VERMONT

State of Vermont
Department of Environmental Conservation

Agency of Natural Resources

WATERSHED MANAGEMENT DIVISION
Lakes and Ponds Section

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June 9, 2016

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Re: EPA RFA #16091

Dear MaryJo, Mario, Mike and Eric,

Enclosed is final version (1.1) of the 2016 Quality Assurance Project Plan for the Lake Champlain Basin Program Project Cyanobacteria Monitoring on Lake Champlain. This new document addresses the comments provided by Dr. Conlon in her June 7th memo.

The scope of work, procedures, protocols and methods are consistent with the previous year's plan. Significant updates are noted below:

1. MaryJo Feuerbach is now the EPA Region 1 LCBP Project Manager.
2. Dr Nora Conlon is now the EPA Region 1 Project QA/QC Officer.
3. Sarah Vose has replaced Andy Chevrefils as the VT Department of Health (VDH) lead.
4. NEIWPCC contact information has been updated.
5. Figure 1 has been updated to reflect the core stations identified for sampling in 2016 and a list of the 2015 sites has been added as Appendix A.
6. Plankton filters will no longer be collected as the VDH has requested whole water samples.
7. A brief discussion of the drinking water sampling program and protocols has been added
8. The VDH has changed manufacturers for the ELISA kits from Abraxis to Beacon.

Please feel free to contact me with any questions,

Angela Shambaugh, Project Manager

QA Project Plan:

Cyanobacteria Monitoring on Lake Champlain
EPA RFA#: 16091

Prepared by:
Angela Shambaugh
Watershed Management Division
Vermont Department of Environmental Conservation

Prepared for:
Lake Champlain Basin Program
54 West Shore Road
Grand Isle, VT 05458

June 2016

Angela Shambaugh 6/15/16
Angela Shambaugh, Project Manager, VT DEC — Date

Sarah Vose, VT Dept. of Health — Date

Mike Winslow, Lake Champlain Committee — Date

Eric Howe 6/15/16
Eric Howe, Project Officer, LCBP — Date

Michael Jennings, Quality Assurance Program Manager, NEIWPCC — Date

MaryJo Feuerbach, Project Officer, EPA — Date

Dr. Nora Conlon, Quality Review Officer, EPA — Date

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Sarah Vose, VT Dept. of Health	Date
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Eric Howe, Project Officer, LCBP	6/15/16 Date
Michael Jennings, Quality Assurance Program Manager, NEIWPCC	Date
MaryJo Feuerbach, Project Officer, EPA	Date
Dr. Nora Conlon, Quality Review Officer, EPA	6/15/2016 Date

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MaryJo Feuerbach, Project Officer, EPA	Date
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MaryJo Feuerbach, Project Officer, EPA	Date
Dr. Nora Conlon, Quality Review Officer, EPA	Date

Table of Contents

A - Project Management

A1 - QAPP Distribution List

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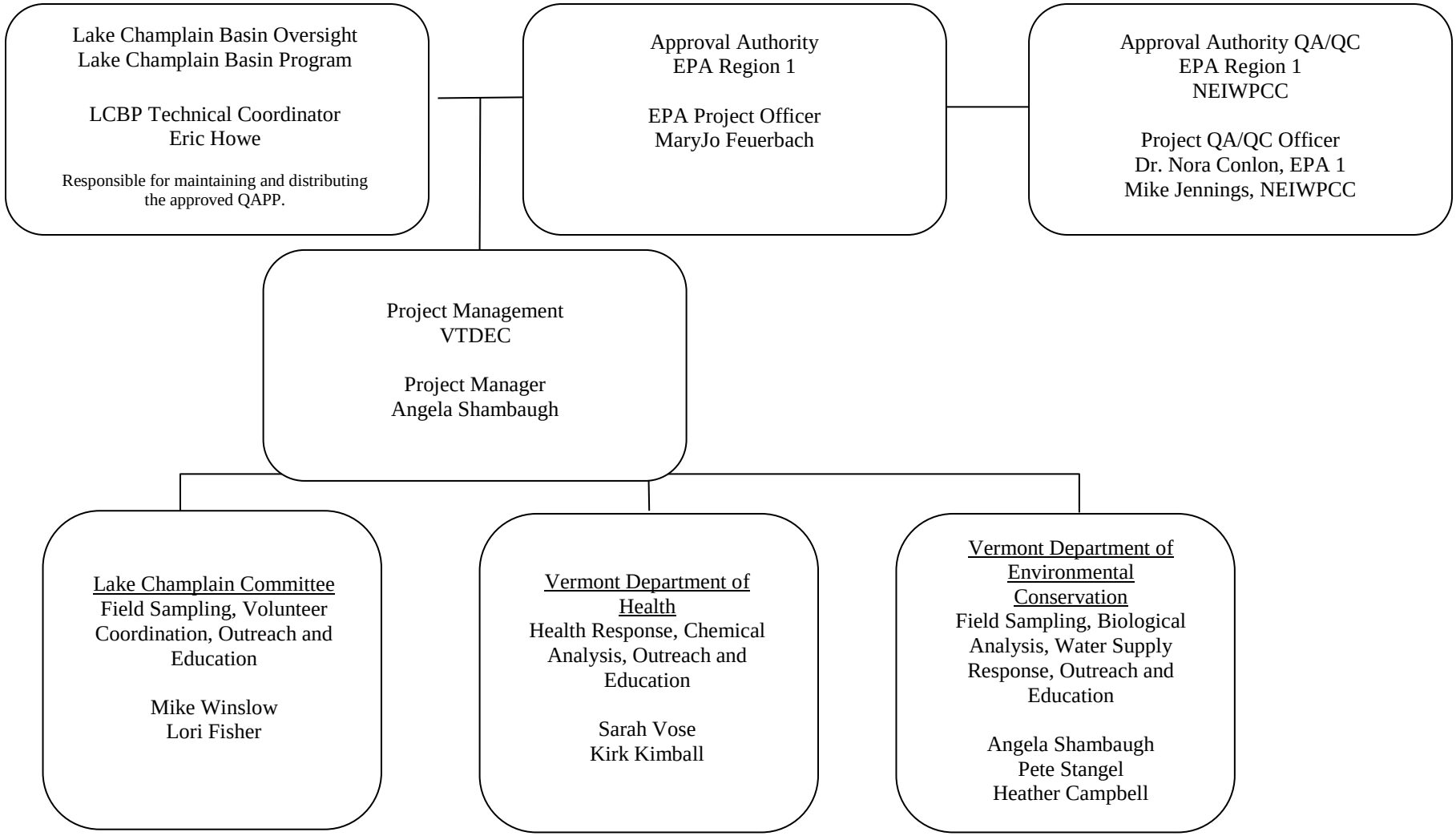
A2 - Project Organization



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. 2015, LTM unpublished data). Cell counts and associated toxins are below levels of concern in most of the lake each year (Shambaugh et al., 2015). 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. Despite the regular presence of these algae in some parts of the lake and sporadic occurrences in others, no confirmed cases of human illness as a result of exposure have been reported (VDH, personal communication).

