Oregon Harmful Algae Bloom Surveillance (HABS) Program

Sampling Guidelines:
Cyanobacterial Harmful Blooms in Recreational Waters

Oregon Health Authority

Public Health Division
Center for Health Protection
Environmental Public Health Section

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Appendices

These sampling guidelines represent a consensus view among experts and stakeholders involved in CyanoHABs issues either through public health protection, resource management, research, or environmental regulation.

The guidelines were reviewed and approved by laboratories that specialize in cyanobacteria analysis. In any case, contact the laboratory prior to sampling to confirm sample collection and preservation requirements.

Background

Cyanobacteria, also known as blue-green algae, are commonly found in many fresh and salt water environments around the world. Some cyanobacteria species are referred to as toxigenic because they have the potential to produce toxins that can harm people, pets and wildlife.

The Oregon Public Health Division (OPHD) is working to gain a better understanding about the occurrence of cyanobacterial harmful blooms (cyanoHABs) in Oregon and their impact on human health. Funding for Oregon's Harmful Algae Bloom program was through a five-year federal grant from the U.S. Centers for Disease Control and Prevention (CDC). That grant ended in September of 2013. Currently program staff implement the highest priority activities, such as issuing and lifting advisories, with no funding.

Part of the cooperative agreement with the CDC included building the capacity of waterbody management agencies to monitor waterbodies in a scientifically sound manner with the goal of protecting public health. The OPHD continues to rely on data from environmental samples collected by waterbody managers to inform advisory decisions. OPHD works in partnership with management agencies when issuing and lifting public health advisories.

Purpose of this document

The purpose of this document is to provide guidance for waterbody managers who intend to monitor waterbodies when potentially harmful algae blooms are detected. This guidance document encompasses sample collection for algae identification, enumeration and toxin analysis of freshwater bodies with the objective of protecting public health, specifically with respect to general recreational uses. This guidance is not intended to encompass sample collection for environmental/ecological assessment or for community water system protection.

Sampling guidance

The goal of this document is to provide guidance for collecting water samples from affected water bodies used for recreation in order to protect public health. Assessing the public health risk posed by toxic cyanobacteria or the potential for development of cyanobacterial blooms is complex. Please use the following considerations when deciding to take a sample.

<u>Visual Assessment: A Precursor to Sampling</u>

Simple visual assessment is an important tool in recognizing cyanobacteria. A visual assessment of the bloom status of the waterbody, such as areas of discoloration or surface scum collection, should be used to guide sampling. Materials to help identify cyanobacteria (e.g., field guides) provide an early warning mechanism to help address concerns about cyanoHABs.

Visual Assessment Steps:

- Determine if a bloom is present based on water color and level of cyanobacteria visible on the surface and/or suspended throughout the water column.
- Describe the location of any areas of concentrated algae or floating mats (e.g., in and around swimming areas).

- Take photos of the areas with concentrated algae or floating mats.
- Record results of the visual assessment on a field data sheet; refer to Appendix A for an example.
- Contact the OPHD if a bloom is present.

Sample collection

Monitoring should focus primarily on the protection of human health and secondarily on the health of pets and livestock. Visual observation of an algae bloom is the precursor for water sampling and is also a key consideration when determining optimal sample locations.

Assessing the potential hazard at recreational waterbodies can be complicated if there are numerous access points that allow people and animals to enter or move around the water. Important factors to consider: cyanobacteria often accumulate along shorelines or as floating mats. Cyanobacteria concentrations rapidly change due to wind or other factors and scums or thick concentrations in the water column can generally be assumed to present the greatest risk to recreational bathers.

Monitoring should include samples that represent worst-case conditions in areas where people and animals are most likely to contact the water. Both the location and number of samples will depend upon the recreational use and available funding. If funding is limited, sampling should focus on near shore waters in areas where wading and swimming might occur. If resources allow, sampling far from shore may be desired in order to assess risks to water skiers and other deep water uses.

When samples are taken can be as important as where. Throughout the day, as temperatures increase, cyanobacteria will move closer to the surface. Also, winds can change throughout the day which influences where the bloom may be found and how well-mixed the water will be. When you plan your sample collection, keep in mind what time of day you would expect recreation to take place. Typically, samples should be drawn during the middle of the day when it is likely to be warmer and calmer.

Follow-up samples

Each bloom is unique and the response will vary according to the characteristics of the bloom and of the affected waterbody. Listed below are some considerations which may influence decisions about when to collect follow-up samples.

