

# Intensive Survey Branch

## **STANDARD OPERATING PROCEDURES MANUAL:**

### *Algal Bloom and Cyanotoxin - Field Collection*



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## ALGAL BLOOM AND CYANOTOXIN - FIELD COLLECTION AND RESPONSE PROTOCOL

Water Sciences Section of the Division of Water Resources

N.C. Department of Environmental Quality

4401 Reedy Creek Road

(T) (919) 743-8519

### APPROVED:

SOP Committee Chair: \_\_\_\_\_ Date: \_\_\_\_\_

Supervisor, Ecosystem Branch: \_\_\_\_\_ Date: \_\_\_\_\_

Quality Assurance Coordinator: \_\_\_\_\_ Date: \_\_\_\_\_

Annual Reviewer				
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## **SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS**

### **Distribution**

Original and Current Copy, Maintained by David Huffman QA Coordinator,  
Ecosystem Branch

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## **SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS**

The following field monitors at this regional office have read this manual.

<b>Signature</b>	<b>Title</b>	<b>RO</b>	<b>Date</b>

## **SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS**

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## SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS

DEQ	Department of Environmental Quality
DWR	Division of Water Resources
WSS	Water Sciences Section
PWSS	Public Water Supply Section
WQROS	Water Quality Regional Operations Section
DHHS	Department of Health and Human Services
LDH	Local Health Departments
EPA	Environmental Protection Agency
USGS	United States Geological Survey
WHO	World Health Organization
PETG	Polyethylene Terephthalate Copolyester NALGENE square media bottles
PPE	Personal Protective Equipment
PFD	Personal Floatation Device
cm	Centimeters
mL	Milliliters
µg/L	Micrograms per liter (parts per billion)
HAB	(Harmful Algal Bloom) - Algal bloom where cyanobacteria are determined to be the dominant algal group
Cyanobacteria	Algal group comprised of photosynthetic prokaryotes (commonly called bluegreen algae). Several species of cyanobacteria are capable of cyanotoxin production.
Algal Bloom	Rapid increase or accumulation in the population of algae in a waterbody.
Cyanotoxin	Group of chemical compounds that can cause acute and chronic health effects in humans and animals.

## **SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS**

### **1.0 Background**

- 1.1 Algae are a diverse group of plant like organisms that utilize chlorophyll-a for photosynthesis. Algae range from microscopic, single celled organisms to macroscopic, multicellular organisms.
- 1.2 Algae are an essential part of healthy aquatic ecosystems and are beneficial as a source of food, shelter, and oxygen production.
- 1.3 Under favorable environmental conditions, algae can rapidly reproduce to form algal blooms.
  - 1.3.1 Algal blooms are often associated with discolored water, surface scums and mats, and fish kills.
  - 1.3.2 Algal blooms are most common during summer months when warm temperatures, extended daylight hours, and excess nutrient availability provide favorable conditions for algal growth.
  - 1.3.3 Algal blooms can affect the physical conditions of a waterbody through changes in pH, light attenuation and dissolved oxygen (DO) concentration. These changes can in turn affect the health and viability of aquatic organisms.
  - 1.3.4 The prevalence of wide-spread, long-lasting algal blooms in aquatic ecosystems has been increasing in North Carolina and worldwide.
- 1.4 Some algal species within the group **cyanobacteria** (commonly called bluegreen algae) have the ability to produce cyanotoxins.
  - 1.4.1 Cyanotoxins are a group of chemical compounds that can cause acute and chronic health effects in humans and animals depending on concentration and length of exposure.
  - 1.4.2 The exact mechanism of cyanotoxin production is unknown. However, the presence of algal species capable of toxin production does not **directly** relate to the presence of cyanotoxins in a waterbody.

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- 1.4.3 The presence of toxins can persist even after a bloom has begun to visually recede as toxins can be released from intracellular material during cell lysis.
- 1.4.4 Chemical analysis of water taken from an affected waterbody is necessary to confirm the presence of cyanotoxins.
- 1.4.5 Algal blooms dominated by cyanobacterial species are defined as [Harmful Algal Blooms \(HABs\)](#).

### **2.0 Scope and Application**

- 2.1 This manual describes the procedures and safety precautions that should be followed when assessing a HAB and the proper collection and handling techniques for phytoplankton and cyanotoxins.
- 2.2 This manual is to be used by trained North Carolina Division of Water Resources (DWR) personnel within the Water Sciences Section (WSS) and Water Quality Regional Operations Section (WQROS).
- 2.3 Currently, there is no national standard reference method for the assessment of HABs or sampling of cyanotoxins. The procedures outlined within this manual are a compilation of methods recommended and used by the US EPA, WHO and other independent state water quality agencies.
- 2.4 The methods and procedures described are applicable to all NC waters of the State.

### **3.0 Department Roles**

- 3.1 The Department of Environmental Quality will act in an advisory capacity to Local Health Departments (LDHs) and the NC Department of Health and Human Services (DHHS) in determination of the potential for exposure to cyanotoxins from HABs in North Carolina.
- 3.2 In its capacity as the Water Quality Regulatory Agency, DEQ-DWR will assess and analyze algal blooms in house and issue press releases with general information as necessary.

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3.3 The DHHS and LDHs are responsible for evaluating health risks and issuing health advisories associated with HABs.

3.4  **DWR personnel should not attempt to issue public health advisories.**

### **4.0 Health and Safety**

4.1 Response to harmful algal blooms presents possible exposure to dermal, respiratory and ingestible toxins. Personnel responding to an algal bloom should observe all necessary precautions.

4.2 All algal blooms should be treated as potentially harmful until verified otherwise. Field personnel should have access to all personal protective equipment (PPE) listed in [Appendix A](#) when evaluating algal blooms in the field.

4.3 Wash hands and equipment (when feasible and with tap water) at the end of sampling to minimize the risk of accidental exposure to cyanotoxins.

### **5.0 Notification of Algal Bloom Activity**

5.1 DWR receives reports of potential algal bloom activity from DWR field personnel, environmental groups, and private citizens/organizations. Notification of potential algal bloom activity may be received by either WSS or WQROS staff.

5.2 WQROS personnel should make every effort to respond to citizen complaints in a timely manner. Once DWR receives reports and information of an algal bloom, personnel will assess the extent of the bloom and potential impacts to the public.