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. An on-line tracking tool developed by the VDH now provides information to the general public in near real-time (http://healthvermont.gov/enviro/bg_algae/weekly_status.aspx#map). In 2016, the program will continue to facilitate communication among the environmental and public health officials, support an appropriate and consistent response during bloom events on Lake Champlain and several Vermont inland lakes, inform the general public and 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 will provide weekly

assessments of cyanobacteria conditions around the lake. Quantitative data (cell counts and toxin levels) will be collected from core locations on a weekly/biweekly basis and analyzed following a modification of the tiered alert protocol developed by UVM (Watzin et al., 2006). Through condition updates posted in near real-time on the VDH cyanobacteria webpage, lake users and the general public will have access to information that enables them to reduce their exposure to potentially toxic cyanobacteria. A weekly email will convey recent status and bloom observations to researchers, health and environmental officials, 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.

In 2015, the VDH coordinated with the Drinking Water and Groundwater Protection Division (DWGWPD) to offer weekly cyanotoxin analyses at no cost to the 22 Vermont public systems drawing drinking water from Lake Champlain. This service will be offered again in 2016.

Project tasks and associated timelines are noted in Table 1.

Table 1. Timeline for project tasks. *as needed

Task	June 2016	July	Aug	Sep	Oct	Nov	Dec	Jan 2017	Feb	Mar	Apr	May
Field Collection	x	x	x	x	*							
Volunteer Monitoring	x	x	x	x								
Drinking water system 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	
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.

A5 – Quality Objectives and Criteria for Measurement Data

Data quality will be measured in terms of accuracy and precision, completeness, represent-

ativeness, 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 algal samples. Analyses at the Vermont DEC will be conducted under the supervision of a taxonomist with more than 25 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 Quality Assurance Project Plan will be provided in electronic format to the partners by the Project Manager (VT DEC).

Field teams and citizen volunteers will document all field-generated data on Field Log Sheets (Appendix F) or online reporting forms (Appendix E), respectively. Digital photographs may provide additional documentation of field conditions during the assessment. Original field sheets will reside with the VT DEC and the LCC, respectively. Summaries of the information will be provided electronically from the LCC and VDH to the VT DEC for inclusion in weekly updates circulated to an established user group and in the long-term database for this project. All data generated by the VDH laboratory will be maintained in an electronic format that can be incorporated into the VT DEC master database for this project. Any photographs of event conditions referenced to specific field sheets and reports may also be provided to the VT DEC for inclusion in the project data files.

B – Measurement/Data Acquisition

B1 – Sampling Design

Sampling locations have been selected to 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 primary objective of this monitoring program is reduction of recreational exposure to cyanobacteria. A secondary goal is to provide pertinent information to water supply facilities. We will achieve this by evaluating both shoreline and mid-lake stations for the presence of cyanobacteria and/or cyanotoxins.

Core locations to be sampled by the VT DEC, VDH and LCC in 2016 are shown in Figure 1. Volunteer sites will be identified during the training sessions. More than 100 sites were regularly assessed by volunteers on Champlain and four Vermont inland lakes during 2015 (Appendix A). We expect that many of these will return in 2016. We also anticipate that additional volunteer sites will be added as a result of education efforts during early summer. Volunteer locations will serve an outreach function, may not be associated with areas of high population or recreational usage, and may be co-located with other program sites. Assessments from six sites on Champlain and one on inland lakes will be supported with funding provided by the VDH. A final list of 2016 field sites, including a map and a table with latitude and longitude, will be provided in the final project report to the LCBP.

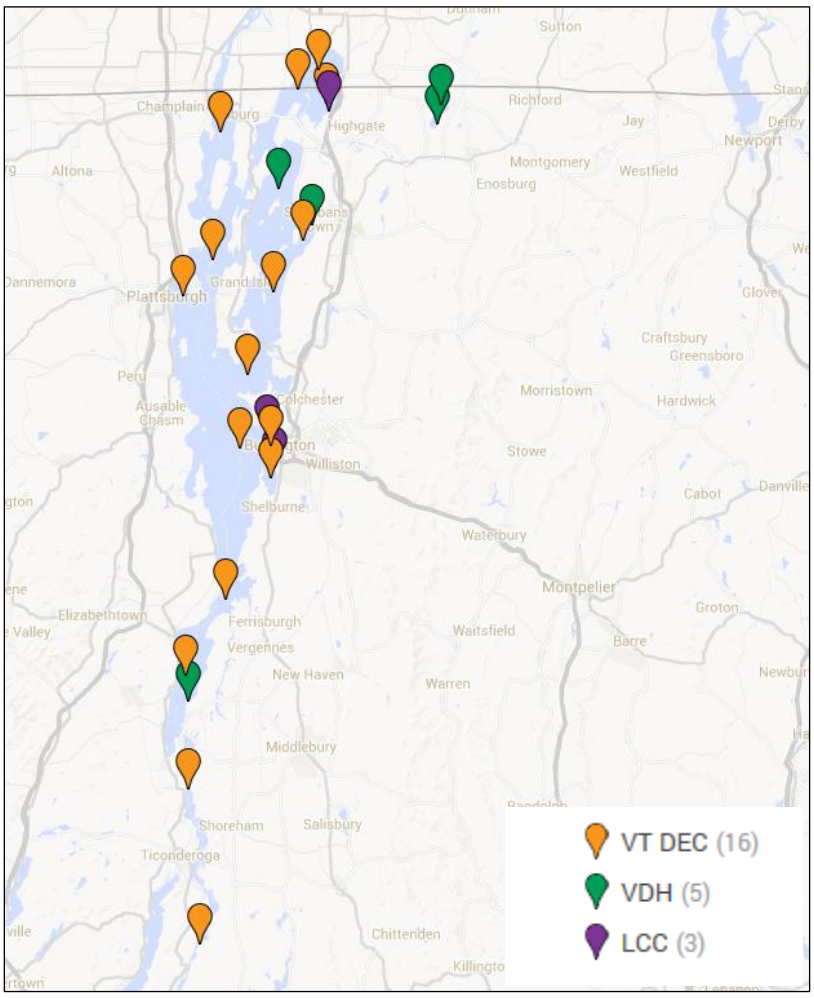


Figure 1. Core Monitoring Locations in 2016 Additional volunteer locations will be identified through the training sessions.

B2 - Allocation of Project Responsibilities

This project is a continuation of the partnership established in 2002. Partners include VT DEC (Watershed Management and Drinking Water/Groundwater Protection Divisions), LCC, and VDH

(Radiation/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	Partners				
	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		
Volunteer Training			x		
Public Health Response				x	x
Drinking Water Supply Response		x		x	x
Drinking Water Supply Testing Program Coordination		x			x
Weekly Email updates	x				
Webpage Maintenance					x
Field/ lab data QA/QC	x		x	x	x
Project Coordination	x				
Maintenance of Central Database	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 collected 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 tracking map 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. Any observations of significant scums or reports of scums will be shared with the stakeholders immediately via the tracking map, email and/or direct outreach by project partners.

Tiered Alert Protocol - Field collections at mid-lake and core shoreline sites will be conducted following a modification of the tiered alert protocol developed by UVM. Sites will be visited at two week intervals as part of the routine water quality monitoring conducted by the VT DEC on Lake Champlain. Observation of a presumed cyanobacteria scum or highly discolored water will trigger collection of toxin and phytoplankton samples regardless of the protocol stage.