- *Are you conducting Toxin-Based Monitoring: Option 4 (see page 6)?* If so, and toxin concentrations are below guideline levels, sample every other week.
- *Are there appearance changes (e.g. size, location, density, color)?* Follow progress of the bloom, conducting visual observations over the course of the bloom.
- *Has there been a report of an animal death or human illness?* Such reports are an important indication that further evaluation, such as toxin analysis, should be done.

- *Does the weather forecast call for rain?* Periods of cool rainy weather may contribute to the collapse of the bloom or run-off may introduce nutrients that can lead to the resurgence of a bloom, especially if warm weather follows the rain.
- *Is the wind or current strong enough to move the bloom from the initial sampling location?* Shifts in wind direction can move a bloom from one location to another and cause blooms to accumulate along shorelines or in protected areas.
- *Is the temperature rapidly falling?* Cyanobacteria blooms generally do not thrive during the winter months due to low water temperatures. Some genera of cyanobacteria can sink into sediment and become dormant until the weather conditions become optimal.

Standardized collection methods

The purpose of standardized collection methods is to ensure that samples are representative of field conditions, and that variability is minimized while striving to meet program goals and manage limited resources.

These instructions will provide samples that reflect the risk of exposure to worst case scenarios when people or animals are in the affected waterbody. The risk of exposure increases depending upon the type of water activities enjoyed. For people, swimming, active water play or waterskiing have the greatest potential risk of exposure. For animals, drinking the water, active water play, eating cells or licking them off rocks or their fur have the greatest risk. (See Appendix B for a list of field equipment and step-by-step instructions for collecting water samples.)

1. *Grab/single point sampling.* This is the most basic collection method used to characterize a bloom. Additional grab samples can be taken at other areas of concern. All results from samples taken at a given waterbody are considered in the public health advisory process. Depending upon the characteristics of the waterbody (size, geography, current, etc.) and the level of monitoring being performed of the waterbody and any resultant advisory applies to the entire waterbody.
2. *Composite sampling*. This is a method used to minimize sample variability. Composite samples use a variety of methods to combine samples taken at a particular point across time, at a given location at many depths, or at many locations at a given depth. Use the following guidelines for composite sampling for the purpose of public health protection from cyanoHABs:
   - Composite sample should combine three samples taken to reflect worst-case conditions. Look for areas with the most scum or thickest cyanobacteria.
   - Do not combine samples from within a bloom with those from outside the bloom (in a “clean” area) to avoid diluting the sample and risking a false negative.
   - Do not combine samples from different blooms or different waterbodies as they are likely to have significantly different species composition and toxin profiles.

Safety

Cyanobacteria can produce potent neurotoxins and hepatotoxins (liver toxins) so exercise care when collecting water samples. In addition, cyanobacteria produce lipopolysaccharides that can irritate the skin. Wear protective clothing such as hip waders and long rubber gloves.

Do not ingest any surface water and avoid inhalation of aerosolized water (e.g., created by a boat motor). Always wash hands, other exposed skin (feet, legs, etc.), glasses, boots and gloves with soap and water after collecting samples. The overarching concern when sampling is personal safety. When selecting sampling locations, look for ease of access and make sure there are no physical barriers to safety.

Lugol's solution should be used to preserve cell enumeration and species identification samples. Wear gloves and eye protection when handling Lugol's. Only add it to the sample(s) in a well-ventilated area e.g., in a fume hood or outdoors. (Lugol's is commonly used for short-term storage of cyanobacteria- from a few months to a year or more.) Contact a laboratory to inquire about how to obtain Lugol's solution and refer to the Material Safety Data Sheet (MSDS) for more information regarding the hazards of this chemical.

Toxin-Based Monitoring Program: Option 4

The Public Health Advisory Guidelines document explains that a monitoring program based on toxins rather than cell counts will likely result in fewer and shorter duration public health advisories, but may have higher sampling costs. Toxin-based monitoring programs also provide the most accurate information in terms of protecting public health, because toxins pose actual risk rather than the potential risk posed by the presence of cyanobacterial cells.

If Option 4 is chosen an advisory will not be issued unless toxin testing shows levels above the guideline values shown in Table 1, even if cell counts are above guideline values. In the interim, the public should be notified that a bloom has been identified and toxin testing is underway or ongoing. The public should be advised to avoid water that is foamy, scummy, thick like paint, pea-green, blue-green or brownish red. Contact OPHD for signage options or templates.

