Change gloves in between each culture. (Examples: Do the daily steps for one culture before doing the daily steps for the other culture, stopper one culture before opening and transferring another, discard the pipettes used for one culture before using a pipette for the other culture, etc.).

6.1.1 On a designated day of the week, wrap clean, foam-plug-stoppered flasks in aluminum foil and autoclave at 15 psi and 121 ˚C for 15 minutes. (Day can be changed due to holidays, weather events, etc., and flasks can be cleaned ahead of time.). Autoclave enough flasks to allow two flasks per culture.

Note: Clean flasks from the previous week's transfer can be found in the drying oven in B205.

6.1.2 Remove the flasks from the autoclave and let cool to room temperature. Remove the aluminum foil.

6.1.3 Label flasks with algal culture name, date, medium batch number, and your initials. To save time, labels can be printed on waterproof labels. Stick one label on each flask.

Note: Two flasks are labeled per culture. The algal culture "name" is a code assigned to the culture that designates the origin of the culture (e.g. "UTEX" for the University of Texas culture, "MUNSON" for the Lake Munson culture, etc.). Fold about 1/4 inch under at each end of the label before applying label to help later removal.

6.1.4 Use a graduated cylinder to pour 100 mL of BG-11 medium into each of the flasks.

6.1.5 Turn on the UV sterilizer in the Microbiology Lab (B212) and sterilize the inside for two minutes prior to loading any flasks. Place the flasks containing media in the UV sterilizer, three at a time, and sterilize each set of three flasks for two minutes. Operate UV sterilizer per instruction manual.

6.1.6 In the Microbiology Lab hood (B212), inoculate each of the sterilized flasks for the first culture with 1 mL of concentrated cells from the previous week's culture using a disposable pipette. Repeat with each of the remaining cultures. Complete the transfer for all flasks of one culture before proceeding to the next culture.

Note: The 6–8 day old cultures are generally in a rapid-growth phase (log phase) and the algae that are transferred to fresh media during the culture transfer are viable and stay in the log phase. Cultures that are 2-3 weeks old have usually depleted most of the nutrients from the culture media and are dying off. Algae in depleted media are generally more susceptible to physiological changes and may form a type that differs from the original culture (Personal communication, D. Schultz retired, U.S. EPA, Athens, Ga.).

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6.1.7 In room B246, remove the oldest culture flasks (≥ 14 days) from their respective maintenance areas on the shaker rack to make room for the newly inoculated culture flasks. There should be three weeks' worth of cultures on the shaker at any given time.

6.1.8 Place the newly inoculated flasks on the culture shaker. The shaker rack is set on low speed (100 ± 10 rpm).

Note: Specific shaker rack location may vary. Shaker-racks are controlled by a wall-mounted electronic timer. The switch on the shaker-rack should be in the on position.

6.1.9 Record the culture transfers in the Microcystis Culture logbook.

6.1.10 Maintain 24-hour lighting over the algae cultures.

Note: Light intensity of 360-440 foot-candles. Monitor light intensity quarterly per SOP TA-06-26.

6.1.11 Tox lab staff monitors the temperature of B246 daily. In the Microcystis Culture logbook, record any sustained deviation of ±2 °C from the preferred temperature of 24 ±3 °C (acceptable range 21 – 27 °C). Sustained deviation means more than two hours at one time or four total hours during a 24-hour period.

6.1.12 Discard the contents of old culture flasks down the sink or the main drain in the Tox lab. Rinse the flasks with tap water and scrub with a brush to remove any algae stuck to the sides of the flask. The algal cells tend to stick to the sides of the flask and if they remain dry until they are washed, it is very difficult to get them properly cleaned.

6.1.13 Flasks must be cleaned per the standard Biology Section glassware procedures (BG-05), which includes washing with a non-phosphate detergent, followed by a tap water rinse, an acid rinse, and a deionized water rinse. The dishwasher under the sink in B205 can perform the cleaning process. The foam stoppers from the discarded cultures can be autoclaved in aluminum foil at 15 psi and 121°C for 15 minutes and kept for reuse.

Note: Phosphate detergents are avoided because of the potential of skewed test results from phosphate-contaminated glassware.