Initial Screening: The first stage of monitoring is designed to locate developing cyanobacteria populations. These samples are qualitative and serve to initiate subsequent tasks. A 3 m vertical plankton net tow (63µm mesh) will be used to concentrate surface waters for microscopic analysis of the algae present. Samples will be screened for the presence of potential toxin- producing cyanobacteria as soon as possible, typically within 12 hours of field collection. Field sampling will begin in early June.

Quantitative Monitoring: Once potential toxin-producing cyanobacteria have been identified in the screening samples, quantitative phytoplankton sampling will be initiated at the next site visit. Phytoplankton collections will be made with a 63µm plankton net. Samples will be enumerated as soon as possible, typically within 12 hours of field collection.

Vigilance Monitoring: If water conditions at the site or previous samples suggest the onset of a bloom, water samples will be collected for toxin and phytoplankton analysis. Whole water samples will be collected to reduce loss of colony fragments through the net. A subsample will be screened by the VDH using enzyme-linked immunosorbent assays (ELISA) for microcystin and cylindrospermopsin within 48 hrs. Samples that consist predominantly of potential anatoxin-producers will be analyzed by the VDH for anatoxin, but this analysis requires significantly more time (several days). For this reason, alert level designations are based on the presence of microcystin or cylindrospermopsin rather than anatoxin. Phytoplankton samples will be enumerated as soon as possible after arrival at the DEC laboratory.

Alert Level: At this level, there is a large amount of accumulated algal biomass in the upper water column. Toxins in this material could reach levels of concern for human and animal health.

The presence of microcystin or cylindrospermopsin below VDH recreational guidelines, or densities of potential toxin-producing cyanobacteria in net plankton samples greater than 4000 cells/ml are designated as Low Alert conditions. Per VDH guidelines established in 2005, ambient toxin concentrations ≥ 6 µg/L microcystin or ≥ 10 µg/L cylindrospermopsin represent a potential threat to human health and will trigger progression to High Alert. If anatoxin-producing species predominate in a bloom, this information will factor into the appropriate communication to public health officials. To date, anatoxin concentrations have not exceeded 0.5 µg per L in samples submitted for analysis, well below Vermont recreational guidelines.

Visible scum or highly discolored water present at tiered alert sites will also trigger progression to High Alert, regardless of toxin concentration or cell density. This is in keeping with the high alert designation for the visual protocol category 3 and the VDH beach guidance which stipulates closure of beaches when visible scums are present. These reports will be added to the interactive map as soon as possible and shared with the email list serve.

The VDH will evaluate recreational activity levels and possible drinking water intakes in the vicinity of sites with alert level conditions, and will arrange for additional sampling as necessary. Should cell densities fall below the alert level threshold during subsequent visits, field crews will follow the Vigilance level monitoring protocol.

SOPs outlining field collection and laboratory processing are documented in Appendices C and D.

Visual Assessment Protocol –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 in June 12th and ending in mid-September 2016. Using the photographs and identification triggers noted in Appendix E, monitors will complete a field form and provide photographic documentation when appropriate. In some cases, field forms and any photographs will be provided directly to the Volunteer Coordinator. When practical, volunteers will upload data and photographs directly to the internal web interface hosted by the VDH. Following review by the LCC coordinator, data are approved for posting to the algae 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.

Qualitative check samples and the VDH Staff Monitoring

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

VDH staff will visit seven Champlain sites and one inland lake site each week (Table 3) and provide cyanobacteria reports using the visual assessment protocol. Weekly whole water samples for cyanotoxins and algae will also be collected and 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.

Table 3. Sampling methodology and collection frequency.

	Station	Location	Latitude (decimal degrees)	Longitude (decimal degrees)	Assessment Methodology*		Assessment Interval	Laboratory Analysis (triggered by protocol or scum observation)	
					Tiered Alert	Visual		Algae Counts	Toxin Analysis
Midlake open water	LTM 02	Champlain - South Lake	43.71483	--73.383	x		biweekly	x	x
	LTM 04		43.95166	-73.40783	x		biweekly	x	x
	LTM07	Champlain - Main Lake South	44.126	-73.41283	x		biweekly	x	x
	LTM09		44.24216	-73.32916	x		biweekly	x	x
	LTM16	Champlain - Main Lake Central	44.42583	-73.232	x		biweekly	x	x
	LTM19		44.471	-73.29916	x		biweekly	x	x
	LTM21		44.47483	-73.23166	x		biweekly	x	x
	LTM25	Champlain - Malletts Bay	44.582	-73.28116	x		biweekly	x	x
	LTM33	Champlain - Main Lake North	44.70116	-73.41816	x		biweekly	x	x
	LTM34	Champlain - Inland Sea	44.70816	-73.22683	x		biweekly	x	x
	LTM36	Champlain - Main Lake North	44.75616	-73.355	x		biweekly	x	x
	LTM40	Champlain - St. Albans Bay	44.78533	-73.16216	x		biweekly	x	x
	LTM46	Champlain - Main Lake North	44.94833	-73.34	x		biweekly	x	x
	LTM50	Champlain - Missisquoi Bay	45.01333	73.17383	x		biweekly	x	x
	LTM51		45.04166	-73.12966	x		biweekly	x	x
	Highgate Springs		44.99176	-73.11338	x		biweekly	x	x

	Station	Location	Latitude (decimal degrees)	Longitude (decimal degrees)	Assessment Methodology*		Assessment Interval	Laboratory Analysis (triggered by protocol or scum observation)	
					Tiered Alert	Visual		Algae Counts	Toxin Analysis
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
	North Hero State Park	Champlain - Inland Sea	44.92078	-73.2402		x	weekly	x	x
Volunteer Sites - shoreline	Not finalized for 2016	lakewide	-	-		X	weekly		
VDH Staff – shoreline^	Arnold Bay, VT	Champlain - Main Lake South	44.14938	-73.36733		x	weekly	X	X
	Alburgh Springs, VT	Champlain - Missisquoi Bay	44.99217	-73.21742		x	weekly	X	X
	Maquam Shore Rd, Swanton VT	Champlain -Inland Sea	44.90378	-73.16709		x	weekly	X	X
	Stephensen Point Fish and Wildlife Access, North Hero VT		44.89484	-73.23253		x	weekly	X	X
	Keeler Bay, South Hero VT		44.65133	-73.30205		x	weekly	X	X
	St. Albans Bay Park	Champlain – St. Albans Bay	44.808658	-73.1444		x	weekly	X	X
	Tri-town Rd, West Addison VT	Champlain - Main Lake South	44.08445	-73.4074		x	weekly	X	X
	Lake Carmi	State park beach	44.96143	-72.87498		x	weekly	X	X

*methodology which will be used to determine tracking map status

^ Note that Prouty Beach on Lake Memphremagog, the state park beach on Lake Elmore, and the public beach on Lake Iroquois will not be sampled by VDH staff in 2016.

Drinking Water Supply Monitoring – Outreach to public facility operators regarding the availability of cyanotoxin testing for 2016 was initiated in March by the DWGWPD. We anticipate that all 22 Vermont facilities will participate in monitoring again this year. Trainings are scheduled for May 24, June 9 and June 16. Attendees will be introduced to basic cyanobacteria ecology, the visual assessment system, and provided with an overview of Vermont's voluntary practice (http://dec.vermont.gov/sites/dec/files/dwgwp/bluegreen/pdf/FINAL_CYANOPRACTICE2015.pdf) for managing cyanotoxin detections during a discussion of cyanobacteria response planning.