The recommended approach to maintaining a toxin-based monitoring program is as follows:

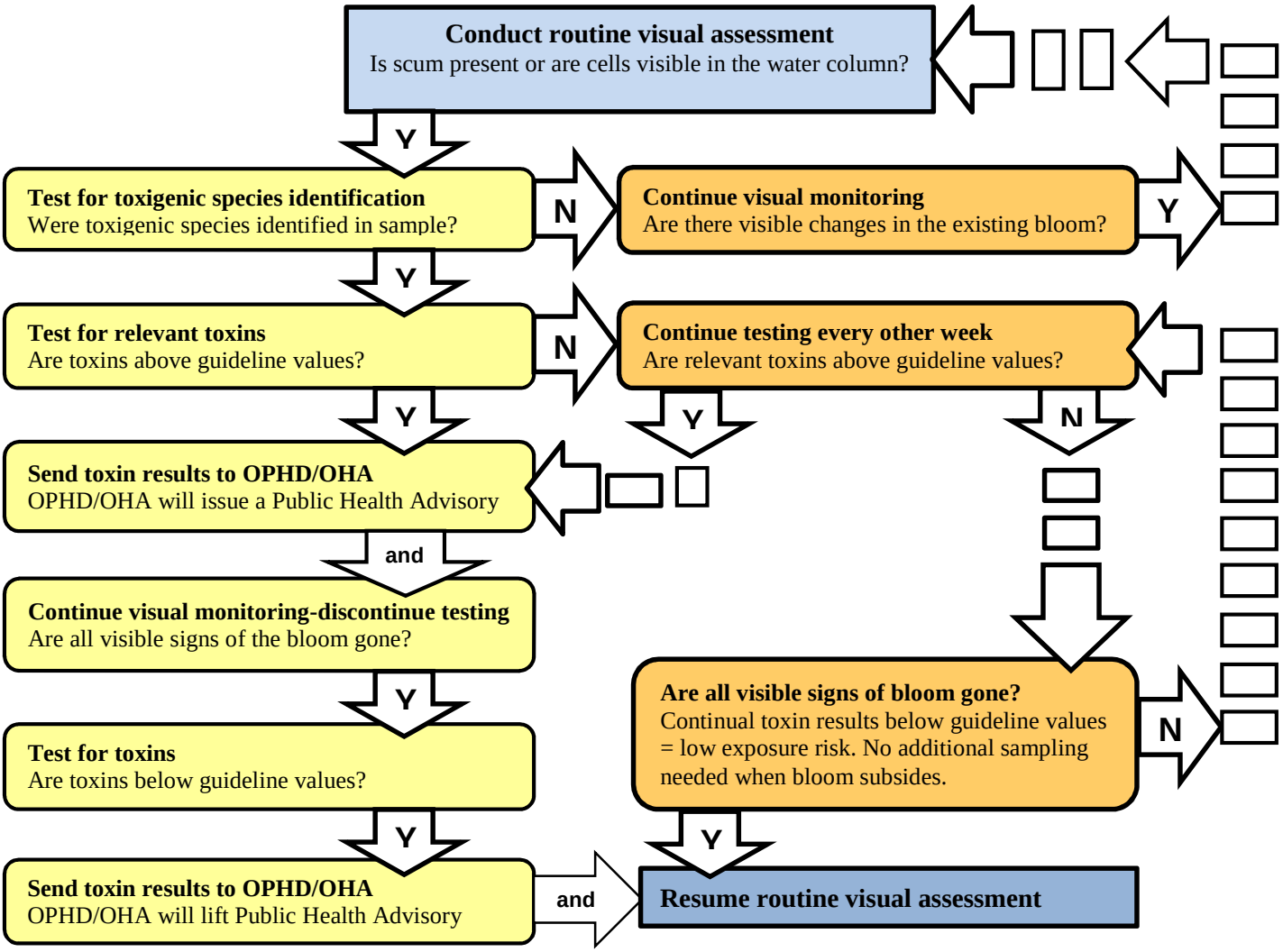

Table 1. Health advisory guideline values for cyanotoxins in Oregon's recreational waters (µg/L)

Guideline Value:	Anatoxin-A	Cylindrospermopsin	Saxitoxin	Microcystin
	20	20*	10*	10

* The guideline values for saxitoxin and cylindrospermopsin have been updated from previous values. See Appendix B of OPHD's *Public Health Advisory Guidelines Harmful Algae Blooms in Fresh Waterbodies* for a discussion of the rationale behind the change.

