CyanoHABs Response Protocol for Public Water Supplies

Microcystis aeruginosa

New Hampshire Department of Environmental Services
Drinking Water and Groundwater Bureau
29 Hazen Drive
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INTRODUCTION

The following describes the New Hampshire Department of Environmental Services (NHDES) protocol for responding to suspected cyanobacteria harmful algal blooms (cyanoHABs) in public water supply sources. An overview of the protocol is provided in the attached flow chart. This protocol uses a tiered approach, with screening and monitoring steps, leading to actions including optimizing treatment and notifying the public if test results indicate that cyanotoxins are or may be present at levels of concern.

This describes the approach for water systems that are not already implementing a customized protocol developed in consultation with NHDES.

Contacting NHDES

Call in the following order in case of a suspected bloom.

Drinking Water and Groundwater Bureau (DWGB)

- (603) 271-3906 (Liz Pelonzi) - Primary contact for source water cyanobacteria issues
- (603) 271-0688 (Pierce Rigrod)
- (603) 271–2513 (DWGB Main Number) (8:00 AM – 4:00 PM weekdays except holidays)

Jody Connor Limnology Center (JCLC):

- (603) 848–8094 (Cyanobacteria Bloom Hotline)
- (603) 271–0698 (Beach Coordinator office)
- (603) 271–8865 (JCLC Director office)

New Hampshire Department of Safety

- (603) 223–4381 (New Hampshire State Police – **outside NHDES business hours**)

Key terms used in the protocol:

- NHDES – New Hampshire Department of Environmental Services
- cyanoHABs – cyanobacteria harmful algal blooms
- JCLC — Jody Connor Limnology Center at NHDES
- DWGB – NHDES Drinking Water and Groundwater Bureau
- PWS – public water system
- USEPA – United State Environmental Protection Agency
- bloom – source water where the suspected bloom appears to be at its worst
- open water – an area of the lake or reservoir between the visible BLOOM and the intake
- raw – raw water entering the treatment plant
- finished – finished water entering the distribution system
- ELISA – enzyme–linked immunosorbent assay (Quantiplate) test for total Microcystins (ADDA) (modified USEPA method 546)
- LC/MS – Liquid Chromatography/Mass Spectrometry for analysis of specific Microcystins, Nodularin, Anatoxin–a, and Cylindrospermopsin (EPA Methods 544 and 545)
- BMAA – beta–Methylamino–L–alanine
- DABA – 2,4–diaminobutyric acid dihydrochloride
- Microcystins – (MC)
- Nodularins – (NOD)
- CMC – Cyanobacteria Monitoring Collaborative
- HDPE – high density polyethylene

- mL – milliliter
- PTFE – polytetrafluoroethylene

MONITORING

1. **All public water systems (PWSs) using surface sources are advised to conduct** at a minimum, **daily visual surveillance** of their source water(s).

2. NHDES recommends that PWSs using sources with a history of suspected cyanobacterial blooms implement monitoring programs that incorporate continuous or frequent monitoring of source water or raw water for:
    a. Phycocyanin
    b. chlorophyll–a
    c. pH
    d. Temperature
    e. Turbidity
    f. Daily observation of weather conditions

3. To facilitate easy monitoring NHDES recommends:
    a. Have sample bottles on hand supplied by NHDES or JCLC
    b. Have samples bottles on hand prepared by another lab following their specific protocol.

4. **When there are visual signs of a bloom** or water quality parameters (e.g., pH, turbidity, taste/odor) indicating a suspected cyanoHABs, the PWS should contact DWGB or JCLC and JCLC will notify DWGB of the confirmed bloom.

5. **Take samples of the bloom, open water, raw, and finished water in brown, 125 – 500 mL HDPE bottles supplied by JCLC**. Refer to "Sample Collection, Preservation, Shipment, and Storage" beginning on page 10.