Once participating systems are identified, a pick-up location and schedule will be set up for each system. Typically, the pick-ups coincide with the VDH shoreline sampling schedule to facilitate laboratory processing. Operators are provided with all bottles and paperwork. Sampling will begin the first week of July and continue for 12 weeks. Both raw and finish water are tested each week, following the procedures outlined in Appendix C.

If any cyanotoxin is detected in either raw or finish water, VDH will notify the DWGWPD immediately so that a confirmation sample can be collected as quickly as possible, following the practice guidelines. DWGWPD works with the facility to evaluate operational conditions and any necessary response. DWGWPD will also work directly with the affected system to implement their cyanobacteria response plan in the event that a confirmation sample returns a positive detection in finish water. Data are published weekly on the DWGWPD website - http://dec.vermont.gov/water/drinking-water/water-quality-monitoring/blue-green-algae/cyanotoxin-monitoring. The weekly email updates will also link to the drinking water data.

B4 – Sample Handling and Custody

Phytoplankton samples will be placed in 75 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 by the VT DEC will be kept on ice and transported to the VDH laboratory. Samples obtained from the volunteer monitoring sites during routine sampling or scum events will be delivered to the VDH laboratory as unfiltered whole water.

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.

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 algae from Lake Champlain. New technicians

will be trained in algal identification. Appropriate taxonomic keys are in house, including John et al. (2002), Joosten (2006), Komarek and Zapomelova (2007, 2008), and Prescott (1982). 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
Once potential toxin-producing cyanobacteria are documented in a sample, the sample may be analyzed for the presence of toxins. Analyses are typically performed on whole water samples.

An Enzyme-Linked ImmunoSorbant Assay (ELISA) antibody technique will be used to test for microcystins and cylindrospermopsin by the VDH in all samples received for toxin analysis. The genera *Aphanizomenon, Anabaena* and *Oscillatoria/Planktothrix* are potential anatoxin producers and samples containing large numbers of these organisms will 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. A copy of 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 priority 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 Laboratories 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

[1]Evaluation 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
[2]Criteria of acceptance	1) S.E. < 10%, analyst comparisons within 5% 2) confirmed agreement on identifications 3) no additional specimens found
[3]Responsibility 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 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 will be maintained by VT DEC and 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 general public by request. Alternately, annual data compilations can be accessed through the VDH's Algae Tracking page - http://healthvermont.gov/enviro/bg_algae/weekly_status.aspx#map

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 a final project report will be submitted to the LCBP Project Officer. The final 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 their weekly submittal, and again by the Project Manager upon receipt. 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 – 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 Program budget supports activities by the VT DEC field team and Project Manager. The LCC has a separate work plan with the LCBP for activities included in the Cyanobacteria Monitoring Program. Funding for VDH cyanobacteria visual monitoring at shoreline sites and cyanotoxin monitoring at drinking water facilities and shoreline sites is provided by the Vermont Climate and Health Program. Funding for training of cyanobacteria monitoring volunteers and support for the Blue Green Algae Tracker is provided by the Vermont Environmental Public Health Tracking Program. Both programs are funded by grants from the Centers for Disease Control and Prevention.

F - References

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Appendix A - List of Sites Sampled in 2015.

Waterbody	Region	Station	Site	Latitude	Longitude
Champlain	Inland Sea	Blockhouse Point Rd.		44.850032	-73.2841
Champlain	Inland Sea	Butler Island		44.839526	-73.2326
Champlain	Inland Sea	Carry Bay	5	44.833592	-73.2899
Champlain	Inland Sea	Cedar Ledge	131	44.846952	-73.2622
Champlain	Inland Sea	City Bay - Rt 2	78	44.815894	-73.2891
Champlain	Inland Sea	Cohen Park St. Albans	174	44.864582	-73.1828
Champlain	Inland Sea	Dunham Bay	186	44.885701	-73.2731
Champlain	Inland Sea	Everest Rd.	185	44.649828	-73.2131
Champlain	Inland Sea	Georgia Shore South	163	44.758833	-73.1794
Champlain	Inland Sea	Grand Isle State Park	11	44.686021	-73.2891
Champlain	Inland Sea	Grand Isle State Park Beach	11	44.686021	-73.2891
Champlain	Inland Sea	Grand Isle State Park Boat Launch	11	44.686021	-73.2891
Champlain	Inland Sea	Keeler Bay Boat Launch	135	44.667908	-73.3199
Champlain	Inland Sea	Keeler Bay East	134	44.654142	-73.292
Champlain	Inland Sea	Keeler Bay, South Hero	207	44.653897	-73.3009
Champlain	Inland Sea	Knight Island	146	44.810722	-73.2581
Champlain	Inland Sea	Knight Point State Park	80	44.768669	-73.2945
Champlain	Inland Sea	Lombard Lane- South Hero	177	44.668999	-73.3105
Champlain	Inland Sea	LTM 34	34	44.708167	-73.2268
Champlain	Inland Sea	Maquam Beach	139	44.920807	-73.1614
Champlain	Inland Sea	Maquam Shore Road, Swanton	209	44.904515	-73.1748
Champlain	Inland Sea	Marycrest Beach	116	44.723362	-73.2815
Champlain	Inland Sea	Milton	81	44.658992	-73.2142
Champlain	Inland Sea	Nichols Point		44.746424	-73.3298
Champlain	Inland Sea	North Hero		44.854333	-73.283
Champlain	Inland Sea	North Hero State Park	23	44.921754	-73.2408
Champlain	Inland Sea	offshore, middle of Keeler Bay		44.664251	-73.308
Champlain	Inland Sea	offshore, west side of Savage Island		44.701533	-73.2555
Champlain	Inland Sea	Pelots Bay	24	44.82537	-73.2991
Champlain	Inland Sea	Sand Bar State Park	57	44.628758	-73.2399
Champlain	Inland Sea	South Alburgh - Squires Bay	182	44.903004	-73.2719
Champlain	Inland Sea	South Hero Fish and Wildlife Boat Access	110	44.636405	-73.2652
Champlain	Inland Sea	Stephenson Point Fish and Wildlife Access	205	44.89486	-73.2315
Champlain	Inland Sea	The Gut	49	44.751374	-73.2903
Champlain	Inland Sea	Van Everest Boat Launch Milton	175	44.705866	-73.2104
Champlain	Inland Sea	Woods Island	145	44.804871	-73.2045
Champlain	Inland Sea	Woods Island campsite 3		44.801543	-73.2116
Champlain	Inland Sea	Woods Island West		44.801543	-73.2116
Champlain	Main Lake Central	Allen Point	189	44.599276	-73.3114
Champlain	Main Lake Central	Beech Bay		44.608883	-73.3158
Champlain	Main Lake Central	Buena Vista Park, Willsboro NY	61	44.403947	-73.3735
Champlain	Main Lake Central	Burlington, VT - Texaco Beach	72	44.487636	-73.2321
Champlain	Main Lake Central	Charlotte Town Beach	76	44.334725	-73.2829
Champlain	Main Lake Central	Community Sailing Center	107	44.48206	-73.2255
Champlain	Main Lake Central	LaPlatte River mouth, Shelburne Bay	55	44.400342	-73.2335
Champlain	Main Lake Central	Law Island		44.560298	-73.3118
Champlain	Main Lake Central	Leddy Park	54	44.500826	-73.2534
Champlain	Main Lake Central	LTM 16	16	44.425	-73.232

