

Project QAPP/Workplan:

**Cyanobacteria Monitoring on Lake Champlain
EPA RFA# 18076**

Prepared by:
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Prepared for:
Lake Champlain Basin Program
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June 1, 2018

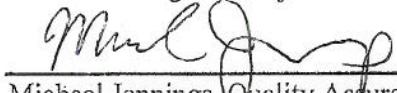
Angela Shambaugh, Project Manager, VT DEC	Date
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Bridget O'Brien, VT Dept. of Health	Date
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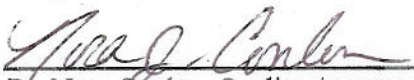
Lori Fisher, Lake Champlain Committee	Date
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Heather Campbell, DWGWP, VT DEC	Date
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 Matthew CH Vaughan, Project Officer, LCBP	2018-06-01
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 Michael Jennings, Quality Assurance Program Manager, NEIWPC	6/4/18
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MaryJo Feuerbach, Project Officer, EPA	Date
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 Dr. Nora Conlon, Quality Assurance Reviewer, EPA	6/7/2018
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Bethany Sargent, Vermont Lake Champlain Project Coordinator, VT DEC	Date
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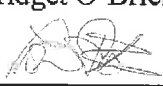
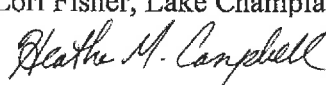
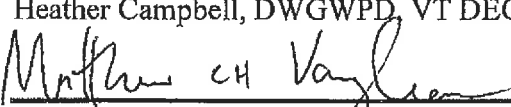

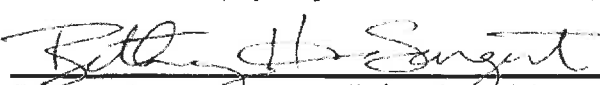
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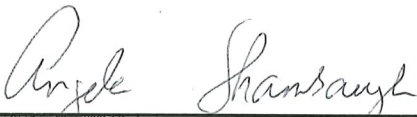
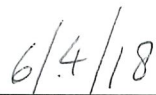
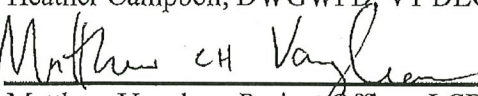
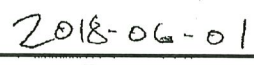
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Table of Contents

A - Project Management.....	3
A1 - QAPP Distribution List.....	3
A2 - Project Organization	4
A3 – Problem Definition/Background	5
A4 – Project/Task Description	5
A5 – Quality Objectives and Criteria for Measurement Data	7
A6 – Special Training Requirements/Certifications	7
A7 – Documentation and Records	7
B – Measurement/Data Acquisition.....	8
B1 – Sampling Design	8
B2 - Allocation of Project Responsibilities.....	9
B3 – Sampling and Analysis Methods	10
B4 – Sample Handling and Custody	13
B5 – Analytical Methods	15
B6 – Quality Control Requirements.....	15
B7 – Instrument/Equipment Testing, Inspection, and Maintenance	17
B8 – Instrument/Equipment Calibration and Frequency	17
B9 – Inspection Acceptance of Supplies and Consumables	17
B10- Data Management	17
C – Assessment/Oversight.....	18
C1 – Assessments and Response Actions	18
C2 – Reports to Management	18
D – Data Validation and Usability.....	18
E – Project Outputs and Deliverables	18
F - Budget.....	19
G - References	19
Appendix A – Core locations to be monitored in 2018.	21
Appendix B – Task Allocation by Partner	22
B1 - Lake Champlain Committee (LCC)	22
B2 - Vermont Department of Health (VDH)	23
B3 - VT DEC, Drinking Water and Groundwater Protection Division (DWGWD)	23
B4 - VT DEC, Watershed Management Division (WsMD)	24
Appendix C –Field Sampling Protocols	27
C1 - Phytoplankton Collection.....	27
C2 - Toxin Sample Collection	28
C3 - Field Sampling Parameters	28
Appendix D – Laboratory Protocols	29
D1 - Plankton for identification and enumeration.....	29
D2 - Anatoxin Analysis – Vermont Department of Health.....	30
D3 - Microcystin by ELISA – Vermont Department of Health	30
D4 - Cylindrospermopsin by ELISA	33
Appendix E – LCC Volunteer Monitoring Protocols.	35
E1 – LCC On-line reporting form.....	35
E2 – LCC Guidance on Determining Cyanobacteria Bloom Intensity	38
Appendix F – Forms	48
F1 - VT DEC Staff Field Form	48
F2 - Plankton Counting Software	49

A - Project Management

A1 - QAPP Distribution List

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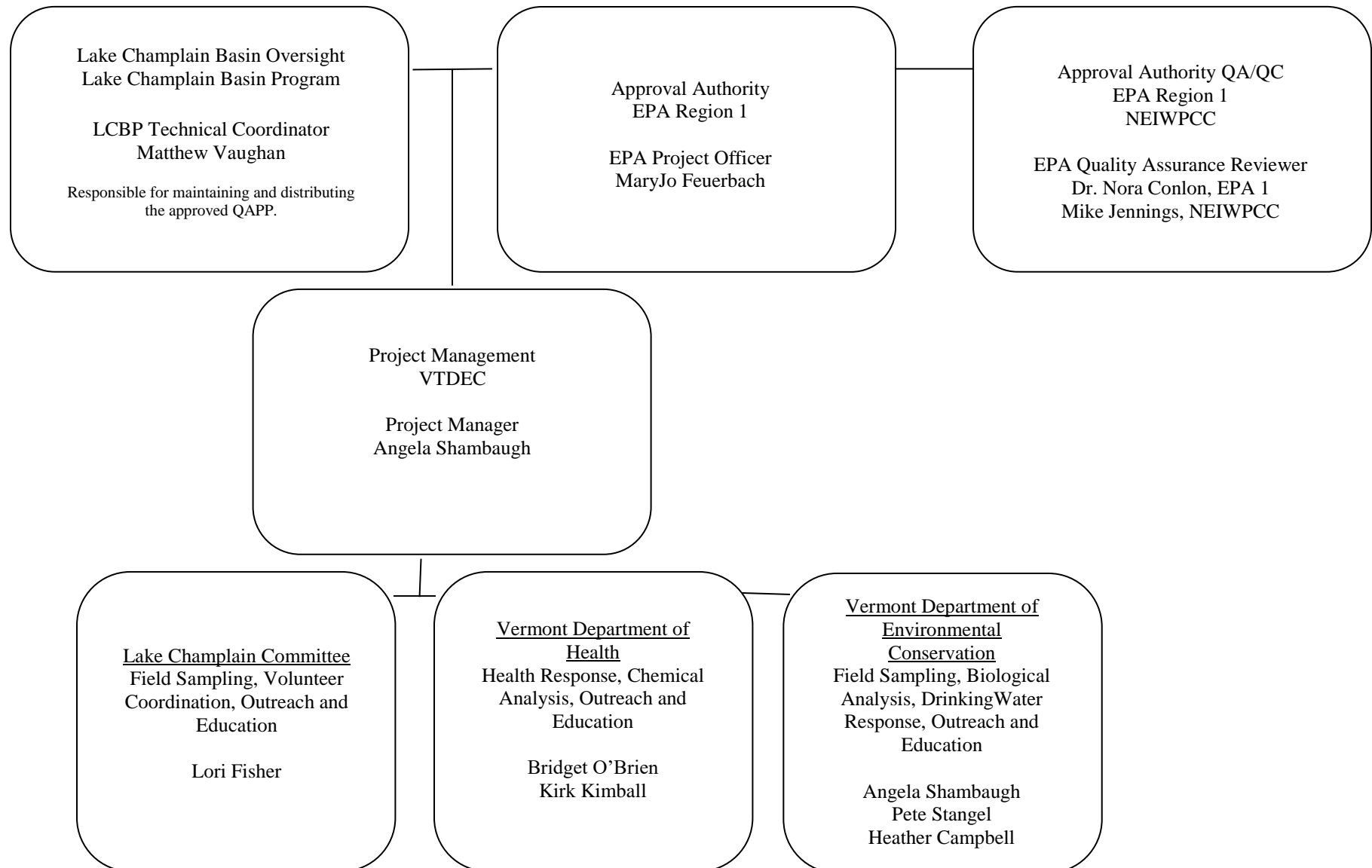
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A2 - Project Organization



A3 – Problem Definition/Background

Lake Champlain is one of the largest lakes in the United States and an important water resource for the states of Vermont and New York, and the province of Quebec. It is primarily a recreational lake, but also serves as an important drinking water source for all three jurisdictions. Cyanobacteria blooms have been documented in the lake since the 1970s, with some areas experiencing extensive annual blooms. In 1999, several dog deaths were attributed to cyanobacteria toxins, raising health and safety concerns regarding drinking water supplies and recreational activities such as swimming, boating and fishing.

Cyanobacteria are a natural and normal component of the phytoplankton in fresh water and are present in Lake Champlain during much of the summer (Shambaugh et al. 2017, LTM unpublished data). Cell counts and associated toxins are below levels of concern for recreation in most of the lake each year (Shambaugh et al., 2017). St. Albans and Missisquoi Bays typically experience annual periods of high cyanobacteria density lasting several weeks, though conditions do not always exceed Vermont's recreational guidance criteria as a result.

Beginning in 2002, the Lake Champlain Basin Program funded an annual cyanobacteria monitoring program which utilized cell density and toxin data to evaluate recreational conditions around the lake. Results were communicated to stakeholders around the region through weekly updates. The University of Vermont (UVM) developed and implemented the program, in cooperation with the Lake Champlain Committee (LCC) and the Vermont Departments of Health (VDH) and Environmental Conservation (VT DEC). It was well received locally and continues to serve as a model at the regional and national level.

Since 2012, oversight of the cyanobacteria monitoring program on Lake Champlain has resided with the State of Vermont, with support from the Lake Champlain Basin Program (LCBP). A visual assessment protocol for use by trained volunteers was added in 2012, allowing the monitoring network to expand to underserved areas of the lake and provide the data necessary to inform recreational and public health response in a fiscally sustainable program. Since 2012, monitoring also occurs on other lakes in the Champlain Basin under the auspices of this program. An on-line tracking tool developed by the VDH now provides information to the public (<http://healthvermont.gov/tracking/cyanobacteria-tracker>). In 2018, the program will continue to facilitate communication among environmental and public health officials, support an appropriate and consistent response during bloom events on Lake Champlain and Vermont inland lakes, inform the public and drinking water suppliers about current cyanobacteria conditions, and educate the public to recognize and avoid blooms.