Sampling frequency for this option is every other week if toxin-producing species are present but the toxin levels are below guideline values. When using Option 4, OPHD requests that sampling results be available within 3 business days after the lab receives the sample. This helps us communicate with the public in a timely manner and demonstrates that action is being taken to protect the public's health. Before hiring a lab to analyze a sample, we suggest contacting them

to discuss turnaround time to ensure that this 3-day standard can be met. For OPHD purposes, electronic versions with official, final results are acceptable.

If levels are found to be above guideline values, then an advisory is issued, advisory signage is posted and sampling can be suspended until the bloom is gone. To lift the advisory, samples must simultaneously show that cell counts and toxin levels are below guideline values.

NOTE: OPHD is interested in copies of all toxin and cell speciation data for tracking purposes. Providing these results is not required for participation in OPHD's cyanoHABs program. However, it helps OPHD build a statewide system for monitoring trends in cyanoHABs in Oregon waters. OPHD takes no action on any data submitted unless results are above threshold values.

Laboratories

The number of laboratories available for species identification, cell counts, and toxin analysis is limited. Laboratories providing cyanobacterial analysis are listed in Appendix C. The accepted method for determining cell counts is Standard Methods Section 10200E and F (also called "SM10200").We recommend contacting your lab for the most current cost of tests and for preservation and shipping instructions for your sample.

Toxin analysis is required to lift public health advisories for cyanoHABs. Cyanotoxin tests currently available through commercial laboratories use a variety of comparable methods. These tests can be costly, depending on the method and equipment used. Lab staff can provide you with the current cost of toxin analysis prior to submitting a sample.

As a rule of thumb, the ELISA method is least expensive for determining levels of microcystin, saxitoxin, and cylindrospermopsin in the bloom. ELISA methods are not currently available for anatoxin-a, however, Abraxis has introduced a micro-titer plate format (96T), receptor-binding assay (RBA) kit for Anatoxin-a. This kit provides two protocols. The EZ protocol requires no sample preparation and has a range of 5 to 500 ppb. If a lower limit of detection is required, the enhanced sensitivity (ES) SPE sample protocol may be performed. The kit provides a real-time, economical, accurate and sensitive alternative for both research and monitoring**.** OPHD does not accept field ready test kits for microcystin or cylindrospermopsin as a basis for lifting an advisory. However, the kits may be useful for monitoring the progress of a bloom.

When testing for cyanotoxins, ensure the lab uses a method with a detection level below the values shown in Table 1 (on previous page). Note that the guideline value for saxitoxin has decreased to 10 µg/L from 100 µg/L. See Appendix B of OPHD's *Public Health Advisory Guidelines Harmful Algae Blooms in Fresh Waterbodies* for a discussion about the change.

It is important to test for toxins that may be produced by the dominant species of cyanobacteria in a bloom. To do so, it is necessary to identify the dominant species present (with or without enumeration). Consult Table 2 to determine the associated toxins.

Table 2.Toxigenic cyanobacteria (data derived from evidence of toxin production presented in Sivonen and Jones, 1999; Carey et al., 2007; Funari and Testai, 2008 and Voloshko, 2008)[1]

	Hepatotoxins			Neurotoxins	
	Microcystin	Nodularin	Cylindro-spermopsin	Anatoxin-a	Saxitoxin
Anabaenopsis	+				
Aphanizomenon (Except A. flos-aquae)			+	+	+
Arthrospira	+				
Cyanobium	+				
Cylindrospermopsis			+		+
Dolichospermum (formerly Anabaena)	+		+	+	+
Gloeotrichia	+				
Hapalosiphon	+				
Limnothrix	+				
Lyngba					+
Microcystis	+			+	
Nodularia		+			
Nostoc	+				
Oscillatoria	+			+	
Phormidium	+			+	
Planktothrix	+			+	+
Raphidiopsis			+	+	
Schizothrix					
Synechocystis	+				
Umezakia			+		

[1]Taxonomy for many types of cyanobacteria is currently being revised. This guidance reflects taxonomy as of January 2015.

Note: Table 2 is at the genus level, not the species level. Not all species of a given genus produce all of the toxins listed for that genus. Once the species involved in a specific bloom have been identified, OPHD recommends that waterbody mangers contact OPHD to determine exactly which toxins could be involved. Also note that for the purpose of public health advisories, Aphanizomenon flos-aquae (AFA)is excluded from calculation of combined cell counts of toxigenic species. See the Public Health Advisory Guidelines (pg 7) for more information.