Waterbody	Region	Station	Site	Latitude	Longitude
Champlain	Main Lake Central	LTM 19	19	44.471	-73.299
Champlain	Main Lake Central	LTM 21	21	44.474833	-73.2317
Champlain	Main Lake Central	LTM 33	33	44.701167	-73.4182
Champlain	Main Lake Central	North Beach	22	44.491058	-73.2404
Champlain	Main Lake Central	Oakledge Park Blanchard Beach	42	44.45744	-73.2255
Champlain	Main Lake Central	Oakledge Park rocky shoreline	44	44.456715	-73.228
Champlain	Main Lake Central	Oakledge Park South Cove	43	44.454958	-73.23
Champlain	Main Lake Central	Peru Boat Launch	159	44.618839	-73.4404
Champlain	Main Lake Central	Phelps Point		44.61843	-73.3459
Champlain	Main Lake Central	Plattsburgh Boat Launch	150	44.69916	-73.4417
Champlain	Main Lake Central	Plattsburgh City Beach	47	44.719494	-73.4308
Champlain	Main Lake Central	Potash Brook	171	44.438672	-73.2206
Champlain	Main Lake Central	Red Rocks Beach	27	44.441999	-73.2241
Champlain	Main Lake Central	Shelburne Beach	48	44.363061	-73.2676
Champlain	Main Lake Central	Shelburne Farms	123	44.404449	-73.2683
Champlain	Main Lake Central	Shelburne Point	125	44.43447869	-73.2512
Champlain	Main Lake Central	Shelburne Shipyard	124	44.434579	-73.247
Champlain	Main Lake Central	South Cove Beach	173	44.450003	-73.2316
Champlain	Main Lake Central	South of Perkins Pier		44.473078	-73.2209
Champlain	Main Lake Central	Starr Farm Beach	108	44.513764	-73.2714
Champlain	Main Lake Central	Sunset/Crescent Beach	132	44.608883	-73.3158
Champlain	Main Lake Central	Teddy Bear Point Cove, Willsboro NY	63	44.442723	-73.3743
Champlain	Main Lake Central	White's Beach in Crescent Bay	114	44.621145	-73.3234
Champlain	Main Lake Central	Willsboro Boat Launch	68	44.39945	-73.3916
Champlain	Main Lake Central	Winooski R. mouth		44.52965	-73.2774
Champlain	Main Lake Central	South of Perkins Pier		44.473078	-73.2209
Champlain	Main Lake North	Alburgh Dunes State Park	35	44.864624	-73.302
Champlain	Main Lake North	Grand Isle Ferry		44.68885	-73.3524
Champlain	Main Lake North	Holcomb Boat Launch	129	44.854684	-73.3316
Champlain	Main Lake North	Horicans Fish and Wildlife Access	127	44.914084	-73.3145
Champlain	Main Lake North	LTM 36	36	44.756167	-73.355
Champlain	Main Lake North	LTM 46	46	44.948333	-73.34
Champlain	Main Lake North	north of Rt 129 Bridge, Alburgh - Isle LaMotte		44.907025	-73.3178
Champlain	Main Lake North	Oliver Bay	45	44.737454	-73.4023
Champlain	Main Lake North	Pelots Point West	130	44.826076	-73.3101
Champlain	Main Lake North	Pt. Au Roche Boat Launch	109	44.804399	-73.363
Champlain	Main Lake North	Pt. Au Roche S.P. Deep Bay	84	44.777511	-73.3789
Champlain	Main Lake North	Pt. Au Roche State Park Beach	26	44.774136	-73.3938
Champlain	Main Lake North	Stoney Point, Isle la Motte	128	44.871482	-73.3594
Champlain	Main Lake North	Treadswell Bay, Beekmantown NY	64	44.760077	-73.4075
Champlain	Main Lake North	Vantines Boat Launch	115	44.719813	-73.3419
Champlain	Main Lake North	Wilcox Dock, Plattsburgh	12	44.708179	-73.4439
Champlain	Main Lake South	Arnold Bay	3	44.149739	-73.3695
Champlain	Main Lake South	Arnold Bay, Panton	206	44.148573	-73.3686
Champlain	Main Lake South	Beggs Park Beach, Essex NY	60	44.308462	-73.3473
Champlain	Main Lake South	Bulwagga Bay	138	44.036878	-73.4548
Champlain	Main Lake South	Bulwagga Bay/Port Henry	138	44.036878	-73.4548
Champlain	Main Lake South	Button Bay Boat Launch	74	44.176162	-73.3523
Champlain	Main Lake South	Button Bay South	183	44.168977	-73.3561

Waterbody	Region	Station	Site	Latitude	Longitude
Champlain	Main Lake South	Button Bay State Park	180	44.180926	-73.3618
Champlain	Main Lake South	Camp Dudley, Westport NY	75	44.143222	-73.4157
Champlain	Main Lake South	Chimney Point	143	44.034809	-73.4226
Champlain	Main Lake South	Converse Bay	184	44.293963	-73.2898
Champlain	Main Lake South	DAR State Park	39	44.054526	-73.4183
Champlain	Main Lake South	Ferrisburgh Stone Beach	137	44.237899	-73.3083
Champlain	Main Lake South	Ferrisburgh Town Beach	117	44.235937	-73.301
Champlain	Main Lake South	Hawkins Bay	105	44.243757	-73.2834
Champlain	Main Lake South	Kingsland Bay State Park	15	44.240302	-73.2987
Champlain	Main Lake South	Lane's Lane Landing	121	44.273405	-73.2889
Champlain	Main Lake South	Long Point	18	44.258135	-73.2776
Champlain	Main Lake South	Long Point South	187	44.252618	-73.2808
Champlain	Main Lake South	Long Pt, (Wood) Ferrisburgh	41	44.256623	-73.2831
Champlain	Main Lake South	LTM 07	7	44.126	-73.4128
Champlain	Main Lake South	LTM 09	9	44.242167	-73.3292
Champlain	Main Lake South	North Harbor	147	44.199725	-73.3588
Champlain	Main Lake South	Panton Shore North	151	44.153539	-73.3643
Champlain	Main Lake South	Port Henry Boat Launch	153	44.052777	-73.4506
Champlain	Main Lake South	Port Henry village beach		44.064703	-73.4496
Champlain	Main Lake South	Summer Point	148	44.218251	-73.338
Champlain	Main Lake South	Town Farm Bay	119	44.269164	-73.2887
Champlain	Main Lake South	Town of Moriah beach		44.050701	-73.452
Champlain	Main Lake South	Tri-Town Road, West Addison	210	44.085383	-73.4079
Champlain	Main Lake South	Westport Boat Launch	59	44.188732	-73.4328
Champlain	Malletts Bay	Camp Kiniya	142	44.606441	-73.2291
Champlain	Malletts Bay	Clay Point	133	44.593928	-73.2318
Champlain	Malletts Bay	LTM 25	25	44.582	-73.2812
Champlain	Malletts Bay	Niquette Bay State Park	67	44.581294	-73.1889
Champlain	Malletts Bay	Rosetti Park	111	44.555009	-73.2528
Champlain	Missisquoi Bay	Alburgh Bridge		44.978469	-73.2159
Champlain	Missisquoi Bay	Alburgh Springs	86	44.993016	-73.2159
Champlain	Missisquoi Bay	Alburgh Springs North	86	44.996014	-73.2173
Champlain	Missisquoi Bay	Alburgh VT - shoreline	208	44.991352	-73.216
Champlain	Missisquoi Bay	areas of shoreline, north of the border		45.050699	-73.0793
Champlain	Missisquoi Bay	Chapman Bay	6	45.00785	-73.2112
Champlain	Missisquoi Bay	Donaldson Point	10	44.993203	-73.1753
Champlain	Missisquoi Bay	Fadden Road - Swanton	181	44.97943	-73.1928
Champlain	Missisquoi Bay	Goose Bay		44.983921	-73.1176
Champlain	Missisquoi Bay	Highgate Cliffs	172	44.996109	-73.093
Champlain	Missisquoi Bay	Highgate Springs	14	44.991767	-73.1134
Champlain	Missisquoi Bay	Jameson Point QE south to the US border		45.028614	-73.0918
Champlain	Missisquoi Bay	Larry Greene Fish and Wildlife Access	87	44.970804	-73.2117
Champlain	Missisquoi Bay	LTM 50	50	45.013333	-73.1738
Champlain	Missisquoi Bay	LTM 51	51	45.041667	-73.1297
Champlain	Missisquoi Bay	midbay - north of the border		45.054528	-73.1051
Champlain	Missisquoi Bay	mouth of the Pike River		45.070439	-73.0966
Champlain	Missisquoi Bay	mouth of the Pike River		45.070439	-73.0966
Champlain	Missisquoi Bay	offshore, north of Rock River Bay		45.004537	-73.1028
Champlain	Missisquoi Bay	open water, north of the Rt 78 Bridge		44.973712	-73.2149