A4 – Project/Task Description

Objectives of Project

Cyanobacteria do not always produce toxins nor is it possible to visually determine if toxins are present. VDH recreational guidance includes criteria based on the presence of visible scum and/or analytical documentation of toxins (VDH 2015). Utilizing a visual monitoring system developed by the LCC from VDH guidance to Vermont communities, volunteers and state staff will provide weekly assessments of cyanobacteria conditions around the lake. Quantitative data (cell counts and

toxin levels) will be collected from core shoreline and open water locations on a weekly/biweekly basis to inform response, outreach and evaluate the overall performance of the visual protocol. Through condition updates posted on the VDH cyanobacteria webpage, lake users and the public will have access to information that enables them to reduce their exposure to potentially toxic cyanobacteria. A weekly email from the Project Manager will convey recent status and bloom observations to researchers, health and environmental officials, drinking water supply managers and monitoring program participants who are responsible for cyanobacteria response at numerous private and public organizations around the lake. Additionally, the data will contribute to the knowledge and perspective about these organisms that has been gained since the program's inception in 2002.

As awareness of cyanobacteria blooms and associated health concerns has increased, both Vermont and New York have increased monitoring and bloom response activities. The cyanobacteria monitoring effort covered by this project continues to be supported by statewide resources brought to bear through the VDH, VTDEC, NYDEC and NYDOH. Examples of this include the CyanoTracker map developed and maintained by the VDH, the toxin testing conducted by VDH, and the inland lake monitoring that occurs in VT and NY outside of the Basin. Activities included under this QAPP will be limited to recreational monitoring in the Champlain Basin directly funded by the LCBP or supported by the partners – open water sampling conducted as part of the Champlain Long-term Water Quality and Biological Monitoring Project, volunteer recruitment and coordination by the LCC, recreational toxin testing by the VDH, and information provided by NY state staff. Inland lake monitoring by Vermont, NY and Quebec outside of the Champlain Basin is not included. Vermont Drinking water facility cyanotoxin testing continues but is not covered by this QAPP.

Project tasks and associated timelines are noted in Table 1.

Table 1. Timeline for project tasks. *if conditions warrant

Task	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Field Collection	x	x	x	x	*							
Volunteer Monitoring	x	x	x	x	*							
Laboratory Analysis	x	x	x	x	x							
Email/Web updates	x	x	x	x	x							
Data entry	x	x	x	x	x							
Data QA/QC	x	x	x	x	x	x	x	x				
Data analysis								x	x	x		
Quarterly Reports		x			x			x			x	
QAPP update memo												x
Final Report												x

The Project Manager has an additional facilitation and technical role outside of the annual summer monitoring activities. On behalf of the State of Vermont and the LCBP, she is tasked with expanding technical expertise and knowledge concerning cyanobacteria control options, taxonomy and monitoring methods; fostering basin and state-wide consistency in monitoring and bloom response; providing technical assistance to the Drinking Water and Groundwater Protection Division and drinking water facilities around Vermont; and supporting coordination at the regional level to develop consistency in monitoring and response activities in the Northeast.

Duration of this Document

This project typically makes only minor changes each year. Beginning with 2018, the QAPP became a five-year document and will expire March 31, 2023. The project manager is responsible for providing an annual memo to the QAPP distribution list by May detailing any changes to the QAPP or workplan. Should a signatory or project team member request a renewed approval process because of a significant change, this QAPP will expire and a revised draft will be circulated for approval.

A5 – Quality Objectives and Criteria for Measurement Data

Data quality will be measured in terms of accuracy and precision, completeness, representativeness, comparability, and the required detection limits for the analytical methods. Acceptance criteria and corrective actions are noted in the methods section of this QAPP where applicable.

A6 – Special Training Requirements/Certifications

Core team members at each partner institution, identified in section A2, are career professionals. Most have been involved in cyanobacteria monitoring program activities for 5 or more years. Core team members are fully trained and experienced in ambient sample collection for both phytoplankton and toxin parameters. They are up-to-date with equipment use and field protocols. No additional specialized training is required for field aspects of this project conducted by core team members. All temporary and seasonal field staff, including members of the volunteer network, are under the supervision of the core team members. VDH Laboratory personnel are supervised by the laboratory director and meet the training and certification requirements specified by the Laboratory.

Taxonomic expertise is required for the analysis of phytoplankton samples. Analyses at the Vermont DEC will be conducted under the supervision of a taxonomist with more than 30 years of experience identifying freshwater plankton from Lake Champlain. Taxonomists will work together to ensure consistency in sample identification and enumeration between analysts.

A7 – Documentation and Records

Current and identical copies of the Project Work Plan will be provided in electronic format to the partners by the Project Manager (VT DEC).

VT DEC will document field-generated data on Field Log Sheets (Appendix F). Online reporting forms are available for volunteers who prefer not to use the reporting web interface. (Appendix E). Digital photographs may provide additional documentation of field conditions during the assessment. Original field sheets and any online forms will reside with the VT DEC and the LCC, respectively. The VDH, LCC and VT DEC are responsible for uploading information to the VDH CyanoTracker including staff reports, reports from volunteers who may lack internet access, and reports received from the public. Downloaded summaries will be included by the VT DEC in weekly updates circulated to an established user group.

VT DEC and VDH maintain central cyanobacteria databases for all of Vermont. Data covered by this QAPP will be maintained as part of those central databases. Photographs of event conditions referenced to specific reports are housed at the VDH and provided to the VT DEC upon request.

LCC provides weekly emails to monitors that summarize weekly reporting results along with reporting guidance and links to useful information. Separate weekly emails are sent to a listserve of over 500 interested citizens and agencies. A third email is sent to media throughout the season. LCC maintains copies of these updates.

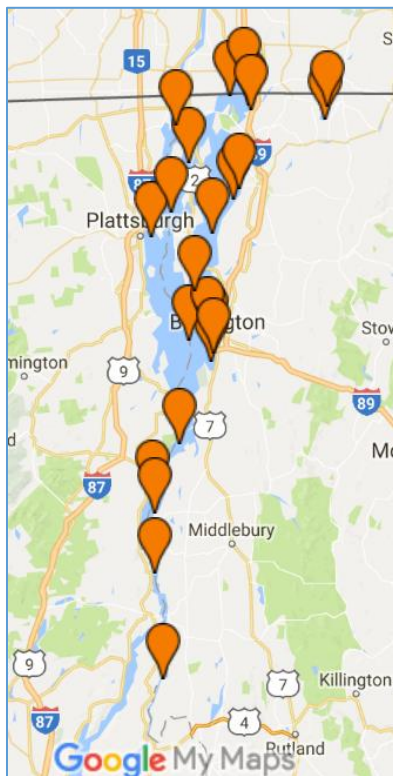
B – Measurement/Data Acquisition

B1 – Sampling Design

Sampling locations represent the range of water quality and cyanobacteria conditions in Lake Champlain. Historically, higher cell and toxin concentrations in the lake have been documented along shorelines, thereby increasing the potential for human exposure during recreational activities (Watzin et al. 2003, 2004, 2006). The LTM open water stations are historical locations providing long-term perspectives on lake water quality. The primary objective of this monitoring program is reduction of recreational exposure to cyanobacteria. We will achieve this by evaluating both shoreline and mid-lake stations for the presence of cyanobacteria and/or cyanotoxins.

Core recreational locations to be sampled by the VT DEC, VDH and LCC in 2018 are shown in Figure 1 and listed in Appendix A. Additional volunteer sites will be identified during the annual training sessions. In recent years, more than 100 volunteers have participated in the program each summer. We expect that many of these will return in 2018. We also anticipate that additional volunteer sites will be added based on LCC's year-round recruitment efforts. Volunteer locations also serve an outreach function. As a result, they may not be associated with areas of high population or recreational usage and may be located in close proximity with other monitoring sites. A final list of 2018 field sites, including a map and a table with latitude and longitude, will be provided in the annual project report to the LCBP.

Figure 1. Core Monitoring Locations in 2018 Additional volunteer locations will be identified through the training sessions.



A secondary goal of the project is to provide pertinent information to drinking water supply facilities. In addition to producing toxins, cyanobacteria can pose treatment challenges for surface water public water systems: pH increases, shortened filter run times, increased chlorine demand, increased turbidity, increased disinfection byproduct formation, or altered taste and odor. Data provided from the recreational monitoring sites, through the weekly email updates, inform drinking water operators around the lake of current conditions and heighten awareness of bloom conditions.

B2 - Allocation of Project Responsibilities

This project is a continuation of the partnership established in 2002. Partners include the VT DEC (Watershed Management and Drinking Water/Groundwater Protection Divisions), the LCC, and the VDH (Radiological/Toxicological and Laboratory Sections). Project responsibilities are summarized in Table 2 and discussed in detail in Appendix B. Data collected by the partners will be uploaded directly to the VDH web interface and utilized by the VT DEC in the weekly email updates.

Table 2. Summary of project responsibilities by partner.

Task	Vermont DEC		LCC	VDH	
	Watershed Management Division	Drinking Water/Groundwater Protection Division		Laboratory	Radiological & Toxicological Program
Field Collection	x		x		x
Toxin Analysis				Anatoxin, microcystin, cylindrospermopsin	
Phytoplankton counts	x				
Volunteer Coordination	x		x		
Volunteer Training	x		x		
Review/approval reports from volunteers and public	x		x		x
Public Health Response				x	x
Drinking Water Supply Response		x		x	x
Weekly Email updates	x		X		
Webpage and Tracker Map Maintenance					x
Field/ lab data QA/QC	x		X	x	x
Project Coordination	x				
Maintenance of Databases	x		x		x
Annual Report	x				
Outreach	x	x	x		x

B3 – Sampling and Analysis Methods

Monitoring and Field Collection

Assessment protocol and frequency are noted for each sampling location in Table 3. The number of samples and the analysis type will be determined by the respective assessment protocol and the extent of cyanobacteria observed on the sampling date. Data and observations will be uploaded to an internal VDH web interface and approved prior to posting to the CyanoTracker by project partners. All low and high alert reports are automatically shared by the tracking software with core team members upon receipt. This facilitates report approval and reduces response time. After review, confirmed observations of significant scums or reports of scums will be shared with stakeholders immediately via the tracking map, email and/or direct outreach by project partners.