5.3 Communication between WQROS, WSS, and DHHS/LDHs will be coordinated by WSS Algal Program staff. All entities should be notified of reported algal bloom activity and updated as the bloom investigation proceeds.

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### **6.0 Initial Bloom Evaluation**

- 6.1 DWR personnel should always use their best professional judgement when responding to a bloom. The procedures in this manual attempt to cover the most common event scenarios but do not address every potential occurrence.
- 6.2 **Prior** to conducting a field investigation, WQROS personnel should obtain as much of the following information as possible:
  - 6.2.1 Location of the bloom and waterbody accessibility
  - 6.2.2 Extent of the bloom, visual descriptions/photos, associated fish kills
  - 6.2.3 Duration of the bloom, when was it first observed, any changes in characteristics
  - 6.2.4 Waterbody characteristics, public or private, recreational and/or drinking water supply
  - 6.2.5 History of algal blooms or HABs
- 6.3 Contact WSS Algal laboratory to discuss current bloom information and any additional sampling requests.
- 6.4 Complete the Algal Bloom Response Checklist ([Appendix A](#)).

### **7.0 Algal Bloom Site Evaluation and Documentation**

- 7.1 Site visits should be conducted between 10 am and 3 pm when algae are most active.
- 7.2 Information on field identification of algal blooms, cyanobacterial blooms, and aquatic plants can be found in the following resources:
  - 7.2.1 Indicators of Algal Blooms and Cyanobacterial Blooms Field Guide ([Appendix B](#))

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7.2.2 The USGS Field and Laboratory Guide to Freshwater Cyanobacteria Harmful Algal Blooms for Native American and Alaska Native Communities (<https://pubs.usgs.gov/of/2015/1164/ofr20151164.pdf>)

7.3 Upon arrival at the bloom site, identify and document the following with photos and the *Algal Bloom Field Evaluation Form* ([Appendix C](#)):

7.3.1 Physical characteristics of the bloom.

7.3.1.1 Percent of waterbody affected (photograph extent of bloom)

7.3.1.2 Water clarity and Secchi depth (if possible)

7.3.1.3 Algal characteristics (surface scum, mats, discolored water) and color

7.3.2 Public access and water intakes

7.3.3 Recreational activities occurring

7.3.4 Sampling points based on the following criteria:

7.3.4.1 Sample near public access areas such as boat ramps, beaches, and drinking water intake sites. This will provide the most relevant data for public exposure risk.

7.3.4.2 Sample where the bloom appears most concentrated. Bluegreen algal blooms can form surface scums or be suspended throughout the water column. Select a sampling location and method that is most representative of the “worst case scenario” for potential exposure to cyanotoxins.

7.3.4.3 Keep in mind that blooms may change location due to wind or wave action.

7.3.5 Document the latitude and longitude of all sampling locations.

## **SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS**

- 7.4 Collect all applicable samples according to the [Sample Collection, Preservation, and Storage guidelines](#) found in [Section 8](#) of this manual. At minimum, water quality parameters and two phytoplankton samples (one preserved and one unpreserved) are required by the Algal Laboratory to complete a bloom investigation.
- 7.5 Label each sample bottle following the example provided in [Appendix A](#). For episodic events, generate a station number for each sample location using the following format: ESWBYMMDD-#
- 7.5.1 ES indicates an Episodic Sample
- 7.5.2 Water Body (WB): select two letters corresponding to the waterbody name (e.g. Clear Lake = “CL”)
- 7.5.3 YYMMDD: year, month, day of site investigation
- 7.5.4 #: designate location # if collecting at multiple locations for the same bloom event
- 7.6 If cyanotoxin samples are collected, perform a preliminary cyanotoxin analysis using the Abraxis Strip Tests ([Appendix E](#)).
- 7.7 Complete the *Algal Bloom Field Evaluation Form* ([Appendix C](#)) and *Central Laboratory Water Sample Collection & Submittal Form* ([Appendix D](#)). If the algal bloom was associated with a **fish kill**, submit a *Fish Kill form*.
- 7.8 Submit a prepared cooler and the *Central Laboratory Water Sample Collection & Submittal Form* (Appendix D) to the courier for delivery to the WSS Chemistry Laboratory at the earliest time possible.
- 7.9 Upload all documentation listed in the Algal Bloom Response Checklist ([Appendix A](#)) to the Algal Bloom Response Sharepoint Site (<https://nconnect.sharepoint.com/sites/DWRAlgalBloomResponse>). Instructions for uploading documents can be found in the document library of the Training page.

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### **8.0 Sample Collection, Preservation, and Storage**

8.1 One of the following collection methods may be utilized when responding to an algal bloom.

8.1.1 **Photic Zone Samples** are composite depth integrated samples taken within the photic zone of the water column. This method is ideal for algae suspended throughout the water column. However, sample collection requires access to a boat or canoe potentially making it impractical for initial bloom investigations.

8.1.2.1 These samples can be taken in waterbodies with a minimum depth of one meter, but ideally with a depth greater than 4-5 meters.

8.1.2.2 Lower the Secchi disc on the shady side of the boat (if possible) until it just disappears then pull the disc up until you see it and record the depth.

8.1.2.3 The Secchi depth is the average of the two recorded depths. The photic zone is twice the Secchi depth.

8.1.2.4 Lower the integrated sampling device to twice the Secchi depth and slowly raise to the surface.

8.1.2.5 Transfer collected sample to the sample bottle. Preserve as necessary.

8.1.3 **Grab Samples** are the most common type of sampling method used for algal bloom investigations and water quality parameters. These samples can be quickly taken from a bridge, dock, shore, or boat.

8.1.3.1 If sampling from a bridge, attach all necessary sample bottles to a cage sampler.

8.1.3.2 Lower the cage sampler using a nylon rope and swing downstream.

8.1.3.3 Drop the sampler into the water just below the surface and pull the rope quickly upstream and out of the water.

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8.1.3.4 If sampling from a dock, shore, or boat, position the bottle towards the flow and away from the collector, boat, or shore.

8.1.3.5 Lower the bottle quickly, mouth down, just below the surface (15 cm). If there is no current, move the bottle away from the collector and tip the bottle up. Leave 3-5 cm of headspace.

8.1.4 **Scoop Samples** can be a surface skim, dip or scraping of sample. Scoop samples should only be used when identification is important.