6. **DWGB or JCLC will visually identify and determine the density of cyanobacteria in the samples.** Based on the cyanobacterial cell counts, DWGB and JCLC staff will determine whether toxin analyses should be done right away (or batched for later) and whether additional sampling should be done, either by the PWS or by NHDES.

7. If immediate action, such as treatment adjustments is required, DWGB will contact the PWS.

8. If toxin producing cyanobacteria are identified and in concentrations above 70,000 cells/mL, DWGB and JCLC will make the appropriate decision as to which of the available toxin testing option(s) seem(s) appropriate (See Tables 1 and 2 for cyanobacteria and associated toxins). If a decision is made to use LC/MS methods, DWGB will consult with JCLC and then the PWS to run such tests and in what frequency.

    Toxin analyses will be conducted at an EPA approved lab that performs ELISA or LC/MS/MS toxin testing. Depending on the results, DWGB may ask the PWS to continue to sample water, will work with the PWS to optimize treatment, and will consider asking the PWS to **issue an advisory**. *Public notification templates are available.*

9. The PWS will notify DWGB once the required action(s) have been completed.

For more information on cyanobacteria and what they might look like, refer to Cyanos.org

CyanoHABs Monitoring and Response Flowchart

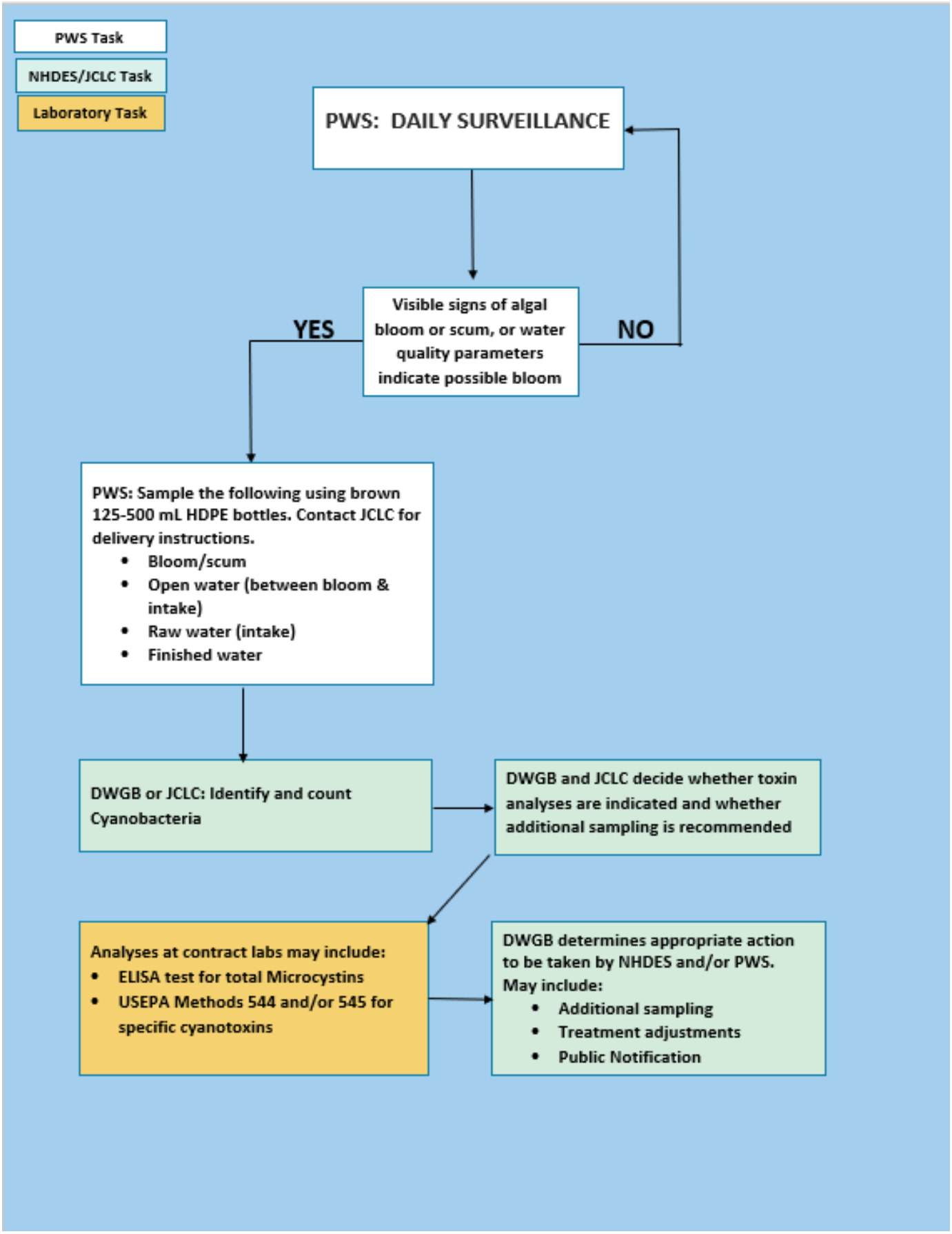

Table 1: Common New Hampshire Cyanobacteria and Associated or Known Toxins

The toxin test that NHDES may advise is first based on the presence of potentially toxic cyanobacteria, then on the concentration of cells (currently 70,000 cells/mL), and the type of toxin they may produce. JCLC will test by ELISA for total Microcystins/Nodularins and Anatoxin–a and/or a lab listed in Table 3 can test by LC/MS methods to determine 7 variants of Microcystins, Nodularins, Anatoxin–a, and Cylindrospermopsin. This is not a complete list of the cyanobacteria or the cyanotoxins.