Volunteer Monitoring

Volunteer monitors will assess cyanobacteria conditions using a three-tiered visual protocol developed by the LCC similar to the VDH guidance for Vermont municipalities (VDH 2015). The

protocol is outlined in Appendix E. After attending a required training session and working in conjunction with the LCC Volunteer Coordinator, volunteers will identify conditions at their site as ‘little to no cyanobacteria (category 1), ‘cyanobacteria present at less than bloom levels’ (category 2), or ‘cyanobacteria bloom in progress’ (category 3).

Monitors will visit their designated shoreline location each week, beginning mid-June and ending in September. Some volunteers may continue to report into mid-October while the project is active and as conditions warrant. Using the photographs and identification triggers noted in Appendix E, monitors will complete a report and provide photographic documentation when appropriate. In some cases, field forms and associated photographs will be provided directly to the LCC. When practical, volunteers will upload data and photographs directly to the secure web interface hosted by the VDH. Internal routing code notifies core project staff whenever category 2 or 3 reports have been submitted. Following review by the LCC, VDH and DEC staff, data are approved for posting to the cyanobacteria tracking map. Category 2 (low alert,) and category 3 (high alert) reports will be verified and posted to the interactive map as soon as possible and shared with the email list serve or via direct contact with affected localities.

Quantitative check samples - At four locations monitored by LCC volunteers, the visual assessment protocol will be supplemented each week with whole water grab samples taken by the volunteer for phytoplankton and toxins (microcystin and anatoxin). These will be used to verify the accuracy of the visual protocol. The visual assessment will be used to populate the interactive online map unless analytical results indicate otherwise. The VDH may request additional sampling at other monitored locations if scums develop.

VDH Monitoring

VDH staff will visit four Champlain and inland lake sites each week (Table 3) and provide cyanobacteria reports using the visual assessment protocol. Whole water samples for cyanotoxins (microcystin and anatoxin) and phytoplankton will also be collected. Results will be shared through the weekly updates as they become available. The visual assessment results will be used to populate the interactive map unless analytical results indicate otherwise.

Monitoring by the VT DEC

In May 2016, Sec. 3. 18 V.S.A. § 1222 (Act 86) established a requirement that public outreach by the VDH shall begin within 1 hour of determining a public health hazard is posed by cyanobacteria. To meet this time requirement and intent of the law, field sampling and analytical activities by the VT DEC were modified in 2017 and identified as ‘open water monitoring protocols’.

Open water monitoring - VT DEC field staff will evaluate conditions at each site following the visual assessment protocol. These reports will be shared with the project manager or VT DEC Volunteer Coordinator as soon as possible for upload to the VDH Cyanobacteria Tracker map. Visual assessments will serve as the report of record unless cyanotoxins indicate otherwise. Phytoplankton samples will be collected during each visit to provide quantitative documentation of cyanobacteria communities. Toxin samples will be collected whenever category 3 conditions are observed. SOPs outlining field collection and laboratory processing of water samples are documented in Appendices C and D. Sampling will begin in early June and continue into early October. Selected sites may be monitored longer if conditions warrant.

Quantitative samples collected by the VT DEC will consist of the following:

1. *Early Season Screening:* A 3 m vertical plankton net tow (63µm mesh) will be used to concentrate surface waters for microscopic examination of the phytoplankton present, recorded as presence/absence only. Field sampling begins in early June with qualitative screening for potentially toxic cyanobacteria.
2. *Routine Quantitative Monitoring:* Once potentially toxic cyanobacteria have been documented at a site, subsequent phytoplankton samples will be analyzed quantitatively. Planktonic algae and cyanobacteria are identified to the lowest practical level and cells counted. Data will be shared with stakeholders through the weekly email updates and used to inform any needed public health response.
3. *Alert Level Monitoring:* A large amount of visible cyanobacteria biomass in the upper water column and/or the presence of surface scums (category 3 of the visual assessment protocol) will trigger the collection of whole water samples for the analysis of phytoplankton density and the presence of cyanotoxins (microcystin, anatoxin, and cylindrospermopsin). At locations where cyanobacteria events are rare, samples may be collected during category 2 events.

Populating the VDH Tracker Map and Coordinating Response

Visual reports from trained volunteers and state field staff will be used to populate the VDH tracking map. Quantitative samples will be available routinely for some locations and may result in the change to the map after analytical results become available in 24 to 48 hours. In the case of alert level reports, VDH and DWGWPD will identify public recreational sites and drinking water facilities, if any, in the vicinity and arrange for additional sampling if necessary. VDH will lead outreach efforts to municipalities and coordinate with the DWGWPD when drinking water facilities are affected.

Reports from volunteers and state field staff will be uploaded to the CyanoTracking map maintained by VDH and categorized as follows:

- a. *Generally Safe conditions*
 - i. Clear water, very small amounts of cyanobacteria, the presence of non-cyanobacteria algae or duckweed, and the presence of run-off related turbidity will indicate Generally Safe conditions (see the visual assessment protocol Category 1).
- b. *Low alert conditions*
 - i. If water conditions suggest the onset of a bloom (Category 2 of the visual assessment protocol), a Low Alert report will be filed for the tracking map but may be upgraded (or downgraded) if analytical results indicate otherwise.
 1. A positive detection of microcystin, anatoxin or cylindrospermopsin below the VDH recreational guidelines (6, 10, and 10 µg/L respectively) will indicate at least Low Alert conditions.
- c. *High alert conditions*
 - i. Visible scum or highly discolored water (Category 3 of the visual protocol) will indicate High Alert conditions, regardless of toxin concentration or cell

- density. This is in keeping with VDH beach guidance which stipulates closure of beaches when visible scums are present.
- ii. Per VDH guidelines, ambient toxin concentrations $\geq 6 \mu\text{g/L}$ microcystin, $\geq 10 \mu\text{g/L}$ anatoxin, and/or $\geq 10 \mu\text{g/L}$ cylindrospermopsin represent a potential threat to human health and will upgrade any report to High Alert.

Response to Changing Climate

Climate change is expected to increase the length of the typical cyanobacteria growing season across the northern Hemisphere. The Champlain Basin has already begun to experience this change as reflected by patterns of winter lake ice formation/melting on Champlain and other lakes. In recent years, significant cyanobacteria blooms have developed after the summer recreational period ended. Project and local resources are stretched at these times since staffing levels drop significantly when part-time summer positions end in early September.

Project partners will work with local health officials and available volunteer monitors to respond to late season cyanobacteria blooms. Routine monitoring may continue at selected lakes if resources are available (e.g. monitoring at the long-term open water stations on Champlain typically continues into mid-October). The VDH CyanoTracker map and condition update portion of the website will remain active as conditions warrant during the fall as they serve as the main public information portal and fill the bloom outreach requirement mandated by Vermont state law. Text on these pages will clearly indicate the reduced monitoring efforts occurring in the fall.

B4 – Sample Handling and Custody

Phytoplankton samples will be placed in 60 ml glass tubes and preserved with acidic Lugols iodine solution to a final concentration of 1% in the field. Samples will be stored in the dark until analysis. Whole water samples collected for toxin analysis will be kept on ice and transported to the VDH laboratory on the day of collection. Toxin sample kits are provided by the VDH. Kits consisting of a field submittal form, a 60mL phytoplankton vial, and two 40ml glass vials with septum tops will be distributed to field staff and volunteers.

Sample labels will be prepared for each field container. Label information will include sample date, description (e.g. whole water), location, preservative if applicable, and sampler's initials. Chain of custody will be maintained for all samples sent to laboratories other than the program partners.

Laboratory processing logs will be maintained for all samples, in paper or electronic format. Information will include date of processing, type of processing, volumes, date of completion, and analyst initials. Partner laboratories will maintain processing and field logs, with periodic review by the respective laboratory supervisor.

Table 3. Sampling methodology and collection frequency.

	Station	Location	Latitude (decimal degrees)	Longitude (decimal degrees)	Assessment Methodology		Assessment Interval	Laboratory Analysis *may be triggered by field conditions	
					Open water	Visual		Algae Counts	Toxin Analysis
Midlake open water	LTM 02	Champlain - South Lake	43.71483	-73.383	x		biweekly	x	*
	LTM 04		43.95166	-73.40783	x		biweekly	x	*
	LTM07	Champlain - Main Lake South	44.126	-73.41283	x		biweekly	x	*
	LTM09		44.24216	-73.32916	x		biweekly	x	*
	LTM16	Champlain - Main Lake Central	44.42583	-73.232	x		biweekly	x	*
	LTM19		44.471	-73.29916	x		biweekly	x	*
	LTM21		44.47483	-73.23166	x		biweekly	x	*
	LTM25	Champlain - Malletts Bay	44.582	-73.28116	x		biweekly	x	*
	LTM33	Champlain - Main Lake North	44.70116	-73.41816	x		biweekly	x	*
	LTM34	Champlain - Inland Sea	44.70816	-73.22683	x		biweekly	x	*
	LTM36	Champlain - Main Lake North	44.75616	-73.355	x		biweekly	x	*
	LTM40	Champlain - St. Albans Bay	44.78533	-73.16216	x		biweekly	x	*
	LTM46	Champlain - Main Lake North	44.94833	-73.34	x		biweekly	x	*
	LTM50	Champlain - Missisquoi Bay	45.01333	73.17383	x		biweekly	x	*
	LTM51		45.04166	-73.12966	x		biweekly	x	*
	Highgate Springs		44.99176	-73.11338	x		biweekly	x	*
LCC Quanti- tative Sites - shoreline	North Beach	Champlain - Main Lake Central	44.492	-73.23983		x	weekly	x	x
	Red Rock Beach		44.44274	-73.22443		x	weekly	x	x
	Highgate Springs- Shipyard	Champlain - Missisquoi Bay	44.97966	-73.10769		x	weekly	x	x
	St. Albans Bay Park	Champlain - St. Albans Bay	44.809457	-73.143504		x	weekly	x	x
Volunteer Sites - shoreline	Not finalized for 2017	lakewide	-	-		x	weekly		
VDH Quanti- tative Sites - shoreline	Alburgh Dunes State Park	Champlain – Main Lake North	44.865709	-73.3013		x	weekly	x	x
	Tri-town Rd, West Addison VT	Champlain - Main Lake South	44.08445	-73.4074		x	weekly	x	x
	Lake Carmi	North Beach	44.65133	-73.30205		x	weekly	x	x
	Lake Carmi	State park beach	44.96143	-72.87498		x	weekly	x	x

B5 – Analytical Methods

Cyanobacteria identification

Phytoplankton samples will be examined with a compound microscope at the magnification necessary to identify cyanobacteria to species when feasible. Lead VT DEC laboratory personnel have many years of experience in the identification of phytoplankton from Lake Champlain. New technicians will be trained in identification. Appropriate taxonomic keys are in house, including John et al. (2002), Joosten (2006), Komarek and Zapomelova (2007, 2008), and Prescott (1982). Several additional on-line resources are also utilized ([AlgaeBase](#), [Phycokey](#), [Freshwater Algae of NW Washington](#)) Taxonomic consistency among staff will be maintained by periodic joint review of organisms utilizing fresh or preserved materials and photographs.