### 8.2 Algae Type and Collection Methods

8.2.1 Algae are classified as one of three types and are collected for identification (unpreserved) and enumeration (preserved). Algal type and collection method are summarized in [Appendix C](#).

8.2.2 **Filamentous Algae** are long strands that can be picked by hand. Collect 2-3 golf ball sized clumps, wrap in a wet paper towel and place in a plastic bag.

8.2.3 **Periphyton** are algae that grow attached to substrate (rocks, soil, sand, etc.). Samples collected can be preserved or unpreserved.

8.2.3.1 Preserved: Collect 50 mL of scrapings (with site water) and place in 500 mL bottle. Add 4 mL of Lugol's solution prepared by the WSS Algal Laboratory.

8.2.3.2 Unpreserved: Collect 2 small (4 inch) pieces of wood, rock, etc. with organism attached and place in bag or jar. Keep wet if possible.

8.2.4 **Phytoplankton** grow suspended in the water column and are the most common algal type collected by DWR.

8.2.4.1 Preserved: Collect a 500 mL [grab sample](#) from the water column. Add 2 mL of Lugol's solution.

8.2.4.2 Unpreserved: Collect a 500 mL [grab sample](#) from the water column.

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### **8.3 Nutrient Samples**

8.3.1 Nutrient samples are collected in conjunction with phytoplankton sampling. A standard nutrient suite measures Ammonia (NH<sub>3</sub>), Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP) & Nitrate + Nitrite (NO<sub>3</sub>-+NO<sub>2</sub>-).

8.3.1.1 Using a 500 mL **clear disposable bottle**, collect a [grab sample](#) at the selected location.

8.3.1.2 Immediately preserve sample with **1:1 H<sub>2</sub>SO<sub>4</sub>** and place on ice. One vial of 1:1 H<sub>2</sub>SO<sub>4</sub> per 500 mL sample is usually sufficient to adjust the pH below 2.

8.3.2 Nutrient levels may not necessarily be elevated during an active bloom.

### **8.4 Chlorophyll-a Samples**

8.4.1 Chlorophyll-*a* has a 24-hour hold-time if not filtered. To avoid degradation of chlorophyll-*a*, samplers should attempt to collect samples and return to the office before the courier arrives.

8.4.1.1 Using a 500 mL **wide mouth HDPE brown bottle**, collect a **grab sample** at the selected location.

8.4.1.2 Immediately place on ice and ship to the laboratory within 24 hours.

### **8.5 Cyanotoxin Samples**

8.5.1 Sampling for cyanotoxin analysis should occur during an algal bloom investigation if cyanobacteria (bluegreen algae) are suspected to be the dominant bloom forming algae.

8.5.2 Sampling should be prioritized if there is a significant exposure risk to humans and pets through recreational activities such as swimming, boating, and fishing or the affected waterbody serves as a drinking water source.

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8.5.3 The use of plastic, intermediary sample containers should be avoided when sampling for cyanotoxins as plastics readily adsorb the toxins.

**PETG plastic bottles are manufacturer prepared for use with cyanotoxins.**

8.5.3.1 Using a 500 mL **PETG bottle**, collect a **grab sample**.

8.5.3.2 If possible, run a preliminary cyanotoxin analysis using the Abraxis Freshwater Strip Tests ([See Appendix E](#)).

8.5.3.3 Place samples on ice and ship to the lab within 24 to 36 hours.

### **8.6 Water Quality Field Parameters**

8.6.1 Whenever possible, a calibrated field meter should be used to measure water quality field parameters.

8.6.2 Dissolved oxygen (DO), conductivity, pH, salinity (if applicable) and temperature help determine if an active bloom is occurring.

8.6.2.1 Follow the manufacturer's recommended guidelines for calibration and best use.

8.6.2.2 At minimum, measure water quality parameters at the surface (15 cm). If possible, take measurements at the depths indicated in [Appendix C](#).

8.6.2.3 Record all measurements on the *Algal Bloom Field Evaluation Form* ([Appendix C](#)) or a field notebook. Submit this data to the WSS Chemistry Laboratory along with any samples collected.

## **9.0 Bloom Sample Analysis**

### **9.1 Sample Submission**

9.1.1 Collectors should submit a cooler to the courier for delivery to the WSS Chemistry Laboratory and alert WSS Algal Laboratory personnel when samples are in transit.

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9.1.2 WSS Algal Laboratory staff should inform WSS Chemistry Laboratory and Receiving staff of incoming samples including the waterbody name, date sampled, collector and associated Regional Office.

9.1.3 Incoming sample notification can be submitted by email to [SampleNotification@ncdenr.gov](mailto:SampleNotification@ncdenr.gov).

### **9.2 Receiving**

9.2.1 Upon arrival at the WSS Chemistry Receiving room, preserved phytoplankton samples and corresponding cyanotoxin samples shall be assigned a lab number (AC #).

9.2.2 Cyanotoxin samples should be delivered to Chemistry Lab staff responsible for cyanotoxin analysis and analyzed as soon as possible.

9.2.3 Preserved and unpreserved phytoplankton samples should be collected by Algal Laboratory personnel for preliminary analysis of dominant algal species.

### **9.3 Sample Analysis**

9.3.1 Algal Laboratory staff should conduct preliminary microscopic screening of bloom samples to identify dominance by toxigenic algal taxa.

9.3.2 A list of the most common toxigenic algal species is provided in [Appendix F](#).

9.3.3 If toxigenic algal taxa are present, Algal Laboratory staff should conduct preliminary cyanotoxin screening using the Abraxis Freshwater Test Strips if not already performed by field staff.

9.3.4 Following preliminary screening, Algal Laboratory staff should perform a full algal density analysis.

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### **9.4 Reporting Results**

- 9.4.1 If preliminary results from cyanotoxin analysis indicate microcystin concentrations **>10 µg/L** (WHO recreational exposure guidelines), WSS Chemistry staff should alert Algal Laboratory Staff via email as soon as possible.
- 9.4.2 If preliminary results do not indicate significant microcystin concentrations, cyanotoxin results should be verified and made available via Laserfische and/or Labworks within 10 days of the sample arrival.
- 9.4.3 It is the responsibility of Algal Laboratory personnel to communicate cyanotoxin concentrations to DHHS.