Common Cyanobacteria Genera of New Hampshire	**Typical Form Observed**	**Associated or Known Toxins**
Anabaena/Dolichospermum	Filaments	Microcystins, Anatoxin–a, Anatoxin–a (S), Saxitoxins, Cylindrospermopsin
Anabaenopsis	Filaments	Microcystins
Aphanizomenon	Rafts of Filaments	Anatoxin–a, Anatoxin–a (S), Saxitoxins, Possibly Microcystins
Aphanocapsa/Aphanothece	Colonies or Single Cells	Microcystins
Coelosphaerium	Colonies	Microcystins
Chroococcus/Gloeocapsa	Colonies	Possibly Microcystins
Gloeotrichia	Macroscopic Colonies	Microcystins
Lyngbya/Phormidium	Benthic Filaments	Microcystins, Lyngbyatoxins, Anatoxin–a
Merismopedia	Rafts of Colonies	Microcystins
Microcystis	Variations of Colonies	Microcystins, Anatoxin–a
Nostoc	Macroscopic Colonies	Microcystins, Nodularins
Oscillatoria/Planktothrix	Filaments	Microcystins, Cylindrospermopsin
Spirulina	Filaments	Microcystins
Synechococcus/Synechocystis	Single Cells, Rarely Colonial	Microcystins and Saxitoxins
Woronichinia	Dense Colonies	Microcystins

Note:

- Some genera grouped here have variations in their taxonomic name or are similar in morphology.
- Species may vary significantly. This is not a complete list of the cyanobacteria.
- More than one type of cyanobacteria and toxin may exist in a typical bloom.
- Microcystins are the most common cyanotoxin in New Hampshire and New England.
- Associated toxins are typical and may change as research evolves.
- Production of some toxins is “turned on” by genetic regulation.
- Toxin tests are also available for Nodularins, commonly produced by marine/brackish cyanobacterium called *Nodularia* (uncommon to New England).
- BMAA, DABA toxins (neurotoxins) have been associated with nearly all cyanobacteria.
- Dermal–toxins, causing rashes on skin can occur with most cyanobacteria.

Table created by Amanda Murby McQuaid

Table 2: Cyanotoxins and Common Modes of Action

(modified from *Handbook of Cyanobacteria Monitoring and Cyanotoxin Analysis, First Ed. 2017*).