New species of concern will be confirmed by an outside source with expertise in cyanobacteria identification. Photographs will be placed in the annual report to serve as a permanent record.

Cyanobacteria enumeration

Quantitative samples will be analyzed with a compound microscope using a Sedgewick Rafter cell (SR cell), utilizing the natural unit protocol developed by UVM (Watzin et al., 2006). Counts will be recorded electronically and final data will be transferred to a Microsoft ACCESS database. Counting protocols are located in Appendix D.

Toxin Analysis

An Enzyme-Linked ImmunoSorbant Assay (ELISA) antibody technique will be used to test for microcystins and cylindrospermopsin by the VDH. Routine shoreline samples and bloom samples will also be tested by the VDH for the presence of anatoxin with liquid chromatography tandem mass spectrometry (LC/MS/MS).

The ELISA kits are commercially purchased and come with calibration standards and defined detection limits. All samples will be performed in duplicate. ELISA test kit instructions and the analytical procedures for LC/MS/MS are located in Appendix D. The microcystin ELISA assay detects several microcystin variants and results are therefore reported as microcystin-LR equivalents. The cylindrospermopsin kit is specific to this toxin and results are reported as cylindrospermopsin.

B6 – Quality Control Requirements

Field Collections – Plankton samples are collected from a highly dynamic environment. Use of an integrated 3m net or composite whole water sample reduces but cannot eliminate the inherent variability. Five percent of the plankton samples collected will be field duplicates and provide information about variability in cell density. Duplicate analyses are not considered priority and will be completed as time permits.

Plankton Counts – 10% of plankton counts will be replicated over the course of the summer. Replicate analyses are not considered a priority and will be completed as time permits. A minimum of 3 plankton samples will be analyzed by each of the VTDEC taxonomists to verify consistency in counting and identification. QC procedures for plankton samples are located in Table 4.

Toxin Analysis – All ELISA samples are processed in duplicate. Data quality evaluations follow the Laboratory's SOP. Blanks and standards are included in each run. Anatoxin analysis follows protocols developed by the VDH laboratory. Each anatoxin run includes standard and blanks. Preparation and analysis procedures for toxin samples are located in Appendix D.

Laboratory supervisors are responsible for review of analytical results. Corrective action will involve identification of the cause of the analytical failure where possible. Response actions will include re-analysis of questionable samples. The VDH Laboratory's Quality Assistance Manual documents practices specific to the VDH laboratory (VDH QSM, 2015). The professional judgment of the Laboratory Supervisor will be relied upon in evaluating results.

Table 4. Quality Control procedures for phytoplankton enumeration.

Parameter	Component
Size of Sample	1 – 5 mls
Apparatus	Sedgewick Rafter counting cell, binocular microscope @200-1000x, ocular micrometer
Data recorded	Taxa identification, abundance by taxa
Criteria for completion of analysis ^{1,2,3}	Qualitative sample – entire chamber scanned Quantitative sample – minimum of 10 and maximum of 100 fields evaluated

¹Evaluation and analysis

- 1) counting error - analyst comparisons
- 2) taxonomic error - analyst comparisons, confirmations by external investigators, voucher specimens via photographs
- 3) pretreatment error - repeat examinations by other analysts

²Criteria of acceptance

- 1) S.E. < 10%, analyst comparisons within 5%
- 2) confirmed agreement on identifications
- 3) no additional specimens found

³Responsibility if unacceptable

- 1) increase number of replicate counts, additional training for analyst(s)
- 2) additional training for analyst(s)
- 3) increase time/repeats for pretreatment examination, additional analyst training

B7 – Instrument/Equipment Testing, Inspection, and Maintenance

Plankton nets will be inspected periodically for tears and repaired as needed. Ropes for the plankton nets and Secchi disk are checked and re-marked annually. Laboratory equipment testing, inspection and maintenance will be conducted in accordance with manufacturer instructions and/or the VDH QSM. Maintenance logs will be kept with the respective instrument. The log will document any maintenance and service of the equipment. A log entry will include the following information:

- Name of person servicing the instrument/equipment
- Date and description of the maintenance procedure
- Date and description of any instrument/equipment problems
- Date and description of action to correct problem

Laboratory instrumentation and equipment operation will follow manufacturer instructions and accepted procedures associated with the selected analytical methods and lab-specific SOPs.

B8 – Instrument/Equipment Calibration and Frequency

Laboratory instrument calibration will follow manufacturer instructions and accepted procedures associated with the selected analytical methods and lab-specific SOPs.

B9 – Inspection Acceptance of Supplies and Consumables

All supplies and consumables for field and laboratory activities will be inspected for cleanliness and condition by qualified laboratory staff prior to use. Supplies or consumables deemed unacceptable will not be used. Any equipment determined to be in an unacceptable condition will be replaced. Supplies and consumables will be stored in accordance with identified storage requirements of each item.

B10- Data Management

Data generated through field and laboratory activities will be stored by the partners, as noted in Section A7, above. Each partner's project supervisor will be responsible for organization and oversight of data generation, disbursement, processing and storage so that the data will be documented, accessible and secure for five years. The Laboratory Director has the same responsibility for laboratory data and information.

Instrumentation used to generate, process and store data will be configured, maintained and operated in accordance with manufacturer recommendations and accepted industry standards. Generated raw data will be stored in formats compatible with the method or instrument of generation. Processed data will be stored in Microsoft Excel or Access, version 2007 or newer. Electronic data will be stored in project directories by each partner on a computer network server that is compatible with this software. Data reported in paper format will be stored in the project files at the partner organizations.

The project data maintained by VT DEC is stored in a Microsoft SQL Server database. Project correspondence and other materials will be maintained electronically whenever possible. Daily backup is provided, and copies of backup files are archived in separate locations. Database security features are employed to prevent editing or deletion of the original data by users other than the authorized database administrators. The data will be available to other government agencies,

researchers, consultants, students, and the public by request. Annual data compilations can be accessed through the VDH's Cyanobacteria Tracking page - <http://www.healthvermont.gov/tracking/cyanobacteria-tracker>

C – Assessment/Oversight

C1 – Assessments and Response Actions

The Project Manager and supervising staff at the partner locations will review all project output. The Project Manager will document, implement, and verify the effectiveness of corrective actions, such as an amendment to the QAPP, and take steps to ensure that everyone on the distribution list is notified.

NEIWPCC may implement, at its discretion, various audits or reviews of this project to assess conformance and compliance to the quality assurance project plan in accordance with the NEIWPCC Quality Management Plan.

C2 – Reports to Management

Quarterly progress reports and an annual project report will be submitted to the LCBP Project Officer. The annual report will include a discussion of the previous summer's monitoring effort, effectiveness and historical perspective. Additional reports or other information related to project status, concerns, completed deliverables, or any other project needs will be provided when requested.

D – Data Validation and Usability

Data quality will be reviewed for logical consistency and errors by each partner before submittal, and again at the end of the field season. The Project Manager will be responsible for overall validation and final approval of the data in accordance with project purpose, use of data, and the criteria included in Section B6 of this QAPP. The project files will include databases, metadata and notation as to the use and limitations of project-specific materials.

E – Project Outputs and Deliverables

Outputs for this project will be:

- The Cyanobacteria Tracker map maintained by the VDH and the online downloadable data file (<http://healthvermont.gov/tracking/cyanobacteria-tracker>)
- weekly email updates to the stakeholder list during the monitoring period
- the VT DEC cyanobacteria density database

Deliverables for this project will be:

- Annual memo outlining any significant changes to project methods
- Quarterly reports
- An annual report for the previous season's activities, due in May

F - Budget

All permanent staff at the VT DEC and the VDH conduct program activities as part of their normal job duties.

- The Champlain Long-term Water Quality and Biological Monitoring Project supports activities by the VT DEC field team, cyanobacteria summer intern, and Project Manager.
- The LCC has a separate work plan with the LCBP for activities included in the Cyanobacteria Monitoring Program.
- Support for the Cyanobacteria Tracker is provided by the CDC funded Vermont Environmental Public Health Tracking Program at VDH.

G - References

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Appendix A – Core locations to be monitored in 2018.