### **10.0 Bloom Communication**

- 10.1 If a bloom is positively identified as a HAB, WSS Algal Laboratory staff should notify/submit samples to the Department of Health and Human Services. Cyanotoxin analysis results should also be shared with DHHS as soon they are available from the WSS Chemistry Laboratory.
- 10.2 Regional Supervisors, WQROS staff, WSS Algal Program staff and the DWR Public Information Officer (PIO) should help draft and coordinate a Press Release for identified HABs.
- 10.3 Additionally, the following actions should be taken:
  - 10.3.1 Notify and provide DHHS, LHDs, and any other interested parties with copies of the press release and supporting materials.
  - 10.3.2 If a water supply is at risk, contact the PWSS and the municipality/utility service.
  - 10.3.3 If the affected waterbody is privately owned, notify the property owner and supply them with information on HABs and precautions

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to take.

### **11.0 Continued Monitoring**

- 11.1 Continued monitoring of the affected waterbody may be necessary based on the results of the initial algal bloom investigation and cyanotoxin analysis. However, staff time and resources may be limited. After the initial bloom investigation, key stakeholders should be identified and, if possible, provided the necessary equipment to continue weekly monitoring (i.e. bottles, test strips, etc.).
- 11.2 Monitoring assistance can be sought from Riverkeepers and/or watershed coalitions.
- 11.3 Waterbody monitoring should continue until visual indicators of the bloom have dissipated and cell densities fall below **20,000 cyanobacteria cells/mL** (WHO 2003).
- 11.4 If cyanotoxin (microcystin) concentrations exceeded 10 µg/L, continue monitoring until two samples, taken at least 1 week apart, contain microcystin concentrations <10 µg/L.
- 11.5 Provide weekly updates to any owners and/or LHDs that post advisories or closure.

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# APPENDIX A

### Algal Bloom Response Checklist

Personal Protective Gear		
<input type="checkbox"/> Nitrile/Shoulder Length Gloves	<input type="checkbox"/> Surgical Mask	<input type="checkbox"/> Waders
<input type="checkbox"/> Field Boots	<input type="checkbox"/> Safety Vest	<input type="checkbox"/> Sunscreen/Insect Repellent
<input type="checkbox"/> Personal Flotation Device (PFD)	<input type="checkbox"/> Sunglasses/Safety Glasses	<input type="checkbox"/> Cellular phone or Radio
General Sampling Equipment		
<input type="checkbox"/> In-situ Meter (calibrated)	<input type="checkbox"/> Cage Sampler (bridge sampling)	<input type="checkbox"/> Lab Line (photic zone)
<input type="checkbox"/> Secchi Disc	<input type="checkbox"/> Abraxis Test Strips (microcystins)	
Bottles and Containers		
<input type="checkbox"/> Disposable (x5)	<input type="checkbox"/> Clear PETG (500 mL) (x2)	<input type="checkbox"/> Amber (500 mL)
<input type="checkbox"/> 1 Gallon Ziploc + Paper Towel	<input type="checkbox"/> Glass beaker (100 mL) (x2)	<input type="checkbox"/> Graduated Cylinder (50 mL)
Preservatives		
<input type="checkbox"/> Lugol's Iodine Solution	<input type="checkbox"/> 1:1 H <sub>2</sub> SO <sub>4</sub>	<input type="checkbox"/> Cooler with Ice
Documentation		
<input type="checkbox"/> Sharpie Marker	<input type="checkbox"/> Field Notebook	<input type="checkbox"/> Labels for Bottles
<input type="checkbox"/> Pen/Pencil	<input type="checkbox"/> Algal Report Form	<input type="checkbox"/> Digital Camera
<input type="checkbox"/> Clipboard	<input type="checkbox"/> Chemistry Lab Sheet	<input type="checkbox"/> GPS device
Equipment Maintenance		
<input type="checkbox"/> Nanopure DI Water (1 L)	<input type="checkbox"/> Phosphate-free Detergent	<input type="checkbox"/> Cutting Piers/Knife
SPATTs Sampling (Special Studies)		
<input type="checkbox"/> SPATTs Resin Disc	<input type="checkbox"/> Mesh Bags	<input type="checkbox"/> Rope
<input type="checkbox"/> Weight	<input type="checkbox"/> 1 Gallon Ziploc (collection)	<input type="checkbox"/> Zipties (7-10 inches)

#### Sample Label Information

<b>Station:</b> ESCL170624	<b>Waterbody:</b> Crystal Lake
<b>Date:</b> 6/24/2017	<b>Time:</b> 11:30 AM
<b>Analysis:</b> Phytoplankton	<b>Preservative:</b> Lugol's
<b>Collector:</b> J. Smith	

<b>Station:</b> ESCL170624	<b>Waterbody:</b> Crystal Lake
<b>Date Deployed:</b> 6/24/2017	<b>Date Retrieved :</b> 7/24/2017
<b>Collector:</b> J. Smith	

Phytoplankton, Cyanotoxin,  
Nutrients, and Chlorophyll-a

SPATTs

#### Reporting Checklist

Courier	Sharepoint Upload
<input type="checkbox"/> Phytoplankton Samples	<input type="checkbox"/> Algal Bloom Form
<input type="checkbox"/> Preserved (Lugol's Solution)	<input type="checkbox"/> Site location and pictures
<input type="checkbox"/> Unpreserved	<input type="checkbox"/> In-situ meter readings
<input type="checkbox"/> Cyanotoxin Samples	<input type="checkbox"/> Abraxis Test Strip results
<input type="checkbox"/> Nutrient Samples (if applicable)	<input type="checkbox"/> Number of dilutions
<input type="checkbox"/> Chl-a Samples (if applicable)	<input type="checkbox"/> Microcystin concentration range
	<input type="checkbox"/> Photo of processed test strip

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### **APPENDIX B**

#### **IDENTIFYING ALGAL BLOOMS**

Algae are responsive to physical and chemical conditions in the aquatic environment. Sometimes their rapid reproduction causes nuisance growths or blooms. Most blooms occur when favorable environmental conditions exist, such as an extended photoperiod during summer months, sufficient nutrients, and slow moving stagnant waters. Several indicators of excessive algal growth can be observed in the field and used to determine the steps necessary to effectively investigate a potential algal bloom. This document can be used as a field guide for identification of algal bloom activity and to distinguish between different types of algal blooms including those that would be categorized as potential harmful.