Waterbody	Region	Station	Site	Latitude	Longitude
Champlain	Missisquoi Bay	Phillipsburg QE south to the US border		45.031555	-73.0897
Champlain	Missisquoi Bay	Phillipsburg QE, south to US border		45.03202	-73.0896
Champlain	Missisquoi Bay	Phillipsburg, QC	58	45.039064	-73.0787
Champlain	Missisquoi Bay	Rock River - Highgate	178	44.989379	-73.0893
Champlain	Missisquoi Bay	Rock River Wildlife Management area		44.997047	-73.0726
Champlain	Missisquoi Bay	Shipyard Road		44.977567	-73.1115
Champlain	Missisquoi Bay	Shipyard, Highgate Springs	30	44.98076	-73.1079
Champlain	Missisquoi Bay	St. Armand		45.05951	-73.0935
Champlain	Missisquoi Bay	Venise-en-Quebec Bay		45.069226	-73.1438
Champlain	South Lake	Allen Bay	52	43.783007	-73.354
Champlain	South Lake	Lapham Bay	141	43.92598	-73.3927
Champlain	South Lake	LTM 02	2	43.714	-73.383
Champlain	South Lake	LTM 04	4	43.951004	-73.407
Champlain	South Lake	McCuen Slang Waterfowl Area	179	44.024305	-73.4016
Champlain	South Lake	Ticonderoga Boat Launch	188	43.854812	-73.3849
Champlain	St. Albans Bay	Black Bridge		44.810209	-73.1518
Champlain	St. Albans Bay	Ferrand Rd. St. Albans	113	44.791711	-73.1425
Champlain	St. Albans Bay	Georgia Beach		44.768331	-73.1626
Champlain	St. Albans Bay	Georgia Beach		44.768331	-73.1626
Champlain	St. Albans Bay	Georgia Shore North	106	44.7587	-73.1792
Champlain	St. Albans Bay	Hathaway Point Rd		44.794823	-73.1659
Champlain	St. Albans Bay	Hathaway Point Road		44.794823	-73.1659
Champlain	St. Albans Bay	Kill Kare State Park	56	44.777702	-73.1808
Champlain	St. Albans Bay	LTM 40	40	44.785333	-73.1622
Champlain	St. Albans Bay	Martha Drive		44.785259	-73.1735
Champlain	St. Albans Bay	Melville Landing	176	44.76174	-73.1676
Champlain	St. Albans Bay	offshore, outer St. Albans Bay towards Ball island		44.766876	-73.1841
Champlain	St. Albans Bay	offshore, St. Albans Bay Park to Lazy Lady Island		44.804147	-73.1464
Champlain	St. Albans Bay	offshore, vicinity of St. Albans town park		44.808192	-73.1462
Champlain	St. Albans Bay	St. Albans Bay Park	31	44.808658	-73.1444
Champlain	St. Albans Bay	St. Albans Boat Launch	32	44.793721	-73.1714
Champlain	Missisquoi Bay	Missisquoi Delta		45.010169	-73.1533
Elmore		Lake Elmore State Park	202	44.540398	-72.5273
Iroquois	Lake Iroquois	Lake Iroquois Southwest	169	44.363273	-73.0856
Iroquois		Lake Iroquois	203	44.378068	-73.0867
Lower Poultney River	South Lake	Lower Poultney River, West Haven		43.570906	-73.3917
Memphremagog		Derby Bay	211	44.994377	-72.1884
Memphremagog		Holbrook Bay	212	44.963922	-72.2397
Memphremagog		Lake Memphremagog	204	44.945012	-72.21
Carmi		Lake Carmi State Park	201	44.960813	-72.8767
Carmi		Lake Carmi State Park South	165	44.956922	-72.8773
Carmi		Lake Carmi, Black Woods	164	44.975297	-72.8855
Carmi		Lake Carmi, Dewing Road	166	44.982139	-72.8535
Carmi		Lake Carmi, Hammond Rd.		44.980168	-72.857
Carmi		Lake Carmi, North Beach	167	44.990535	-72.8703
Carmi		Lake Carmi, Westcott Shore	168	44.957115	-72.894
Carmi		Vics Crossing Road		44.98544	-72.8607

Appendix B – Task Allocation by Partner

B1 - Lake Champlain Committee (LCC)

The LCC will serve as the primary connection with the volunteer monitors around the lake. Volunteers will target shoreline locations during the high recreation activity period from mid-June to September, providing a weekly visual assessment of cyanobacteria conditions and supplementary photographic documentation. LCC will serve as the oversight point for these data, which they will review and share with the other partners. The LCC will work with selected volunteers to collect water and algae samples when appropriate and deliver 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 that have successfully completed the training
  - Develop and refine supportive materials for volunteers
    - Reporting/field sheet
    - Reference and guidance materials
    - Photography assistance and guidance
- Outreach and education
  - Hold workshops for the general 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
  - Offer similar workshops to 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
    - Respond to bloom inquiries and requests for information from the general public
- 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 to the VDH web interface each week
  - Provide a summary of the volunteer network operation for inclusion in the annual report
  - Maintain an electronic database of volunteer reports and photographs

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 and cylindrospermopsin samples and will analyze anatoxin samples as conditions warrant.