Waterbody	Station	Latitude	Longitude
Champlain - Inland Sea	LTM 34	44.70817	-73.22683
Champlain - Main Lake Central	LTM 16	44.42500	-73.23200
Champlain - Main Lake Central	LTM 19	44.47100	-73.29900
Champlain - Main Lake Central	LTM 21	44.47483	-73.23167
Champlain - Main Lake Central	LTM 33	44.70117	-73.41817
Champlain - Main Lake Central	North Beach	44.49106	-73.24037
Champlain - Main Lake Central	Red Rocks Beach	44.44200	-73.22413
Champlain - Main Lake North	Alburgh Dunes State Park	44.86462	-73.30196
Champlain - Main Lake North	LTM 36	44.75617	-73.35500
Champlain - Main Lake North	LTM 46	44.94833	-73.34000
Champlain - Main Lake South	LTM 07	44.12600	-73.41283
Champlain - Main Lake South	LTM 09	44.24217	-73.32917
Champlain - Main Lake South	Tri-Town Road, West Addison	44.08538	-73.40791
Champlain - Malletts Bay	LTM 25	44.58200	-73.28117
Champlain - Missisquoi Bay	Highgate Springs	44.99177	-73.11338
Champlain - Missisquoi Bay	LTM 50	45.01333	-73.17383
Champlain - Missisquoi Bay	LTM 51	45.04167	-73.12967
Champlain - Missisquoi Bay	Shipyard, Highgate Springs	44.98076	-73.10788
Champlain - South Lake	LTM 02	43.71400	-73.38300
Champlain - South Lake	LTM 04	43.95100	-73.40700
Champlain - St. Albans Bay	LTM 40	44.78533	-73.16217
Champlain - St. Albans Bay	St. Albans Bay Park	44.80866	-73.14436
Lake Carmi	Lake Carmi State Park	44.96081	-72.87674
Lake Carmi	Lake Carmi, North Beach	44.99054	-72.87031

Appendix B – Task Allocation by Partner

B1 - Lake Champlain Committee (LCC)

The LCC will serve as the primary connection with the volunteer monitors around lakes Champlain, Bristol Pond, Carmi, and Iroquois. Volunteers will target shoreline locations during the high recreation activity period from mid-June to at least early September (and through the early fall where possible), providing a weekly visual assessment of cyanobacteria conditions and supplementary photographic documentation. The LCC will work with selected volunteers to collect water and cyanobacteria samples when appropriate and coordinate the delivery of them to the VDH Laboratory in Colchester VT.

Tasks

- Recruitment and training
 - Recruit volunteers
 - Develop and conduct annual training session(s) for volunteers
 - Work with volunteers to ensure accuracy and quality of field assessments
 - Maintain a list of volunteers who have successfully completed the training
 - Develop and refine supportive materials for volunteers
 - Reference and guidance materials
 - Photography assistance and guidance
 - Water sampling guidance and protocols
 - Weekly email updates
- Outreach and education
 - Hold workshops for the public at beaches, state parks and other locations prior to and during the summer recreational season
 - provide general information about cyanobacteria and associated health concerns,
 - provide tips for visual identification of blooms and recognizing when there is cause for concern,
 - Provide contact information for reporting blooms
 - Recruit new volunteers
 - Participate in similar workshops for drinking water facility operators, beach managers and town health officers
 - Contribute to annual outreach efforts in the Champlain Basin
 - Post information and materials on the LCC website - <http://www.lakechamplaincommittee.org> and through social media as warranted
 - Respond to bloom inquiries and requests for information from the public
 - Provide weekly emails to a list-serve of interested citizens and agencies during the monitoring season
- Coordination of sample collection, pick-up and delivery
 - Quality control sites
 - Scum as requested by VDH
- Reporting and documentation
 - Provide scum observations to the partners as received
 - Review and approve volunteer reports on the VDH web interface

- Provide a summary of the volunteer network operation for inclusion in the annual report

B2 - Vermont Department of Health (VDH)

The VDH has public health authority and will lead public health response efforts. The VDH Laboratory will provide analysis of microcystin, cylindrospermopsin, and anatoxin samples as conditions warrant.

Tasks

- Public Health Response
 - Update and maintain web-based public information including the online tracker map and supplementary materials on the VDH cyanobacteria pages - <http://healthvermont.gov/health-environment/recreational-water/cyanobacteria-blue-green-algae>
 - Issue health alerts or warnings as conditions warrant
 - Issue a general recreational safety reminder prior to the summer season
 - Initiate contact with municipalities and provide public health assistance to areas affected by cyanobacteria blooms
 - Coordinate any additional sampling
 - Work in conjunction with DWGWPDP to respond to any drinking water concerns
 - Respond to public inquiries about cyanobacteria and health
- Laboratory
 - Conduct microcystin analyses
 - For routine weekly shoreline sampling at selected sites
 - As warranted in response to bloom events and emergency situations
 - Conduct cylindrospermopsin analyses
 - As warranted in response to bloom events and emergency situations
 - Conduct anatoxin analyses
 - For routine weekly shoreline sampling at selected sites
 - In response to bloom events and emergency situations
 - Reporting and documentation
 - Provide data to VDH project staff for inclusion in the tracking database
 - Share data with DEC staff for the weekly email updates and as needed during bloom events
 - Maintain an electronic database of results

B3 - VT DEC, Drinking Water and Groundwater Protection Division (DWGWPDP)

DWGWPDP has oversight of public water supplies in the Champlain Basin, and will work with the VDH to respond to drinking water supply concerns.

Tasks

- Public Health response
 - Initiate contact and provide operational guidance to water suppliers located in the vicinity of cyanobacteria blooms
 - Assist drinking water facilities in the event of a toxin detection

- public notification language,
 - additional sampling
 - implementation of cyanobacteria response plan.
- Education and Outreach
 - Provide outreach and general guidance to drinking water suppliers
 - Post informational material on the DWGWPD website - <http://dec.vermont.gov/water/drinking-water/water-quality-monitoring/blue-green-algae>
 - Respond to inquiries about drinking water supply concerns

B4 - VT DEC, Watershed Management Division (WsMD)

The WsMD will be responsible for overall project management, field collections at mid-lake stations on Champlain, algal identification, and communication of results. Field activities associated with this project will begin in June and continue through mid-September or later, as conditions warrant.

Monitoring Tasks

- Field Collection – Lake Champlain
 - Provide field sheets and supporting photographic documentation
 - Collect phytoplankton and toxin samples following the open water protocol at the Champlain Long-term Water Quality and Biological Monitoring Project lake sites.
 - Collect water and algae samples from scums observed along transit routes as appropriate
 - Same-day delivery of toxin samples, if collected
- Laboratory analysis
 - Process phytoplankton samples following the rapid assessment protocol
- Reporting and Documentation
 - Provide qualitative and quantitative data for inclusion in the CyanoTracker
 - Provide information for the annual monitoring program summary
 - Maintain an electronic database of field and analytical results
- Weekly email updates
 - Maintain central email notification list
 - Provide a weekly/biweekly update of cyanobacteria conditions to stakeholders via email.
- Outreach and Education
 - Post information and materials on the Department's website – <http://dec.vermont.gov/watershed/lakes-ponds/learn-more/cyanobacteria>
 - Assist in training workshops
 - Respond to public inquiries
- Annual Report Activities
 - Conduct final review of the year's data and reports
 - Finalize the database and reports file in conjunction with partners
 - Provide an annual report each winter
 - Summarize the previous year's efforts
 - Observed conditions
 - Occurrence and severity of toxins

- Public health
 - reported health impacts, if any
 - public health response and outreach efforts
 - Discuss pertinent trends and observations
- Coordinate the development of the work plan for the following year
 - Provide an annual memo of significant changes to project methods
- Project Database
 - Maintain an electronic database of all data provided by the partners
 - Maintain copies of project documentation and outreach materials
 - QAPPs and metadata materials
 - Outreach and education materials
 - Weekly emails
 - Respond to requests for project data

Support and Coordination of Cyanobacteria Response Activities

- Basin-wide
 - Provide expertise and technical support for the Champlain Basin Program in the following areas
 - Taxonomy and identification
 - Control options
 - Ecology
 - Environmental Impacts
 - Current research
 - Cyanotoxins
 - Monitoring technologies
 - Facilitate basin-wide consistency in cyanobacteria monitoring, assessment, and response
 - Outreach
 - Develop and distribute basin-wide information materials
 - Work in conjunction with public health authorities to encourage monitoring at basin beaches and recreational areas
- Vermont
 - Provide technical expertise and technical support for the Agency of Natural Resources in the areas noted above
 - Facilitate statewide consistency in cyanobacteria monitoring, assessment and response
 - Outreach
 - Develop and distribute outreach materials
 - Work in conjunction with Agency staff, municipalities and watershed associations to develop local monitoring programs
- The Northeast
 - Coordinate with New England states and New York to develop consistency in
 - Monitoring
 - participate in EPA Cyanobacteria Monitoring Methods workgroup
 - Response
 - Participate in the NEIWPCC Harmful Algal Bloom workgroup

- Outreach and messaging
 - Participate in the NEIWPCC Harmful Algal Bloom workgroup
- Provide data and information in support of a regional assessment of historical and current bloom frequency

Appendix C –Field Sampling Protocols

Sampling procedures for this project are based on handbooks published for the International Biological Programme, specifically IBP Handbook No. 12, “Methods for Measuring Primary Production in Aquatic Environments” (Vollenweider, 1969). All samples will be placed in appropriate containers, preserved in the field, and transported to the VT DEC or VDH laboratory. Table C1 lists sampling equipment for phytoplankton and toxin collections.

C.1. Equipment and Preservatives
1. 60 mL glass sample vials - algae
2. Lugols solution
3. squirt bottles
4. plankton net – 63 um mesh
5. sample labels
6. coolers and ice
7. marking pens and pencils
8. field collection sheets/field notebook
9. VDH sample bottles for toxin analysis

C1 - Phytoplankton Collection

C.1.1. Phytoplankton Net Samples

- Rinse plankton net three times with lake water at location
- Drop opening of 63 µm plankton net to 3 m depth and pull smoothly to the surface.
- Rinse the collected material down into the bucket using a squirt bottle.
- Fill one 60ml glass test tube to half or two-thirds full with collected material, using lake water as needed to rinse the material into the container.
- Add 0.5mL Lugols per test tube. Samples with large amounts of algae may require additional preservative.
- Label tube with date, location, and depth
- Store sample cool and in the dark for transportation to the laboratory

C.1.2. Whole Water Collection

- Collect a water sample by carefully placing a bucket or large bottle at the surface and tipping slightly to fill.
- Mix well and decant an aliquot into a 60mL glass test tube. The remaining water should be saved for toxin analysis.
- Record location and date.
- Add 0.5mL of Lugols solution to the phytoplankton tube
- Store sample cool and in the dark for transportation to the lab

C2 - Toxin Sample Collection

C.2.1. Surface Water for the VDH Laboratory

- Collect a surface water sample as outlined in B.1.2.
- Mix well and dispense sample into pre-cleaned bottles
- Label and place on ice for transport to the Laboratory.