#### **VISUAL INDICATORS:**

##### **Surface Scums**

Surface scums have the appearance of spilled paint forming a film across the water's surface. They can appear in a variety of colors including bright green, red, brown, or even blue. Surface scums are formed by algae that are able to swim or float to the surface where sunlight is readily available for photosynthesis. Common scum forming algal groups include cyanobacteria, euglenoids, and green algae.



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### **Algal Mats**

Algal mats are dense, macroscopic growths of algae that generally float on the surface of the water, but can also be found growing along the bottom. Algal mats form from an accumulation of filamentous algae. Algal groups such as green algae, cyanobacteria, and diatoms contain filamentous species capable of forming algal mats.



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### **Discolored Water**

Algae suspended throughout the water column can cause the water to appear green, brown, red, or even blue. At high densities, all algal groups have the ability to discolor the water. However, a variety of non-algal, environmental factors can also cause water discoloration including suspended minerals and organic matter.



**DISSOLVED OXYGEN AND PH:**  
Elevated photosynthetic activity of high density algal blooms has an observable effect on the dissolved oxygen and pH of a waterbody. A healthy and productive algal bloom typically produces dissolved oxygen concentrations  $\geq 120\%$  saturation and a pH of  $\geq 8$ . If the bloom has died off and begun to decay, it is typical to observe dissolved oxygen concentrations  $\leq 30\%$  saturation.

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### **IDENTIFYING POTENTIALLY HARMFUL ALGAL BLOOMS (HABS)**

An algal bloom that is dominated by cyanobacteria (bluegreen algae) is defined as a potentially harmful algal bloom. Some species of bluegreen algae have the ability to produce toxins (called cyanotoxins) that present a potential health risk to humans and animals that come into contact with the bloom. While microscopic identification is required to verify the presence of bluegreen algae, there are several simple field techniques that can be used as preliminary indicators of a HAB.

#### **VISUAL INDICATORS OF BLUEGREEN ALGAL BLOOMS:**

**Surface films/discolored water that appears bright green to deep bluegreen in color**



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### **VISUAL INDICATORS OF BLUEGREEN ALGAL BLOOMS:**

**Blue and white surface scums associated with decaying bluegreen algae**



**Bluegreen flecks and “grass clippings”**



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### **Special Exception: *Lyngbya Wollei***

*Lyngbya wollei* is a filamentous, bluegreen algae that forms thick floating mats. These mats appear similar to filamentous green algae. However, *Lyngbya wollei* can be distinguished by a strong, musty smell that is generally not associated with green algal mats. *Lyngbya wollei* may appear dark brown or black in color, earning it the common name “Black Mat Algae”.



## SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS

# APPENDIX C

### Sample Collection & Field Evaluation Form For Algae, Aquatic Plants and Related Organisms

SAMPLE INFORMATION						
Sampler Name(s): <input type="text"/>		Agency: <input type="text"/>				
Date: <input type="text"/>	Time: <input type="text"/>	Station #: <input type="text"/>				
Waterbody/Address: <input type="text"/>		Basin: <input type="text"/>	County: <input type="text"/>			
Latitude: <input type="text"/>	Longitude: <input type="text"/>	HUC: <input type="text"/>				
Sample Type: <input type="checkbox"/> Filamentous Algae <input type="checkbox"/> Periphyton <input type="checkbox"/> Phytoplankton <input type="checkbox"/> Aquatic Plant <input type="checkbox"/> Unknown						
Collection Method: <input type="checkbox"/> Photic Zone <input type="checkbox"/> Grab <input type="checkbox"/> Scoop Attached: <input type="checkbox"/> Map <input type="checkbox"/> Photographs (PLEASE)						
Other Samples Collected: <input type="checkbox"/> Nutrients <input type="checkbox"/> Chlorophyll- <i>a</i> <input type="checkbox"/> Cyanotoxins: <input type="checkbox"/> Other:						
Algal Bloom Response? <input type="checkbox"/> Yes <input type="checkbox"/> No		Fish Kill Response? <input type="checkbox"/> Yes** <input type="checkbox"/> No				
ENVIRONMENTAL CONDITIONS						
Weather Conditions: <input type="text"/>						
Water Clarity: <input type="checkbox"/> Clear <input type="checkbox"/> Turbid <input type="checkbox"/> Tannic <input type="checkbox"/> Green <input type="checkbox"/> Other (Explain) <input type="text"/>						
Characteristics: <input type="checkbox"/> Filaments <input type="checkbox"/> Balls <input type="checkbox"/> Flecks <input type="checkbox"/> Surface Film <input type="checkbox"/> Other <input type="text"/>						
Algal Color: <input type="text"/>		Algal Abundance: <input type="text"/>		% Coverage		
Secchi Depth: <input type="text"/> meters		Bottom Depth: <input type="text"/> meters				
CHEMICAL AND PHYSICAL SAMPLE DATA						
Depth (m)	Cond (µS)	Temp (°C)	DO (mg/L)	DO (%sat)	pH (SU)	Salinity (ppt)
0.15 (surface)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
1.0	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
2.0	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
3.0	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
4.0	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

**NOTES:**

°C = degrees Celsius      Cond = conductivity      m = meter      ppt = parts per trillion  
 %sat = percent saturation      DO = dissolved oxygen      µS = microSiemens      SU = standard units

#### SAMPLE INSTRUCTIONS

Sample collection should follow Standard Operating Procedures for the Collection and Analysis of Aquatic Algae (2016) and the Standard Operating Procedures Manual: Physical and Chemical Monitoring (2013). Sample quantities, handling requirements, and preservation methods are below. All samples should be kept on ice or in a refrigerator for storage and shipping.

Sample Type	Preservation Method	Quantity if Preserved	Quantity if Unpreserved
Filamentous Algae	Wet paper towel, plastic bag	N/A	2-3 golf-ball sized clumps
Periphyton	500 mL jar, 4mL Lugol's	50 milliliters of scrapings	2 small (4-inch) rocks/wood
Phytoplankton	500 mL jar, 2mL Lugol's	500 mL	500 mL
Aquatic Plant	Wet paper towel, plastic bag	N/A	3-5 stems, leaves, flowers, fruits
Cyanotoxin	500 mL PETG bottle	N/A	500 mL

Send this form along with the sample and any supplemental information to the address included on the reverse. Copies of this and other sample collection forms can be obtained by calling (919) 743-8400 or visiting [www.deq.nc.gov](http://www.deq.nc.gov).

# SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS

## APPENDIX D

### Water Sample Collection & Submittal Form

North Carolina Division of Water Resources Laboratory (Water Sciences Section)										Water Sample Collection & Submittal Form										Visit ID: (optional)		Tag ID		Lab Use Only:							
Location Description:										Location Code:										Laboratory Sample Number:		Date Received:		Time Received:		Received By:					
County:					Collector:					Priority:		Water Matrix:		Location Type:						Delivery Method:		State Courier		Hand Delivered		Other:					
DWR Region: (must be county)					DWR Office: (or agency name)					Ambient		Surface		River/Stream		Lake		Conductivity		Temperature on Arrival (°C):		Salinity (ppt):		Other:		Other:					
River Basin:					Date:					Routine		Ground		Estuary		Canal		Dissolved Oxygen (ppm):		Conductivity (µmhos/cm):		Solubility (pp):		Other:		Other:					
Notes:					Time:					Compliance		Waste		Stormwater		Water Supply		Dissolved Oxygen (ppm):		Conductivity (µmhos/cm):		Solubility (pp):		Other:		Other:					
Chlorinated					De-chlorinated in Field					Sampling Method:		Grab Other:		Composite		Filtered in Field		Dissolved analysis: Enter "DES" in test-tubes for parameters		Sample Depth:		Temperature on Arrival (°C):		Salinity (ppt):		Other:					
Collector's Contact										Field Parameters (optional)										Preservative: Y		Water Temp (°C):		pH (n.u.):		Dissolved Oxygen (ppm):		Conductivity (µmhos/cm):		Salinity (ppt):	
LAB COMMENTS :																															
Microbiology Parameters:							Wet Chemistry Parameters:							Metals Parameters:							Metals Parameters Cont:										
Alkalinity as CaCO <sub>3</sub> , to pH 4.5/8.3							Bromide							Aluminum (Al)							Strontium (Sr)										
BOD: Biochemical Oxygen Demand, 5-day							Chloride							Antimony (Sb)							Thallium (Tl)										
cBOD: Carbonaceous BOD, 5-day							Fluoride							Arsenic (As)							Tin (Sn)										
Coliform: Fecal MF							Sulfate							Barium (Ba)							Titanium (Ti)										
Coliform: Total MF							Chlorophyll a							Beryllium (Be)							Vanadium (V)										
Specific Conductance, at 25 °C							Color: ADM 1							Cadmium (Cd)							Zinc (Zn)										
TOC - Total Organic Carbon							Color: Platinum Cobalt							Calcium (Ca)							Mercury (Hg) low level										
Turbidity							COD: Chemical Oxygen Demand							Chromium (Cr), Total							Boron (B)										
Other Parameters:							Cyanide, Total							Cobalt (Co)							Organics Parameters:										
pH							Formaldehyde							Copper (Cu)							Acid Herbicides										
Hardness, Total as CaCO <sub>3</sub> - by filtration							Hexavalent Chromium (Cr6)							Iron (Fe)							Organochlorine Pesticides										
Nutrients Parameters:							MBAS (surfactants)							Lead (Pb)							Organonitrogen Pesticides										
Ammonia as N (NH <sub>3</sub> -N)							Oil and Grease, HEM, Total Recoverable							Lithium (Li)							Organophosphorus Pesticides										
Nitrate-Nitrite as N (NO <sub>3</sub> +NO <sub>2</sub> -N)							Phenols, Total Recoverable							Magnesium (Mg)							PCBs (polychlorinated biphenyls)										
Total Kjeldahl Nitrogen as N (TKN)							Residue: Total (Total Solids)							Manganese (Mn)							Semi-Volatile Organics (SVOCs)										
Total Phosphorus as P (TP)							Residue: Volatile/Fixed, Total							Mercury (Hg)							TPH Diesel Range										
Nitrite as N (NO <sub>2</sub> -N)							Residue: Sedimentable							Molybdenum (Mo)							Volatile Organics (VOCs)										
Nitrate as N (NO <sub>3</sub> -N calculated)							Residue: Suspended (Suspended Solids)							Nickel (Ni)							1,4-Dioxane										
Orthophosphate as P (PO <sub>4</sub> )							Residue: Volatile/Fixed, Suspended							Potassium (K)							TPH Gasoline Range										
Cyanotoxins:							TDS - Total Dissolved Solids							Selenium (Se)							Biological:										
Microcystin							Silica							Silver (Ag)							Phytoplankton / Algae										
							Sulfide							Sodium (Na)																	
							Tannin & Lignin																								
Preservative Legend (circle above as needed): (A) cool <10°C, (B) cool <5°C, (C) 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub> (when chlorine is present), (D) H <sub>2</sub> SO <sub>4</sub> to pH <2, (E) HNO <sub>3</sub> to pH <2, (F) HCl to pH <2, (G) H <sub>3</sub> PO <sub>4</sub> to pH <2, (H) 10N NaOH to pH >10, (I) pH >8.3-8.7, (J) zinc acetate & NaOH to pH >9, (K) pH 8-9 ascorbic acid (when chlorine is present), (L) EDTA, (M) Na <sub>2</sub> SO <sub>3</sub> to pH <2, (N) 6% EDTA, (O) ammonium sulfate buffer pH >8.3-8.7, (P) ascorbic acid (when chlorine is present), (Q) Lugol's, (S) Na <sub>2</sub> SO <sub>3</sub> to pH <2, (V) analyzed within 6 minutes of sample collection, (Z) filtered in field within 6 minutes																															
Required: 1) Collector Initials and Date, 2) Circling of Utilized Preservative next to each Parameter																															
Revision: 2/9/2016																															

## **SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS**

# **APPENDIX E**

## **ABRAXIS ALGAL TOXIN STRIP TEST (MICROCYSTINS) FOR RECREATIONAL WATERS**

### **1.0 Overview**

- 1.1 The Abraxis Freshwater Strip Tests provide a rapid assessment for the detection algal toxins in freshwater systems.
- 1.2 Abraxis currently produces four kit varieties specific to one of three cyanotoxins: Microcystins (Recreational and Drinking Water), Anatoxin-a, and Cylindrospermopsin.
- 1.3 Instructions within this document are specific to the *Abraxis Algal Toxin Strip Test (Microcystins) for Recreational Waters* and should not be applied to other toxin kits.
- 1.4 Guidance on the use of other algal toxin kits can be found on the ABRAXIS website, <https://www.abraxiskits.com/products/algal-toxins/>.