Tasks

- Public Health Response
  - Update and maintain web-based public information maps and supplementary materials on the VDH cyanobacteria pages - http://healthvermont.gov/enviro/bg_algae/bgalgae.aspx
  - Issue health alerts or warnings as conditions warrant
    - Issue a general recreational safety reminder prior to the summer season
  - Initiate contact and provide public health assistance to areas affected by cyanobacteria blooms
  - Work in conjunction with DWGPD to coordinate the drinking water monitoring program and respond to any drinking water concerns
  - Respond to public inquiries about algae and health
- Laboratory
  - Conduct microcystin analyses
  - Conduct cylindrospermopsin analyses
    - for routine tiered alert samples
    - as warranted in response to bloom events and emergency situations
  - Conduct anatoxin analyses
    - As warranted by the presence of potential anatoxin producers
    - In response to bloom events and emergency situations
  - Reporting and documentation
    - Provide data to VDH project staff for inclusion in the tracking database
    - Maintain an electronic database of results

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

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

Tasks

- Public Health response
  - initiate contact and provide operational guidance to water suppliers located in the vicinity of cyanobacteria blooms
  - Work with VDH to coordinate the drinking water monitoring program
    - respond to drinking water concerns, i.e. public notification language, additional sampling.
- Education and Outreach
  - Organize workshops for water suppliers
  - Provide outreach and general guidance to public water suppliers
  - Post informational material on the DWGWPD website
  - Respond to inquiries about water supply concerns

B4 - VT DEC, Watershed Management Division (WsMD)

The Watershed Management Division will be responsible for overall project management, field collections at mid-lake stations on Champlain, algal identification and communication of results. The Lake Champlain Long-term Water Quality and Biological Monitoring Program (LTM) staff will collect quantitative samples from the established LTM sites during routine biweekly site visits. Field activities associated with this project will begin in June and continue through mid-September or later, as conditions warrant.

Monitoring Tasks

- Field Collection
  - Provide field sheets and supporting photographic documentation
  - Collect phytoplankton and toxin samples following the tiered alert protocol at mid-lake open water 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 according to the rapid assessment protocol
- Reporting and Documentation
  - Provide qualitative and quantitative data for inclusion in the on-line tracking map
  - 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://www.anr.state.vt.us/dec/waterq/lakes/htm/lp_cyanobacteria.htm
  - Assist in LCC-organized 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
  - Provide an annual report to partners each winter
    - Summarize the current year's efforts and conditions
      - Observed conditions
      - Occurrence and severity of toxins
      - Public health
      - reported health impacts, if any
      - public health response and outreach efforts
    - Discuss trends and observations over time
  - Coordinate the development of the work plan for the following year
- 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. 75 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 - Kit BGA-1, microcystin, and Kit ANA-1, anatoxin

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 75ml 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 1mL Lugols per 100 mL sample. Samples with large amounts of algae may require additional preservative.
- Label tube
- 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 1L bottle at the surface and tipping slightly to fill.
- Mix well and decant an aliquot into a 75mL glass test tube. The remaining water should be saved for toxin analysis.
- Record collection depth, location and date.
- Add 1 mL of Lugols solution per 100 mL of sample to each 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 (Kit BGA-1 and/or Kit ANA-1)
- 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	1
LC/MS/MS samples	a	A	3	2

Processing: a - whole water
b – net plankton

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

Containers: 1 - 75 mL glass test tube
2 – 40ml glass vials, septum-top (VDH toxin analysis)
3 – 60 or 80 ml polyethylene bottle (VDH toxin analysis)

Holding times: 1 – 48 hours
2 - 6 months

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

C4 - Drinking Water System Sampling Procedure

Facilities are asked to sample raw water prior to any anti-fouling treatments, if possible.

VERMONT
DEPARTMENT OF HEALTH

VERMONT DEPARTMENT OF HEALTH LABORATORY
195 Colchester Ave. P.O. Box 1125
Burlington, Vermont 05402-1125
Phone Number (802) 863 7336 (800) 660-9997 (VT only)

Instructions for Collection and Packaging of Drinking Water Samples for Cyanotoxin Analyses (Routine/Weekly)

Please Read Instructions Carefully Before Collecting Samples

Routine/Weekly Kit Contents:

- 1 sample submittal form (Request for Cyanotoxin Analyses)
- 2 40mL sample vials with septum caps pre-labeled
- Ziploc Bag

Kits can be stored at room temperature until needed

Sample Collection Instructions:

1. Identify the raw water tap; it is the intake stream just before it is filtered and disinfected.
2. Uncap one of the vials and collect a sample from the raw water source. The vial should be as full as possible, but it is acceptable if there is some air space. No gloves required unless the raw water appears to be contaminated with a bloom. Write collection information on label with waterproof marker. Be sure to identify sample as ***RAW***.
3. Identify the finished drinking water tap; it is the point where the water enters the distribution system.
4. Uncap the second vial and collect a sample from the tap. The vial should be as full as possible, but it is acceptable if there is some air space. Write collection information on label with permanent marker. Be sure to identify sample as ***FINISHED***.
5. Provide the requested information on the submittal form for each sample. Place completed form and samples in the Ziploc bag.
6. Samples should be collected the same day as the drop-off day. Keep samples cool during transportation to designated drop-off location. Make sure to keep samples refrigerated if storing more than 2 hours before sample drop-off. If samples need to be collected a day prior, they should be kept under refrigeration. Samples should be collected on Monday or Tuesday so that a routine schedule is established.

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):

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

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: 60 cells
  - 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 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 (filtered or unfiltered) into a labeled 15ml conical tube.

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% trifluoracetic acid
- Bring sample to dryness with the Turbo Vap at < 40^{o}C.
- Reconstitute with 200 µL of 15% acetonitrile containing 0.05% trifluoracetic 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 algal concentrations 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* = (average OD of calibrator, control or sample x 100) ÷ 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 ± SD*	%RSD	%Bo**	MCYN conc. (ppb)
Negative Control	1.478 1.552	1.515 ± 0.052	3.4	100	N/A
0.1 ppb Calibrator	1.255 1.194	1.225 ± 0.043	3.5	80.8	N/A
0.3 ppb Calibrator	0.941 0.932	0.937 ± 0.006	0.68	61.8	N/A
0.8 ppb Calibrator	0.626 0.602	0.614 ± 0.017	2.7	40.5	N/A
2.0 ppb Calibrator	0.389 0.386	0.302 ± 0.008	0.54	25.6	N/A
Sample	0.634 0.610	0.622 ± 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.
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Beacon Analytical Systems Inc.