Cyanotoxin	Mode of action and/or symptoms
Microcystins (nearly 100 variants)	Hepatotoxic, targets the liver and digestive organs, tumor promoting, inhibition of protein phosphatases. Acute gastroenteritis, chronic tumor promotion.
Nodularins (similar in structure to Microcystins)	Similar to Microcystins, but not as toxic and common in brackish or marine systems.
Anatoxin–a	Neurotoxic, inhibits acetylcholine receptors (neurotransmitter). Fast–acting and may cause seizures or death (i.e., common for dogs or other animals to ingest and die).
Anatoxin–a (S)	Neurotoxic, similar to anatoxin–a
Saxitoxins	Neurotoxic, blocking voltage gate of sodium ion channels. More common to marine organisms.
Cylindrospermopsins	Toxic to multiple organs, neurotoxic and genotoxic, affecting neurons and genes.
Lyngbyatoxins	Tumor promotion
BMAA/DABA	Neurotoxic, chronic exposure may be linked to neurodegenerative diseases such as ALS. (Though individuals may have a genetic precursor).
Note: • Dermal–toxins, causing rashes on skin, and can occur with most cyanobacteria. Usually depends on the individual in contact. • Synergistic effects of the cyanotoxins may also occur. • Many of the cyanotoxins cause gastroenteritis–like symptoms, while others may cause seizure–like or possibly neurodegenerative symptoms. • Exposure can occur through drinking, food, dietary supplements, inhalation, and/or by dermal contact, and has occurred by hemodialysis (with contaminated water).	
Table created by Amanda Murby McQuaid	

LABORATORY METHODS

USEPA Methods for Cyanobacteria Toxin Analysis

USEPA Method 544: Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

JCLC does not perform this analysis. See Table 3.

- Analytical method to determine six microcystins (including MC-LR) and nodularin in finished drinking water

USEPA Method 545: Determination of Cylindrospermopsin and Anatoxin–a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI—MS/MS)

JCLC does not perform this analysis. See Table 3.

- Analytical method to determine cylindrospermopsin and anatoxin-a, in finished drinking water

USEPA Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by ADDA–Enzyme–Linked Immunosorbent Assay (ADDA—ELISA)

- Analytical method to determine “total” microcystins (MC) and nodularins (NOD) in finished drinking water

Table 3: Labs Conducting ADDA–ELISA and LC/MS/MS Toxin Analysis using USEPA Methods 544/545/546

Other labs listed by USEPA are available for similar services. Consult USEPA's list of Laboratories that Analyze for Cyanobacteria and Cyanotoxins.

GREENWATER LABORATORIES*	(386) 328–0882 Greenwaterlab.com	205 Zeagler Dr. Palatka, FL 32177	**USEPA Method 545:** $350/sample **USEPA Method 546:** $200/sample Lab **DOES NOT** do USEPA Method 544	**Sample Protocol:** Amber glass bottles** Keep cold **Shipping Protocol:** Ship on Ice
ALLOWAY LABORATORY*	(800) 873-2835 Allowaylab.com	1776 Marion-Waldo Road Marion, OH 43302	**Ohio EPA DES 701.0** (ELISA) Method $110.00/sample - Regular turn-around time (7 days) $220.00/sample – Expedited turn-around time (48 hours)	**Sample Protocol:** Amber glass bottles** Keep cold **Shipping Protocol:** Ship on Ice
EnviroScience In., Toxicity Lab*	(330) 688-0111 www.enviroscienceinc.com	5070 Stow Road Stow, OH 44224	**Ohio EPA DES 701.0** (ELISA) Method $125.00/sample - Regular turn-around time (3-4 business days) Cost (TBD) Expedited turn-around time (1-2 business days)	**Sample Protocol:** Amber glass or polyethylene terephthalate glycol (PETG) container. Keep cold **Shipping Protocol:** Ship on Ice
New Jersey Center for Water Science and Technology*	(973) 655-7117 wum@montclair.edu	Dr. Meiyin Wu Montclair State University; NJ Center for Water Science and Technology; Center for Environmental and Life Sciences 100; Montclair, NJ 07043	**USEPA Method 546** $190.00/sample – Regular turn-around time (10 day) Can do 2-3 days if requested Can possibly do weekend analyses **USEPA Method 544** $375.00/sample	**Sample Protocol:** Amber glass bottles** Keep cold **Shipping Protocol:** Ship on Ice **48 hour hold time between sample collection and lab's receipt**