### **2.0 Limitations**

- 2.1 DWR is currently evaluating the use of the Abraxis test strips as a tool for monitoring potentially harmful algal blooms (HABs) in North Carolina.
- 2.2 The accuracy and reliability of these test results has yet to be confirmed by DWR. Therefore, discretion is advised when interpreting the results of a test strip.
- 2.3 The kits are not designed to act as a confirmed quantitative analytical test, but rather function as a preliminary qualitative indicator of cyanotoxins.
- 2.4 Additional cyanotoxin testing using a more sophisticated methodology (i.e., ELISA) should be run in conjunction with the test strips to confirm any preliminary results.

## **SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS**

### **3.0 Sampling**

#### 3.1 When to Sample

- 3.1.1 Sampling for cyanotoxin analysis using the Abraxis test strips should occur during an algal bloom investigation if cyanobacteria (bluegreen algae) are suspected to be the dominant bloom forming algae. Refer to [Appendix B](#) of the *Algal Bloom and Cyanotoxin—Field Collection and Response Protocol* for information on field identification of bluegreen algal blooms.
- 3.1.2 Sampling should be prioritized if there is a significant exposure risk to humans and pets through recreational activities such as swimming, boating, and fishing.
- 3.1.3  Samples should be collected between the hours of **10:00 am and 3:00 pm** when possible. Bluegreen algae are typically the most active during this time and tend to float to the surface.

#### 3.2 Where to Sample

- 3.2.1 Near public access areas such as boat ramps and beaches. This will provide the most relevant data to public exposure risk.
- 3.2.2 Where the bloom appears to most concentrated. Bluegreen algal blooms can form surface scums or be suspended in the water column. Select a sampling location that is representative of the “worst case scenario” for potential exposure to cyanotoxins.
- 3.2.3 Keep in mind that blooms may change location due to wind or wave action.

#### 3.3 How to Sample

- 3.3.1 All blooms should be treated as potentially harmful until proven otherwise. Be sure to observe all necessary safety measures before collecting for cyanotoxin analysis.

## **SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS**

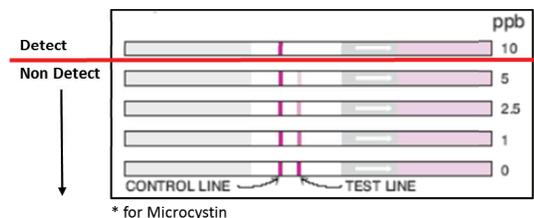
- 3.3.2 Cyanotoxin samples should be collected in a **500 mL PETG bottle** and can be sampled as either a photic zone, grab, or surface skim depending on the distribution of the bloom.
- 3.3.3 Collect the sample so that it is representative of the phytoplankton concentrations found at the sample site (try not to dilute or concentrate the sample during collection).
- 3.3.4 Pour off ~50 mL of sample from the PETG bottle into a clean 100 mL beaker to begin the Test Strip Analysis procedure. The remaining sample can be couriered to the WSS Chemistry Laboratory for an ELISA test for cyanotoxins.
- 3.3.5 Two phytoplankton samples should also be collected at the site (one preserved with Lugol's solution and one unpreserved) and sent to the algal lab for identification and enumeration of the phytoplankton community.

### **4.0 Analysis**

- 4.1 The Abraxis Strip Test can be processed and analyzed in the field, or samples can be held on wet ice and analyzed at the regional office.
- 4.2 Directions for processing cyanotoxin samples are included on [page 26](#).
- 4.3 Check that the test kits have not expired and allow all testing materials and water sample to come to room temperature before running the analysis.
- 4.4 Results of the test strips should be read between 5-10 minutes after processing is complete.
- 4.5 Results will be interpreted as either detect or non-detect for microcystins.

## SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS

4.5.1 A test result of detect will be reported at >10 ppb, indicated by only the control line being present.



4.5.2 A test strip showing both the control line and test line will be reported as a non-detect < 10 ppb.

4.5.3 A test strip that shows the test line with no control line is void and the analysis should be run again.

4.6 Document each test strip with a photo to include with your site assessment.

4.7 When a test strip indicates microcystin concentrations > 10 ppb, it will be necessary to perform a sample dilution and retest in order to determine an approximate cyanotoxin concentration range.

4.7.1 Use a graduated cylinder to measure 25 mL of DI water and 25 mL of your cyanotoxin sample and add to clean 100 mL beaker. This will result in a 50% dilution.

4.7.2 Run the analysis on the diluted sample. If the second analysis results in a detect for microcystins (> 10ppb), perform a second dilution by combining 25 mL of the 50% dilution sample and 25 mL of DI water and rerun the analysis.

4.7.3 Repeat until the test strips result in a non-detect for microcystins.

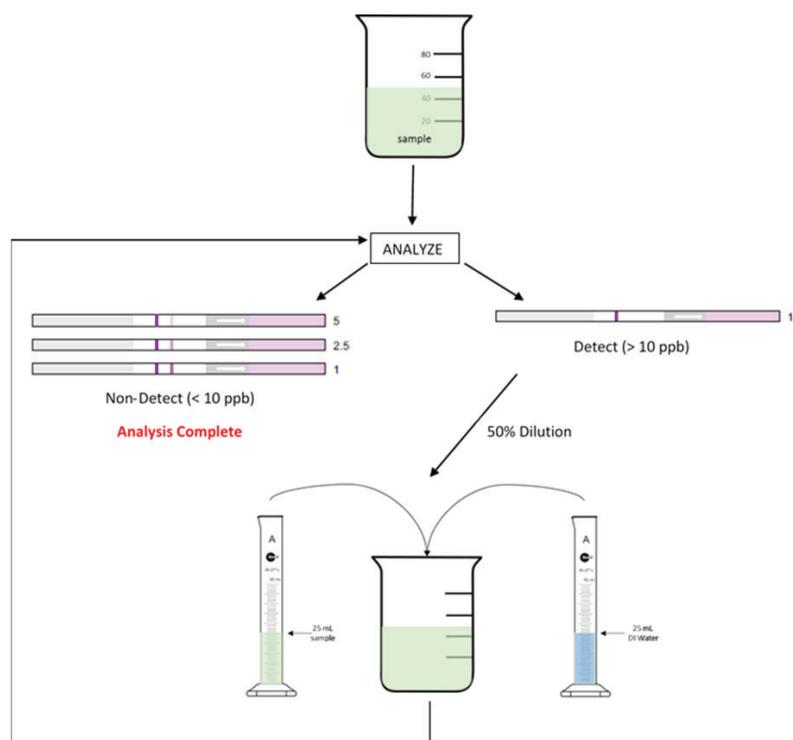
4.7.4 Use Table 1 to determine the range of microcystins present in the original sample based on the number of dilutions necessary to receive a non-detect result.