C3 - Field Sampling Parameters

Table C2. Field preservation and processing procedures for water quality samples.

Parameter	Field Processing	Preservation	Container	Holding Times
Live Phytoplankton	a,b	A	1	1
Preserved Phytoplankton	a,b	B	1	2
ELISA samples	a	A	2	3
LC/MS/MS samples	a	A	2	2

Processing: a - whole water
b – net plankton

Preservation: A - no addition, sample kept cool
B - Lugols added

Containers: 1 - 60 mL glass test tube
2 – 40ml glass vials, septum-top (VDH toxin analysis)

Holding times: 1 – 48 hours
2 - 6 months
3 – 72 hours

Sample containers will be purchased from Fisher Scientific or provided by the VDH laboratory.

Appendix D – Laboratory Protocols

D1 - Plankton for identification and enumeration

D.1.1. Sample Preparation

- thoroughly mix sample by shaking gently
- using a pipette, withdraw an aliquot and place it in a Sedgewick-Rafter cell. The cover slip should be moved into place as the cell fills. If bubbles are present, refill the chamber
- allow the sample to settle for 10 - 15 minutes
- record the volume of the aliquot (most chambers use 1 mL) and the volume of the concentrated tow samples

D.1.2. Qualitative Samples

- at 200x, scan over the entire chamber, moving from left to right
- record all taxa observed

D.1.3. Quantitative Samples

- scan the chamber and verify even distribution of plankton
- using fields or strips, count at 200x. Record plankton densities following protocols outlined in C.1.4.
- record whether fields or strips were used, the magnification, and concentrate volume
- record the number of fields/strips evaluated and the number of units observed
- calculate the algal density using the following equation (APHA 2005):

$$\text{number of organisms per mL} = \frac{C \times 1000 \text{ mm}^3}{A \times D \times N}$$

where C = number of organisms counted

A = area of the field or strip used, mm

D = depth of the field or strip, mm

N = number of fields or strips counted

For net plankton samples, the number of cells per mL must be multiplied by the necessary correction factor to account for sample concentration.

D.1.4. Enumeration Protocols

- Taxa should be identified to the lowest possible level
- Taxa represented by single-celled organisms should be counted as single cells, e.g. 3 individual diatom cells would be documented as 3 cells.
- Taxa represented by multi-celled colonies estimated using the following size categories, where a single colony may be represented by a combination of categories (e.g., a colony of 350 cells would be a sum of 3 medium and one small):
 - fragments: count each cell
 - small: 50 or 60 cells (taxon specific)
 - medium: 500 cells
 - large: 1000 cells
- After calculating algal densities, multiply the number of fragments per mL by 1, the total in the small category by a factor of 50 (60), the medium category by a factor of 500 and the large category by a factor of 1000 to obtain a conservative estimate of the number of cells.

- Taxa which should be identified following the multi-cell protocol include - Colonial and filamentous cyanobacteria (e.g. *Microcystis*, *Anabaena*, *Coelosphaerium*, *Woronichinia*)
 - Colonial diatoms (*Fragilaria*, *Tabellaria*, *Aulocoseira*)
- Exceptions
 - *Gloeotrichia* represents a unique counting group because its spherical colonies are significantly larger than most of the other colonies. For this genus, fragments were counted as 20 cells, quarters of colonies as 2500 cells, half colonies as 5000 cells, and full colonies as 10,000 cells.
 - filamentous cyanobacteria (*Aphanizomenon*, *Limnothrix*) will be evaluated using the micrometer grids to estimate filament length. Total cell length will be converted to approximate cell density using median cell lengths or literature values.

D2 - Anatoxin Analysis – Vermont Department of Health

D.2.1 Preparation of Water Samples

- Measure 10mls of water (unfiltered) into a labeled 15ml conical tube. Samples with large amounts of planktonic biomass will be diluted.

D.2.2 Concentration of Prepared Samples

- Condition each SPE extraction cartridge with 4ml each of methanol and Milli-Q reagent grade water
- Add the prepared sample to a labeled cartridge using a clean pipet and allow gravity flow to remove the water.
- Wash the cartridge with 3 ml of 50% methanol solution prepared with Milli-Q reagent water.
- Dry under vacuum for 5 minutes
- Add the prepared sample to a labeled cartridge using a clean pipet and allow gravity flow to remove the water.
- Elute into a clean labeled 15 mL conical centrifuge tube with 8 mL of methanol containing 0.05% trifluoroacetic acid
- Bring sample to dryness with the Turbo Vap at < 40°C.
- Reconstitute with 200 µL of 15% acetonitrile containing 0.05% trifluoroacetic acid.
- Vortex and analyze on the LC/MS/MS

D3 - Microcystin by ELISA – Vermont Department of Health

D.3.1 Processing Whole Water Samples for Microcystin by ELISA

- Whole water samples receive no processing prior to analysis
- Samples containing visibly high biomass will be diluted before analysis.
- Samples exceeding the highest standard concentration provided with the kit will be diluted and re-analyzed.

D.3.2 Instructions for Microcystin by ELISA using Beacon test kits

CALCULATE RESULTS

1. After you read all of the wells, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:

$$\%B^* = \frac{\text{(average OD of calibrator, control or sample} \times 100)}{\text{average OD of negative control}}$$

2. Graph the %Bo of each calibrator on the Y (linear) axis against its microcystin concentration on the X (log) axis using semi-log graph paper. Draw the best-fit line through the calibrator points.
3. Determine the Microcystin concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.
4. Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

Quality Control

1. The value of the 1.0 ppb control should fall within the following range:

1.0 ppb Microcystin control 0.80 – 1.30ppb

SAMPLE CALCULATIONS

Well Contents	OD	Average OD \pm SD*	%RSD	%Bo**	MCYN conc. (ppb)
Negative Control	1.478 1.552	1.515 \pm 0.052	3.4	100	N/A
0.1 ppb Calibrator	1.255 1.194	1.225 \pm 0.043	3.5	80.8	N/A
0.3 ppb Calibrator	0.941 0.932	0.937 \pm 0.006	0.68	61.8	N/A
0.8 ppb Calibrator	0.626 0.602	0.614 \pm 0.017	2.7	40.5	N/A
2.0 ppb Calibrator	0.389 0.386	0.302 \pm 0.008	0.54	25.6	N/A
Sample	0.634 0.610	0.622 \pm 0.017	2.7	31.5	0.909

Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

Beacon
Analytical Systems Inc.



Microcystin Plate Kit

Cat.# 20-0068

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Microcystin Plate Kit is an immunological laboratory test for the quantitation of Microcystins in water.

BEACON ANALYTICAL SYSTEMS, INC.

82 Industrial Park Road

Saco, ME 04072

Tel. (207) 571-4302

Fax (207) 602-6502

www.beaconkits.com

USE PRINCIPLES

The Beacon Microcystin Plate Kit uses a polyclonal antibody that binds both Microcystins and a Microcystin-enzyme conjugate. Microcystins in the sample compete with the Microcystin-enzyme conjugate for a limited number of antibody binding sites. In the assay procedure you will:

- Add Microcystin-enzyme conjugate and a sample containing Microcystins to a test well, followed by antibody solution. The conjugate competes with any Microcystins in the sample for the same antibody binding sites. The test well is coated with anti-rabbit IgG to capture the rabbit anti-microcystin added.
- Wash away any unbound molecules, after you incubate this mixture for 30 minutes.
- Add clear substrate solution to each well. In the presence of bound Microcystin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every well, and each well receives the same number of Microcystin-enzyme conjugate molecules, a sample containing a low concentration of Microcystins allows the antibody to bind many Microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of Microcystins allows fewer Microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to Microcystin concentration.

Darker color = Lower concentration
Lighter color = Higher concentration

MATERIALS PROVIDED IN THE BEACON MICROCYSTIN PLATE KIT

- 1 plate containing 12 strips of 8 wells coated with sheep anti-rabbit antibodies
- 1 vial of Negative Control (0.0 ppb Microcystin-LR)
- 1 vial each of 0.1, 0.3, 0.8 and 2.0 ppb Microcystin-LR Calibrator
- 1 vial 1.0 ppb Microcystin control.
- 1 vial of Microcystin-HRP Enzyme Conjugate
- 1 vial Rabbit anti-microcystin antibody solution
- 1 vial of Substrate
- 1 vial of Stop Solution
- 1 vial 100X Wash Solution

You also need these items:

- Microtiter plate reader
- Tape or Parafilm®
- Watch or timer
- Clean running water or a wash bottle containing tap or deionized water.
- Orbital shaker (optional)

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon Microcystin Plate Kit does not differentiate between Microcystin-LR (used as kit calibrators) and other microcystin variants, but detects their presence at varying degrees. The following table shows the relative values for the percent cross-reactivity (%CR) versus Microcystin-LR. All concentrations are in parts per billion (ppb).

Variant	%CR
Microcystin-LR	100
Microcystin-RR	73
Microcystin-YR	58
Microcystin-LA	2
Microcystin-LF	3
Microcystin-LW	4
Nodularin	126

PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents and samples to reach ambient temperature before you begin the test.
- Do not use kit components after the expiration date.
- Do not mix reagents or test strips from kits with different lot numbers.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
- The assay is not specific for microcystin and will react with related structures. See table in Performance Characteristics for specific information.
- Samples found to have or expected to have concentrations of microcystin greater than 2.0 ppb should be diluted prior to analysis.

ASSAY PROCEDURE

1. Bring all kit reagents and samples to be run to room temperature.
2. Remove the required number of antibody coated strips from the re-sealable foil bag. Be sure to re-seal the bag with the desiccant to limit exposure of the strips to moisture.
3. Prepare 1X wash solution by diluting the 100X concentrate, i.e. 5 mL of the 100X plus 495mL deionized water in 500 ml wash bottle.
4. Add 50 µL of Enzyme Conjugate to each well.
5. Pipet 50 µL of calibrators, control and samples into the appropriate wells. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
6. Add 50 µL of Antibody Solution to each well.
7. Swirl the plate rapidly to mix the contents and cover the wells with tape or Parafilm. Alternately, the plate may be incubated on a rotator for continuous mixing during incubation.
8. Incubate for 30 minutes.
9. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with 1X wash solution, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much water as possible.
10. Add 100 µL of Substrate to each well.
11. Cover the wells and incubate for 30 minutes.
12. Add 100 µL of Stop Solution to each well in the same order of addition as the Substrate.
WARNING: Stop Solution is 1N hydrochloric acid. Handle carefully.
13. Read the plate on a microtiter plate reader at 450nm. If the plate reader has dual wavelength capability, read at 450nm minus 605 or 650nm.
14. If the microtiter plate reader has data reduction capabilities, use either a semi-log linear or 4-parameter curve fit. If manual data reduction is required, proceed with next section.