Shipment of samples

Samples should be shipped the same day as collected. Ship the samples in a cooler or a cardboard box lined with plastic bags (to prevent leakage) and newspaper (for insulation).

For toxin samples, include ice bags, ice packs, or other cooling products (e.g., blue ice) to ensure the samples stay cold. If shipping algae identification, enumeration and toxins samples to the same laboratory, you may package them in the same cooler to save shipping costs.

Collection volume, storing methods, holding times and shipping guidance is provided in Appendix B. Prior to sample collection, discuss such details with the laboratory that will be performing the analyses.

Program contact information

Email: habhealth@state.or.us
Phone: (971) 673-0440 Toll free: (877) 290-6767 and press 4
Website: www.healthoregon.org/hab

Appendix A: Field assessment sheet

Please gather the following information for each location assessed, if applicable.

General Information

Assessment date ___/___/_____mm/dd/yyyy Time _____AM / PM*(circle one)*

Name of waterbody _____ County _____

Assessment location description/number _____

Assessment Location Latitude_____ Longitude _____

Visual Assessment

Water clarity *(circle all that apply):* Clear Cloudy Hazy Opaque Don't know

Water color *(circle all that apply):* Green Blue-green Brown Rust Milky White
Red Pink Clear Don't know

Visible bloom *(circle one)*: Yes No Don't know Visible scum *(circle one)*:Yes No Don't know

Reason for visual assessment: Monitoring Fish kill Health event response Other _____

Recreational Area Assessments

Total number of people in the recreational area _____Of those, number of people in the water _____

Total number of boats in use _____ Number of people waterskiing/boarding _____

Total number of dogs in the recreational area_____ Of those, number of dogs in the water _____

Other Observations and Comments *(e.g., sketch bloom location in respect to recreational areas)*

Sample Collector Information

Name _____ Position _____

Phone Number _____ Agency/Organization _____

Appendix B: Step-by-step sample collection instructions

(Contact the laboratory prior to sampling to confirm collection and preservation requirements)

Sampling Equipment Checklist (confirm with laboratory)

- ☐ *Field Assessment Sheet*
- ☐ *Arm-length Disposable Waterproof Gloves*
- ☐ *Hip Waders*
- ☐ *Eye Protection/Safety Glasses*
- ☐ *Waterproof Permanent Marker*
- ☐ *4 liter stainless steel bucket (if compositing sample)*
- ☐ *Cooler or insulated shipping box*
- ☐ *Ice Pack(s)*
- ☐ *GPS Unit (recommended)*
- ☐ *500 mL HDPE Plastic Sample Bottles*
- ☐ *Lugol's Preservative Solution*
- ☐ *Long-handled Stainless Steel Stirring Spoon (if compositing sample)*

Sampling Instructions

Sample Event Preparation

1. Prepare Field Assessment Sheets (Appendix A). Use one sheet per sample location to describe ambient conditions. This will help to interpret results, as well as provide sample collection contact information. Use pencil or waterproof permanent marker when completing the sheet.
2. Label each bottle with the sample location and either "Cell & Species" or "Toxins" as needed. Use pencil or waterproof permanent marker when completing the labels.

Collecting Samples- *Wear disposable gloves and any other necessary personal protective equipment.*

1. Select a sampling area that represents a distinct cyanoHAB and/or distinct risk of exposure (for example, a swim beach). Within that sampling area, select the location (or three locations, if compositing samples) with the highest concentration of cyanobacteria.
2. If taking the sample from the shoreline, wade or boat slowly to the sampling location. If wading or boating, approach on the downwind side and avoid agitating the water or sediment.
3. Disturb the water at the sample location for approximately ten seconds to simulate conditions created by a swimmer or wader coming into contact with the cyanobacteria.
4. Remove the cap from the sampling bottle, tilt the bottle approximately 45 degrees and allow the bottle to fill as you submerge it 3 to 6 inches below the surface.
5. Replace the cap and remove any cyanobacteria adhered to the outside of the bottle. Turn the bottle end-over-end 3-4 times to mix.
6. If creating a composite sample:
    a. Because cyanotoxins are organic compounds, sampling equipment used to collect and combine samples should be made of fluorocarbon polymers (such as Teflon®); metals (such as stainless steel); or glass.
    b. Rinse the bucket with fresh water or with lake water relatively free from cyanobacteria.
    c. Pour the three sample bottles representing the sampling area into the bucket.
    d. Use a long-handled stainless steel stirring spoon to thoroughly mix the sample. Do not stir vigorously as this type of mixing may rupture (lyse) the cyanobacteria cells. If lysing occurs, cell enumeration becomes very difficult to perform with accuracy. This is less of an issue for

toxin samples since cyanobacteria cells in the sample will be lysed at a later stage of processing.

e. Pour into a rinsed sampling bottle. Replace the cap and remove any cyanobacteria adhered to the outside of the bottle. Turn the bottle end-over-end three or four times to mix.