6.1.14 Once clean, place flasks in the drying oven in B205.

6.2 Agar Plates Culture Maintenance Methods

6.2.1 Once a month, get two previously prepared BG-11 agar-media plates per culture from the Biology walk-in refrigerator (B227). Examine the plates for contamination and discard if any is observed. Place plates bottom up in the hood in the Microbiology Lab (B212).

6.2.2 Under the hood remove lid and shake off excess condensate. Replace lid.

6.2.3 Label the bottom of the BG-11 agar plate with the following: "*Microcystis aeruginosa*," culture name, transfer date, BG-11 batch # and flask #. To save time, labels can be printed on waterproof labels.

Note: Agar prep and expiration dates have been written on the container in which the agar plates are stored in the walk-in refrigerator.

6.2.4 Place two of the one week old flasks of each algal culture in the hood beside the agar plates.

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6.2.5 For each of the flasks, pour (or pipette with sterile 10 mL pipette) 10 mL of the appropriate algae on to the agar dish labeled for the appropriate culture. Do not allow the algae to overflow the agar plate. Replace the plate lid.

6.2.6 Allow the agar plates with culture additions to sit undisturbed for two hours.

6.2.7 After two hours, slowly pour off most of the liquid in the agar plate into a beaker. Leave a thin layer of liquid on the agar. Replace the plate lid and seal the rims of inoculated plates with Parafilm®.

Note: Warning! Be careful not to cross-contaminate cultures. Leaving a thin layer of liquid on the agar ensures the presence of the algae. Settled algae should be visible as a green-colored thin mass.

6.2.8 Place the inoculated agar plates on paper towels beside the culture shaker table in room B246. Do not stack the newly inoculated plates on the old plates (or vice versa). Discard the previous month's plates in the Biohazard trash located in the Microbiology Laboratory (B212). Discard the contents of the beaker in step 6.2.7 down the sink while diluting with water.

Note: Stacking the inoculated plates increased the chance of cross-contamination and creates less than optimal growing conditions by blocking light from the lower agar plates.

6.2.9 Record the agar plate inoculation in the Microcystis Culture logbook.

7. QUALITY CONTROL AND DOCUMENTATION

7.1 Use a pen with black ink for all logbook entries.

7.2 Microcystis Culture logbook

7.3 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error, and initial and date the correction. If the correction requires an explanation, write a comment and reference the error.

7.4 Refer to the DEP Laboratory Quality Manual.

8. SAFETY

8.1 Lab coat, latex or vinyl gloves, and eye protection. Proper laboratory protection is required when working with chemicals and unprocessed samples.

8.2 Avoid breathing fumes or exposing co-workers to them. Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of chemicals or cultures. Work in a well-ventilated area.

8.3 Refer to the Material Safety Data Sheet (MSDS) for information about reagents and chemicals in this SOP.

8.4 Refer to the DEP Laboratory Health and Safety Plan.

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9. POLLUTION PREVENTION AND WASTE MANAGEMENT

9.1 Refer to the DEP Laboratory Health and Safety Plan.

10. REFERENCES

10.1 DEP Laboratory Quality Manual, http://www.dep.state.fl.us/labs/library/progplan.htm

10.2 DEP Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

10.3 The *Selenastrum capricornutum* Printz Algal Assay Bottle Test (EPA-600/9-78-018)

10.4 SOP AB-12, *Preparation of BG-11 Medium, Medium Stock Solutions, and Agar Plates*

10.5 SOP BG-05, *Biology Wash Party Procedures*

10.5 SOP TA-06.26, *Measurement of Light Intensity for AGP Cultures, Animal Cultures, Toxicity Incubators and the Bioassay Laboratory*

Appendix of Changes

01/10/2011 Reformatted the layout following SOP LB-001. In step 6, added the “every 2 month” recurrence for examining a slide of the culture. In step 6.2.3, changed the expiration date from 6 months to 3-4 months.

01/14/2013 Reduced using 4 flasks to two flasks per culture and 3 shaker tables to one. Culture shaker table was relocated from B246A to B246.

12/24/2014 Formatted for 508 compliance.

02/10/2016 Updated names of and moved the DEP Laboratory Quality Manual and Laboratory Health and Safety Plan links to the reference section.

02/28/2017 Standardized SOP naming conventions. Updated links. Added an SOP to the References section.

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