Available on-line at

<http://www.beaconkits.com/welcome/PDF/Microcystin%20plate%20Brochure%20%2820091208%29.pdf>

D4 - Cylindrospermopsin by ELISA

D.4.1 Processing Whole Water Samples for Cylindrospermopsin by ELISA

- Whole water samples receive no processing prior to analysis
- Samples exceeding the highest standard concentration provided with the kit will be diluted and re-analyzed

D.4.2 Instructions for Cylindrospermopsin by ELISA using Beacon test kits

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample containing less color than a calibrator well have a concentration of Cylindrospermopsin greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.

2. Quantitative interpretation requires graphing the absorbances of the calibrators (Y axis) versus the log of the calibrator concentration (X axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the X-axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.1 ppb or >2 ppb, respectively. Alternatively, Beacon can supply a spreadsheet template, which can be used for data reduction. Please contact Beacon for further details.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD \pm SD*	%RSD	%Bo**	Cylindrospermopsin Conc. (ppm)
Negative Control	1.487 1.444	1.466 \pm 0.030	2.07	100	N/A
0.1ppb Calibrator	1.153 1.155	1.154 \pm 0.001	0.12	78.7	N/A
0.5 ppb Calibrator	0.699 0.703	.701 \pm 0.003	0.4	47.8	N/A
2 ppb Calibrator	0.268 0.261	0.265 \pm 0.005	1.87	18	N/A
Sample	0.487 0.467	0.492 \pm 0.008	1.44	33.6	0.9355

Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

Beacon
Analytical Systems Inc.



Cylindrospermopsin Plate Kit

Cat. # 20-0149

Instructional Booklet

READ COMPLETELY BEFORE USE.

BEACON ANALYTICAL SYSTEMS, INC.

82 Industrial Park Road
Saco, ME 04072
Tel. (207) 571-4302
Fax (207) 602-6502
www.beaconkits.com

INTENDED USE

The Beacon Cylindrospermopsin Plate Kit is a competitive ELISA for the quantitative analysis of Cylindrospermopsin in water.

USE PRINCIPLES

The Beacon Cylindrospermopsin plate kit is a competitive enzyme-labeled immunoassay. The Cylindrospermopsin -HRP enzyme conjugate is pipetted into test wells followed by calibrators or sample extracts. Cylindrospermopsin Antibody Solution is then pipetted into the test wells to initiate the reaction. During the 45-minute incubation period, Cylindrospermopsin from the sample and Cylindrospermopsin HRP conjugate compete for binding to Cylindrospermopsin antibody. The Cylindrospermopsin antibody is captured on the walls of the test well. Following this 45-minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Cylindrospermopsin, Cylindrospermopsin HRP conjugate and free Cylindrospermopsin antibody. After this wash step, a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 45-minute incubation, the reaction is stopped and the amount of color in each well is read. The color of the unknown samples is compared to the color of the calibrators and the Cylindrospermopsin concentration of the samples is derived.

MATERIALS PROVIDED IN THE BEACON CYLINDROSPERMOPSIN PLATE KIT

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8°C.

- 1 plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.
- 4 vials each containing 2 mL of Cylindrospermopsin calibrators corresponding to 0, 0.1, 0.5, 2 µg/L (ppb) of Cylindrospermopsin.
- 1 vial containing 7 mL Cylindrospermopsin HRP Enzyme Conjugate.
- 1 vial containing 7 mL of Polyclonal anti-Cylindrospermopsin antibody.
- 1 vial containing 14 mL of Substrate.
- 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
- 1 Instructional Booklet

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon Cylindrospermopsin cannot differentiate between Cylindrospermopsin and related compounds, but detects their presence to differing degrees.

COMPOUND	% CR
CYLINDROSPERMOPSIN	100 %
MICROCYSTIN-LR	< 1%
NODULARIN	< 1%

MATERIALS REQUIRED BUT NOT PROVIDED

- Methanol
- Pipet with disposable tips capable of dispensing 50 µL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter.
- Timer
- Vortex mixer
- Wash bottle
- Laboratory quality distilled or deionized water.
- Graduated cylinder, 100 ml or larger.
- Glassware for sample collection and dilution.

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Cylindrospermopsin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Cylindrospermopsin Plate Kits with different Lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
3. Do not use reagents after expiration date.
4. Reagents should be brought to room temperature, 20 – 28°C (62 – 82°F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
5. Cylindrospermopsin is a toxin and should be treated with care.
6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts reach room temperature prior to running the test. Fill a wash bottle with lab grade water.
2. Place the appropriate number of test wells into a micro well holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
3. Using a pipet with disposable tips, add 50 µL of Enzyme conjugate to the appropriate test wells.
4. Add 50 µL of Calibrators or sample extract to each well. Be sure to use a clean pipet tip for each.
5. Dispense 50 µL of Antibody Solution into each test well.
6. Shake the plate gently for 30 seconds and incubate the test wells for 45 minutes.
7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory grade water and dump. Repeat 3X for a total of four washes.
8. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
9. Dispense 100 µL of Substrate into each well.
10. Incubate the wells for 45 minutes.
11. Dispense 100 µL of Stop Solution into each test well.
12. Read and record the absorbance of the wells at 450 nm using a strip or plate reader.

Available on line at

[http://www.beaconkits.com/welcome/PDF/Cylindrospermopsin%20plate%20Brochure\(20110207\).pdf](http://www.beaconkits.com/welcome/PDF/Cylindrospermopsin%20plate%20Brochure(20110207).pdf)

These on-line pages may be updated as protocols evolve.

Appendix E – LCC Volunteer Monitoring Protocols.

E1 – LCC On-line reporting form

- <https://www.lakechamplaincommittee.org/get-involved/volunteers/cyanobacteriamonitors/cyanobacteria-monitor-reporting-form/>

Reporting Cyanobacteria on Lake Champlain

Cyanobacteria blooms can be easily confused with other natural phenomena. Please consult our guide to [Recognizing Cyanobacteria in Lake Champlain](#) before reporting a bloom. If there is a bloom, avoid direct contact ([see Vermont Department of Health link](#)).

Also, our [guide to categories of cyanobacteria bloom intensity](#) and our [instructions for photographing cyanobacteria blooms and taking water samples](#) will be helpful in filling out the form below.

CYANOBACTERIA REPORTING FORM

Please Complete Form Below

Lake (Lake Champlain, Lake Carmi, Lake Iroquois, Shelburne Pond etc.)*

Region*

- ☐ Champlain - Missisquoi Bay
- ☐ Champlain - St. Albans Bay
- ☐ Champlain - Inland Sea
- ☐ Champlain - Malletts Bay
- ☐ Champlain - Main Lake North
- ☐ Champlain - Main Lake Central
- ☐ Champlain - Main Lake South
- ☐ Champlain - South Lake
- ☐ Lake Carmi
- ☐ Lake Iroquois
- ☐ Lake Memphremagog
- ☐ Lake Morey
- ☐ Lake Willoughby
- ☐ Maidstone Lake
- ☐ Peacham Pond
- ☐ Salem Lake
- ☐ Seymour Lake
- ☐ Shadow Lake
- ☐ Shelburne Pond

☐ Tinmouth Pond

Municipality of observation

Site #*

Site name*

Type of report

☐ Routine weekly

☐ Supplemental

Routine weekly reporting day

☐ Sunday

☐ Monday

☐ Tuesday

☐ Wednesday

☐ Thursday

☐ Friday

☐ Saturday

☐ Filing supplemental report (outside of routine weekday)

Date of observation*

Time of observation (military time)*

Please choose the category (see links above) that best describes conditions and intensity of any bloom present*

☐ 1a - No cyanobacteria present - clear water

☐ 1b - No cyanobacteria present - brown and turbid conditions

☐ 1c - No cyanobacteria present - other plant material

☐ 1d - Little cyanobacteria present - Generally safe conditions

☐ 2 - Cyanobacteria present, but at less than 'bloom' levels - Low alert conditions (include photos)

☐ 3 - Cyanobacteria bloom in progress - High alert conditions (include photos)

Additional details (required for categories 1d, 2 or 3)

Photo - water surface close-up

Photo - water surface broad view

Photo - water sample in clear container

Extent of cyanobacteria bloom on open water (Evaluate the area within 100 yards of where you are).

☐ No Bloom

☐ Very Limited

☐ <50% cover

☐ Between 50 and 75% cover

☐ Coverage greater than 75%

Cyanobacteria Color

- ☐ None
- ☐ Green
- ☐ Turquoise
- ☐ Reddish
- ☐ Yellow
- ☐ Other (please add details below)

Add cyanobacteria color details here

Water temperature

Water surface

- ☐ Calm
- ☐ Rolling
- ☐ White-caps

I am using LCC's cyanobacteria report because: (check all that apply)

- ☐ I am a first time monitor
- ☐ This is the only reporting form I'm aware of
- ☐ I had problems using the VDH cyanobacteria data tracker form (please provide details below)
- ☐ I didn't have access to my user name and password
- ☐ LCC's form is more convenient

Please describe the problems you experienced using the VDH cyanobacteria tracking form. Include the device

you used to access the form, your internet browser, and screen size and resolution.