RHODE ISLAND STATE HEALTH LABORATORIES*	Evan K. Philo Principal Lab Scientist/Food Testing Coordinator (401)–222–5553 Evan.Philo@health.ri.gov	RI State Health Laboratories RI Dept. of Health 50 Orms Street Providence, RI 02904	**Combined USEPA Methods 544/545** $250/sample **USEPA Method 546:** $50/sample	**Sample Protocol:** Amber glass bottles** Keep cold. **Shipping Protocol:** Ship on ice

*** Call laboratory to confirm they can expedite the sample(s) analyses prior to shipping.**

** **PWS's are responsible for obtaining the required sampling bottles and preservatives and having them available on-site.** Amber glass bottles and preservatives can be supplied, if necessary, upon request by DWGB.

Contact:

Liz Pelonzi, Source Protection Specialist at (603) 271–3906; liz.pelonzi@des.nh.gov
Pierce Rigrod, Supervisor, Source Water Protection Program at (603) 271–0688; pierce.rigrod@des.nh.gov

SAMPLING

This sampling protocol outlines how to collect cyanobacteria and cyanotoxin samples at PWS source waters, finished waters, and other sampling locations. This protocol does not address sample collection for site specific monitoring plans. Consult with DWGB on recommendations for a routine cyanobacteria monitoring plan or refer to USEPA Cyanobacteria Monitoring Collaborative (CMC) at cyanos.org.

Safety Precautions

- Wear protective gear such as gloves if handling a suspected harmful cyanobacteria bloom.
- Precautions should be taken to avoid mouth and eye contact.
- Wear eye protection and a mask to further prevent exposure.
- Chest waders should be worn if collecting a cyanotoxin sample when wading off the shore to protect skin from contact with cyanotoxins.
- Always wash your hands and rinse thoroughly after handling.

Recommended Safety Supplies

For cyanobacteria and cyanotoxin sampling at public water systems, the recommended safety supplies include:

- Disposable gloves or reusable arm-length gloves
- Goggles
- Mask
- Chest Waders

General Sampling Supplies

Cooler with packed ice for sample storage under 12 hours and/or refrigerate if up to 24 hours until delivery. (Do not allow bottles to float in warm water or melted ice water)

High density polyethylene (HDPE) brown bottles of at least 125 mL capacity

Analytical Method Specific Supplies

Cyanobacteria screening for Microcystins/Nodularins
at NHDES JCLC:

- HDPE brown bottles of at least 125 mL capacity.
  - HDPE bottles will be supplied upon request by calling Liz Pelonzi at (603) 271-3906 or the Cyanobacterial Bloom Hotline at (603) 848-8094.

For cyanobacteria toxin sampling for lab analysis using USEPA Methods 544/545/546:

- 500–mL amber glass bottles (1–2) fitted with polytetrafluoroethylene (PTFE)–lined screw caps **and;**
- USEPA Method specific preservatives.
    - Sodium Thiosulfate (0.1g/L) - **Method 546**
    - Ascorbic Acid (0.1g/L) and Sodium bisulfate (1g/L)- **Method 545**
    - Trizma (7.75 g/L); 2-Chloroacetamide (2 g/L); Ascorbic acid (100 mg/L); Ethylenediaminetetraacetic acid trisodium salt (0.35 g/L)-**Method 544**
    - **Confirm with lab for specific protocols**