### 5.0 Reporting

5.1 Report all algal bloom investigations to the WSS Algal Laboratory

## SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS

- 5.2 Submit a prepared cooler to the courier for delivery to the WSS Chemistry Laboratory at the earliest time possible.
- 5.3 Email a summary of the site visit to Algal Program staff with all forms and pictures attached.



**TABLE 1. MICROCYSTINS RANGE INDICATED BY NUMBER OF DILUTIONS NECESSARY TO PRODUCE “NON-DETECT” READING**

Number of Dilutions to Non-Detect	Microcystins Range (ppb)
0	< 10
1	10-20
2	20-40
3	40-80
4	> 80

# SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS

## Algal Toxin Strip Test (Microcystins) Recreational Water

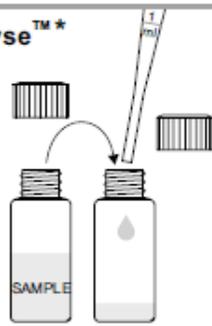
### 1. Collect Sample



Collect 1 to 2 mL of sample.

### 2. Transfer/QuikLyse™\*

Using the graduated pipette provided, transfer 1 mL of SAMPLE to the lysis vial containing the dried lysis reagent.



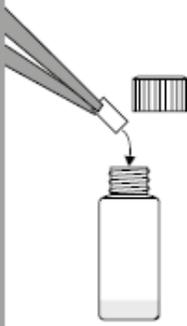
\*QuikLyse™ reagents may be used in a method of U.S. Patent 9,739,777

Cap and shake for 2 minutes.

Let rest for 8 minutes.

### 3. Add Reagent Paper/QuikLyse™\*

Using the forceps provided, add 1 reagent paper to the lysis vial.

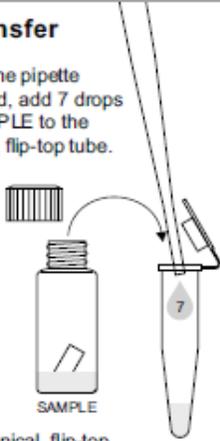


Cap and shake for 2 minutes.

Let rest for 8 minutes.

### 4. Transfer

Using the pipette provided, add 7 drops of SAMPLE to the conical, flip-top tube.



(The conical, flip-top tube contains dried reagents.)

### 5. Shake and Incubate

Close the conical, flip-top tube and shake for 30 seconds.



(Dried reagents will dissolve, turning the sample purple.)

### 6. Test

Insert test strip into conical, flip-top tube with arrow pointing down. (sample pad down).



Incubate for 10 minutes.



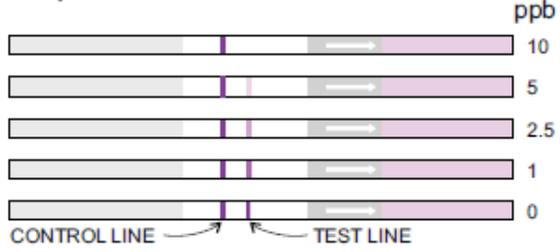
### 7. Dry

Remove test strip. Lay flat and allow to continue developing for 5 minutes.




### 8. Interpret

ppb



CONTROL LINE      TEST LINE

INTERPRET TEST

CONTROL LINE	TEST LINE	INTERPRETATION
NO CONTROL LINE PRESENT	NO TEST LINE PRESENT	INVALID RESULT
CONTROL LINE PRESENT	NO TEST LINE PRESENT	> 10 ppb
CONTROL LINE PRESENT	MODERATE INTENSITY TEST LINE PRESENT	BETWEEN 0 AND 10 ppb

For Ordering or Technical Assistance Contact:  
 ABRAXIS, INC. 124 Railroad Drive, Warminster, PA 18974 Phone: 215-357-3911 Fax: 215-357-5232 www.abraxiskits.com

## SOP FOR SAMPLING OF ALGAL BLOOMS AND CYANOTOXINS

# APPENDIX F

## CYANOBACTERIA KNOWN TO PRODUCE MAJOR CLASSES OF CYANOTOXINS

TOXIN	PUBLISHED PRODUCERS	
MICROCYSTINS	<b>Chroococcales:</b>	<i>Microcystis spp.</i>
	<b>Oscillatoriales:</b>	<i>Oscillatoria spp.</i> <i>Planktothrix spp.</i> <i>Arthrospira sp.</i>
	<b>Nostocales:</b>	<i>Anabaena spp.</i> <i>Nostoc sp.</i> <i>Anabaenopsis sp.</i> <i>Gloeotrichia sp.</i> <i>Rivularia spp.</i>
NODULARINS	<b>Nostocales:</b>	<i>Nodularia sp.</i>
ANATOXIN-A	<b>Oscillatoriales:</b>	<i>Arthrospira sp.</i> <i>Oscillatoria sp.</i>
	<b>Nostocales:</b>	<i>Anabaena spp.</i> <i>Aphanizomenon sp.</i> <i>Cylindrospermum sp.</i> <i>Raphidiopsis sp.</i>
SAXITOXINS	<b>Oscillatoriales:</b>	<i>Lyngbya wollei</i> <i>Planktothrix sp.</i>
	<b>Nostocales:</b>	<i>Aphanizomenon sp.</i> <i>Anabaena sp.</i> <i>Cylindrospermopsis sp.</i>
CYLINDROSPERMOPSINS	<b>Nostocales:</b>	<i>Cylindrospermopsis sp.</i> <i>Aphanizomenon sp.</i> <i>Anabaena spp.</i> <i>Raphidiopsis sp.</i>

\*Adapted from Metcalf & Codd, 2012