Last Name*

First Name*

Email*

Street Address*

City*

State*

Zip Code*

Telephone*

Submit

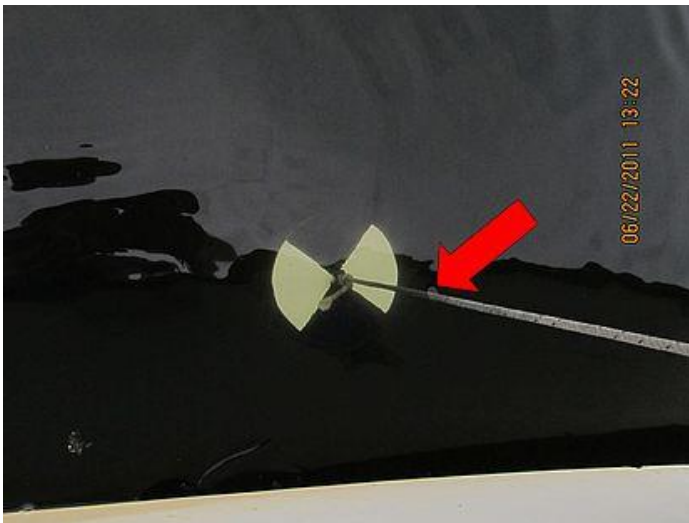
E2 – LCC Guidance on Determining Cyanobacteria Bloom Intensity

<https://www.lakechamplaincommittee.org/get-involved/volunteers/cyanobacteriamonitors/algaebloomintensity/>

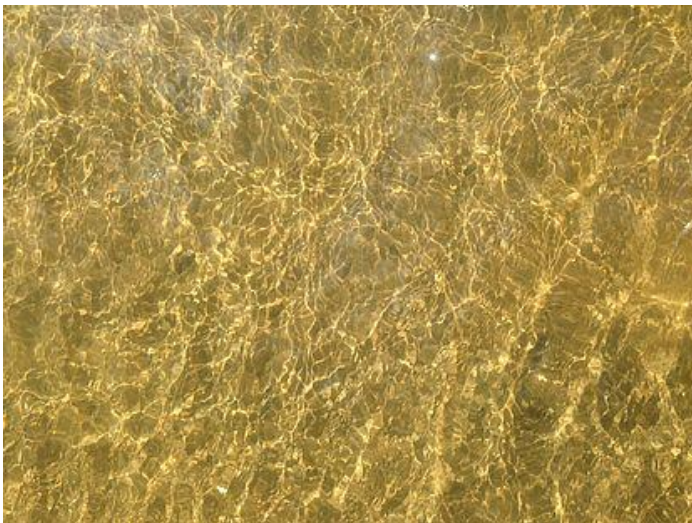
-

Category 1a: No cyanobacteria present - clear water

Any organisms floating in water column are clear (e.g. insect 'skins') rather than green. Leafy or grass-like plants (including duckweed) may be present. Foam may be present.



Objects sitting lower in the water column are clearly visible (red arrow indicates water surface)



Overall appearance of water is clear

Category 1b - No cyanobacteria present - brown and turbid conditions

Brown turbid low visibility through water column



Brown and cloudy does not indicate presence of cyanobacteria

Category 1c - No cyanobacteria present - other plant material

Other material that doesn't count as cyanobacteria might include:

- Long strands that tangle around paddles or boat hooks
- Small bright mustard yellow (pollen) or grass green (duckweed) particles
- Algae attached to rocks or the lake bottom.



Green dots are duckweed; stringy algae are not cyanobacteria



From a distance duckweed may look like green algae or cyanobacteria



Stringy algae attached to the bottom are not cyanobacteria



Duckweed up close

Category 1d - Little cyanobacteria present - Generally safe conditions

Green floating balls may be visible, but only on close inspection and in densities so low that they do not impair recreational enjoyment of the water. There are no surface or near shore accumulations of cyanobacteria.



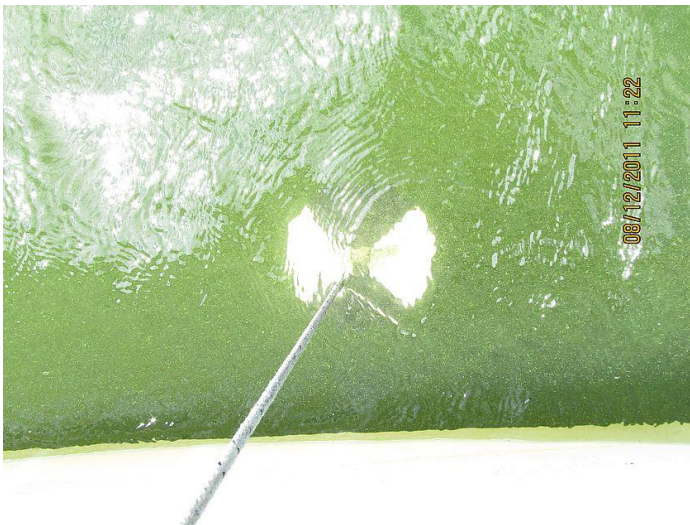
Water appears perfectly clear



But close inspection shows some cyanobacteria present

Category 2: Cyanobacteria present, but at less than 'bloom' levels - Low alert conditions

Numerous green balls (pinhead size or larger) floating in water column, but not accumulated at water surface. Possible small (smaller than a softball) patches of algae accumulation. Open water color **not** green. Possible narrow band of cyanobacteria accumulation at shoreline.



Some cyanobacteria in water but not a uniform layer



Open water not green



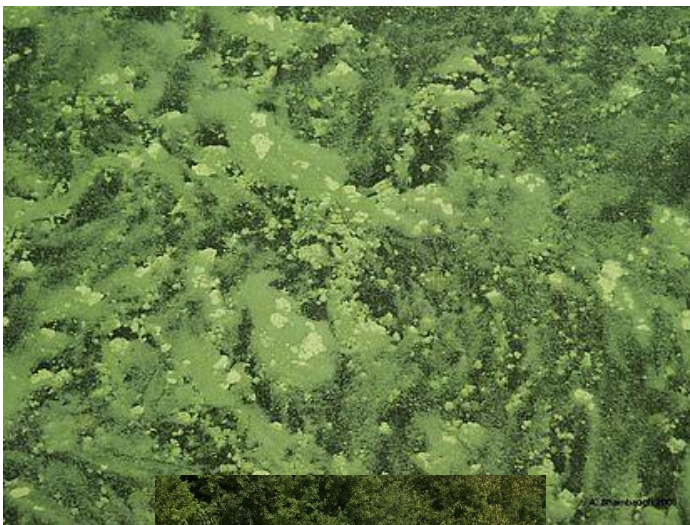
Band of cyanobacteria at shoreline

Category 3: Cyanobacteria bloom in progress - High alert conditions

Extensive surface scum on water – color may range from green to electric blue (not yellow/pollen). Usually accompanied by a thick accumulation at shoreline. Open water appears green.



Continuous layer of cyanobacteria at the surface - not stringy



Thick surface scum present



Open water surface green to turquoise

<https://www.lakechamplaincommittee.org/get-involved/volunteers/cyanobacteriamonitors/bga-photos/>

Instructions for Photographing Cyanobacteria Blooms & Taking Water Samples

Please take digital photographs of the water when [category 2 or 3 bloom conditions](#) are observed.

We need three photographs:

1. A close-up of the water surface,
2. A broad view of water in the vicinity, and
3. A close-up of a water sample in a clear container and placed against a background that provides contrast such as a sheet of paper or a wall. Darker colors provide more contrast.



1. Use your camera's date stamp, or hold up a card in the photo with time, date, and location and category if possible.



broad view.

2. Photograph both a close-up and a



3. For close-ups, take a sample of water in a clear container and photograph against a contrasting background. Over about 1/2 hour cyanobacteria will rise toward the surface; detritus will sink.

When collecting a water sample to photograph take care to avoid exposure to cyanobacteria. Wear gloves, don't wade or immerse yourself in the water and wash any exposed portions of your body

immediately after collecting the sample. After sampling wash the gloves before reusing them. It is okay not to collect a physical sample for photography if you are uncomfortable doing so.

For best results, collect samples in water about knee deep. Invert the container before submerging it into the water and then tilt it once it is under water. This gives a more representative sample by collecting more than just the surface scum.

All photographs should include the time, date, and location. This information can be added by using the date stamp in your camera or by holding a piece of paper with the relevant information in the picture. Name the photograph file with the year-month-day-photographer's name-location-photo type-category.

Example photo file name: 2017-07-15_JaneDoe_DonaldsonPt_Closeup_category2

Appendix F – Forms**F1 - VT DEC Staff Field Form****VT DEC - Cyanobacteria Monitoring Project – 2018**

FieldID	Station	Date	Sample type (circle one)	Volume or depth	Analysis (circle one)	Type (Circle one)	Visual Assessment
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	
			Ww - grab Ww - hose net		cyanotoxins phytoplankton	bloom routine	

F2 - Plankton Counting Software

Plankton samples will be counted utilizing 'Counter', an electronic counting program used by the Long-Term Monitoring Program. Data are exported as an EXCEL spreadsheet.

Counter - 2011 bacill, chryso, crypto

File Sample View Help

LabId: 184 FieldId: 42622 Station: 19

QA: A SampleDate: 10/12/2011 ConcentrationVolume: -1

TowLength: 7.2 Microscope: Olympus Inverted Magnification: 200x

VolumeSettled: 1 UnitEvaluated: full grid Analysis Type: Utermohl

Analyst: ads Date Analyzed: 4/2/2012 Remarks: settled 3/29

1 Asterionell... #col q	1 Asterionell... #cells w	2 Aulo sp 1 #col e	8 Aulo sp 1 #cells r	0 Aulo sp 3 #col t	0 Aulo sp 3 #cells y	0 Fragilaria spp #col u	0 Fragilaria spp #cells i	0 Chryso flag #cells o	-1 Rhizosolenia #cells p
0 M pseudoco... #cells a	12 Cryptomonas #cells s	1 Aulo helical #col d	1 Aulo helical #cells f	0 Fcrotonensis #col g	0 Fcrotonensis #cells h	0 D bavaricum #col j	0 D bavaricum #cells k	5 Pennaes sm #cells l	
0 D divergens #col z	0 D divergens #cells x	0 Pennaes md #cells c	108 Chroomonas #cells v	4 Crypto flag 2 #cells b	10 Central sm #cells n	1 Centralmd #cells m	15 No of fields field count .		

Dinobryon divergens

Total Count: 168

Start | Cyano QAPP v3.doc [Co... | draft work plan & QAPP -... | Counter - 2011 bacill, ... | 1:57 PM