- **PWS's are responsible for obtaining the required sampling bottles and preservatives.** The amber glass bottles and preservatives can be supplied, if necessary, upon request by DWGB , contact:
    - Liz Pelonzi, Source Protection Specialist at (603) 271–3906 or liz.pelonzi@des.nh.gov
    - Pierce Rigrod, Supervisor, Source Water Protection Program at (603) 271–0688 or pierce.rigrod@des.nh.gov

Sample Collection, Preservation, Shipment and Storage

Sample Collection Procedure for Grab Samples and Surface Skim Samples

- **Grab samples:** samples that are collected from a sample tap or by submerging a bottle in water at an attainable location by hand. Wearing gloves, sample by submerging bottle slowly through the water and swing arm in a u–shaped orientation. Recommended for:
    - Water surface
    - Beach or shoreline at knee depth (or about 1 meter)
    - Raw or finished water (from a sample tap)

- **Surface skim:** using a collection bottle to skim the surface of the water. Recommended for:
    - Dense surface bloom or shoreline accumulation

Sample Collection Procedure for EPA Methods 544 and 545

- Open the cold water tap and allow the system to flush until the water temperature has stabilized (approximately 3 to 5 minutes). Collect samples from the flowing system. Fill sample bottles, taking care not to flush out the sample preservation reagents.

- After collecting the sample, cap the bottle and agitate by hand until preservative is dissolved. Note that 2–chloroacetamide is slow to dissolve, especially in cold water. Samples must be chilled during shipment but should not be frozen.

- Shipping instructions are provided in Table 3.

Sample Collection Procedure for EPA Method 546

- Open the tap and allow the system to flush for approximately 5 minutes. Fill each bottle, taking care not to flush out the sodium thiosulfate. Invert bottle(s) several times to mix the sample with the reducing agent. Sample must be chilled during shipment but should not be frozen.
- Shipping instructions are provided in Table 3.

JCLC Sample Submittal Procedure

Samples will be analyzed free of charge. At JCLC samples will be analyzed for:

- Cyanobacteria ID and cell count

1. Take a photo(s) of the bloom and submit it to Liz Pelonzi at liz.pelonzi@des.nh.gov or the JCLC Harmful Algal and Cyanobacterial Bloom Program Coordinator at HAB@des.nh.gov

2. Collect a sample(s) from the options listed below, using a 125mL brown HDPE bottle(s).
    a. Grab sample
    b. Surface skim
    **Avoid collecting samples from areas where the bottom sediment has been disturbed.*

3. Label and store water samples properly
    a. Label 125 mL brown HDPE bottle(s) to include:
        i. Waterbody and location (coordinates if possible)
            1. Bloom
            2. Open water (away from bloom)
            3. Intake, Raw water
            4. Finished water
            5. Or other location within source
        ii. Date and time
        iii. Type of sample (indicate if this was a surface bloom or other)
        iv. How sample was stored (e.g., on ice or refrigerated) **DO NOT FREEZE.**

4. Place in packed ice in cooler or refrigerate (deliver within 12 hours of event) .
    a. Bring samples to JCLC, 29 Hazen Dr., Concord, NH during business hours (8:00 a.m.–4:00 p.m.) or by prior arrangement.

5. When dropping off the sample(s), fill out a requisition form at the JCLC for confirmation of sample delivery and details of bloom event with appropriate contacts.
    a. Your name, contact and concern
    b. Location – waterbody, beach or specific bloom area or depth
    c. Date, time and weather
    d. Details or description of bloom– surface scum or throughout water column, surface area, magnitude of area, color and odors, etc.
    e. Submit photos, if possible, to Liz Pelonzi at liz.pelonzi@des.nh.gov or HAB@des.nh.gov
    f. How sample was collected and stored prior to delivery

If additional toxin analysis for Microcystins, Nodularin, Cylindrospermopsin and Anatoxin–a using USEPA Methods 544, 545, or 546 is required, DWGB will request the system take additional samples. Laboratories that conduct these methods are listed in Table 3 above.

If using USEPA Methods, follow USEPA sampling procedure instructions.

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