Sample preservation – (*confirm with laboratory)*

1. Cell Enumeration & Species Identification Samples: *Handle preservatives only with good ventilation. Wear gloves and safety glasses.*
    a. Remove the cap and pour off enough of the sample to leave one inch air space for mixing the Lugol's solution.
    b. Using a pipette, calibrated dropper, or medicine cup to measure the correct amount of preservative, add 5 mL Lugol's Solution (or other preserving agent recommended by the lab) to the sample.
    c. Close the cap tightly and turn the bottle end-over-end three or four times to mix.
    d. Place samples on ice in a cooler or insulated box and arrange overnight shipping of the samples to the laboratory.

A. Toxin Samples:

    1. Place the bottle(s) on ice as soon as possible in a cooler or insulated box and arrange overnight shipping of the sample to the laboratory.

Appendix C: Available laboratories for cyanobacteria testing

When processing water samples for toxins as part of a toxin based monitoring program, OHA recommends sampling data be available within 3 business days after receipt of a sample. This helps OHA and the waterbody managers to meet the need for timely communication with the public, and demonstrates that action is being taken to protect the public's health. For OHA purposes, electronic versions with official, final results will suffice.

Aquatic Analysts
43 Telegraph Lane, Friday Harbor, WA 98250
(Identification and enumeration)

Attn: Jim Sweet
phone: (503) 869-5032
jwsweet@aol.com

Aquatic Services
42184 Tweedle Lane, Seaside, OR 97138
(Consulting, identification and enumeration)

Attn: Wayne W. Carmichael, PhD
phone: (937) 620-4603, (503) 755-0711
wayne.carmichael@wright.edu

Beagle Bioproducts, Inc
959 Schrock Road
Columbus, OH 43229
(Toxin testing)

Attn: Stephanie A. Smith, PhD
ph: (614) 519-0154 (cell)
www.beaglebioproducts.com
stephanie.smith@beaglebioproducts.com

CAHFS Toxicology Laboratory
University of California, School of Veterinary Medicine
West Health Sciences Drive, Davis, CA 95616
(Toxin testing)

Attn: Birgit Puschner
ph: (530) 752-6322
fax: (530) 752-3361
bpuschner@ucdavis.edu

CH2M HILL Applied Science Laboratory
1000 NE Circle Blvd., Suite 10350, Corvallis, OR 97330
(Toxin testing - Microcystin)

Attn: Lab Customer Service Support
ph: (541) 768-3120 fax: (541) 766-2852
asl@ch2m.com

EcoAnalysts, Inc.
1420 S. Blaine St., Suite 14, Moscow, ID 83843
(Toxin testing, identification and enumeration)

ph: (208) 882-2588
fax: (208) 883-4288
eco@ecoanalysts.com

GreenWater Laboratories/Cyano Lab
205 Zeagler Drive, Suite 302,Palatka, Florida 32177
(Toxin testing, identification and enumeration)

ph: (386) 328-0882
fax: (386) 328-9646,
markaubel@greenwaterlab.com

King County Environmental Laboratory
322 West Ewing Street, Seattle WA 98119
(Identification, enumeration, toxin testing)

Attn: Fran Sweeney
ph: (206) 684-2358
Francis.sweeney@kingcounty.gov

LSSU Environmental Analysis Laboratory
Lake Superior State University
650 W. Easterday Avenue, Sault Ste. Marie, MI 49783
(Identification, enumeration, toxin testing)

Attn: Ben Southwell
ph: (906) 635-2076
bsouthwell@lssu.edu

PhycoTech
620 Broad Street, Suite 100, St. Joseph, MI 49085
(Identification and enumeration)

ph: (269) 983-3654
fax: (866)728-5579
info@phycotech.com

WATER Environmental Services, Inc.
9515 Windsong Loop NE, Bainbridge Island, WA 98110
(Identification and enumeration)

Attn: Maribeth Gibbons, Pres.
ph: (206) 842-9382
mvg.water@gmail.com