Microcystin Plate Kit

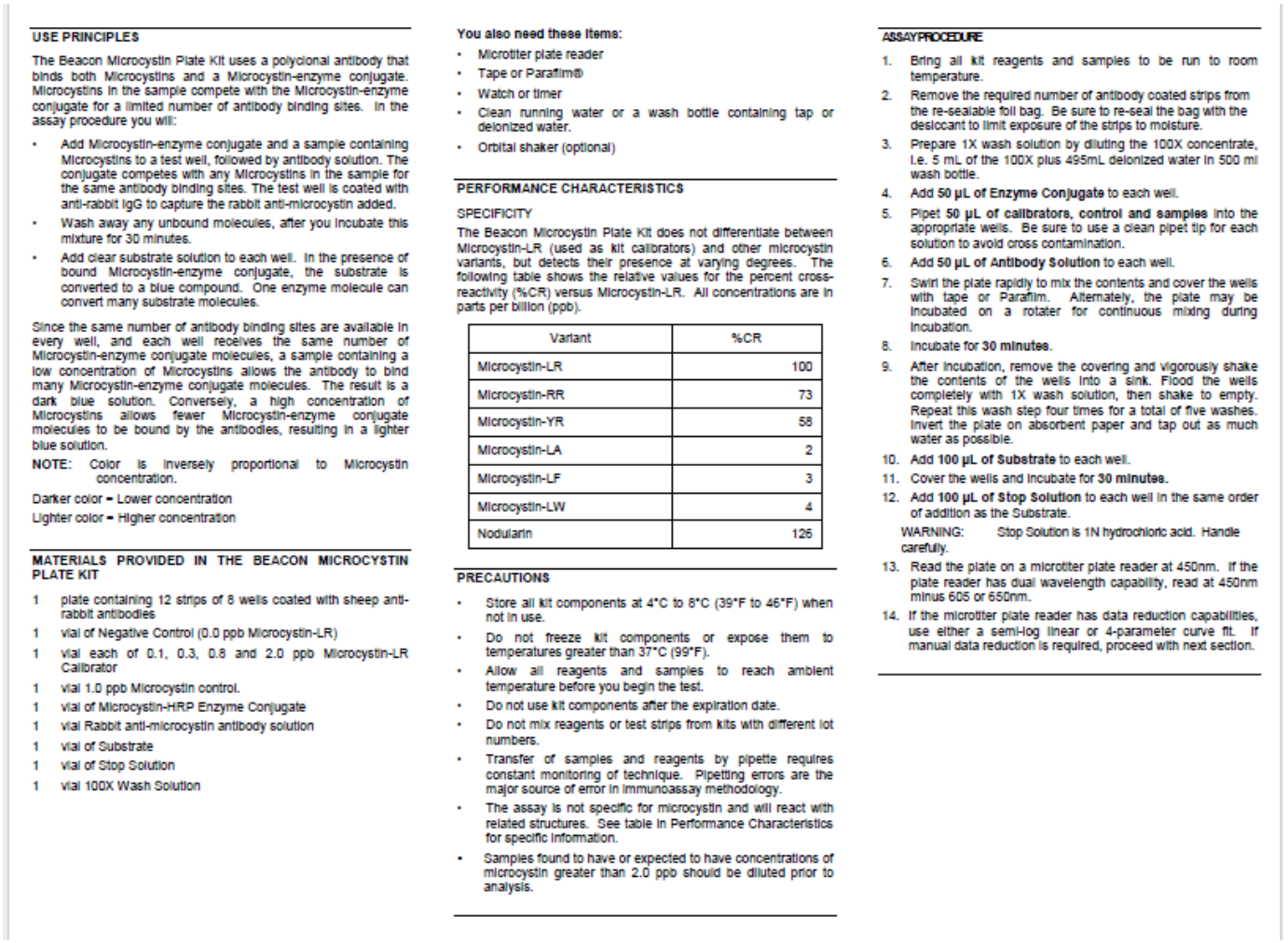
Cat.# 20-0068

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.



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 rotater 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 Microcystin 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 ± SD*	%RSD	%Bo**	Cylindrosper-mopsin Conc. (ppm)
Negative Control	1.487 1.444	1.466 ± 0.030	2.07	100	N/A
0.1ppb Calibrator	1.153 1.155	1.154 ± 0.001	0.12	78.7	N/A
0.5 ppb Calibrator	0.699 0.703	.701 ± 0.003	0.4	47.8	N/A
2 ppb Calibrator	0.268 0.261	0.265 ± 0.005	1.87	18	N/A
Sample	0.487 0.497	0.492 ± 0.006	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

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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.

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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.
82 Industrial Park Road
Saco, ME 04072
Tel. (207) 571-4302
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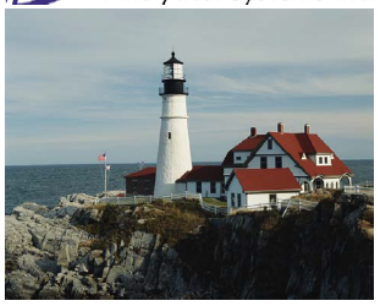
Beacon Analytical Systems Inc.



Cylindrospermopsin Plate Kit

Cat. # 20-0149

Instructional Booklet

READ COMPLETELY BEFORE USE.

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 – 8ºC.

1 plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating dessicant.

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 – 28ºC (62 – 82ºF) 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 dessicant.
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
These on-line pages may be updated as protocols evolve.

Appendix E – Volunteer Monitoring Protocols.

E1 - On-line reporting form

- http://www.lakechamplaincommittee.org/get-involved/volunteers/bgamonitors/bga-report/

Algae Reporting Form

ALGAE REPORTING FORM - 2016

Please Complete Form Below

Type of report

- ○ Regular weekly
- ○ Supplemental

Site name/water body or section of Lake Champlain or GPS coordinates*

Municipality of observation

Date of observation*

Time of observation*

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

- ○ 1a - Little or no blue-green algae present - clear water
- ○ 1b - Little or no blue-green algae present - brown or turbid water
- ○ 1c - Little or no blue-green algae present - other material present
- ○ 1d - Little blue-green algae present but enjoyment of water not impaired
- ○ 2 - Blue-green algae present -less than bloom levels - enjoyment of water slightly impaired (include photos)
- ○ 3 - Blue-green algae bloom in progress - enjoyment of water substantially impaired (include photos)

Photo - water surface close-up

Choose File No file chosen

Photo - water surface broad view

Choose File No file chosen

Photo - water sample in clear container

Choose File No file chosen

Extent of algae 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%

Algae Color

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

Add algae color details here

Water temperature

Water surface

- ○ Calm
- ○ Rolling
- ○ White-caps

I am using LCC's blue-green algae 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 blue-green algae 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 blue-green algae tracking form. Include the device you used to access the form, your internet browser, and screen size and resolution.

Full name*

Email*

Address

Telephone

Submit

E2 - Determining Algae Bloom Intensity

https://www.lakechamplaincommittee.org/get-involved/volunteers/bgamonitors/algaebloomintensity/

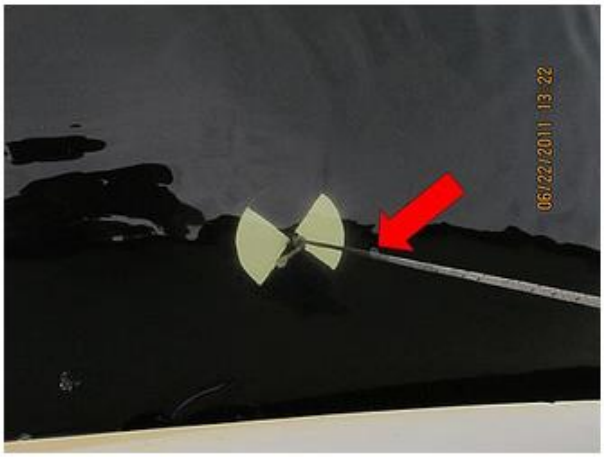
General Instructions

Observations should be made at the same location once per week. Observations must be made between 10:00 AM and 3:00 PM. At that time the algae have had a chance to rise from lower in the water column, but cells are not yet likely to have ruptured from the heat of mid-day. Only observations submitted online **by noon on Wednesday** will be included in the weekly report. Anyone providing reports should include information on the extent and type of algae and plant growth, the color of the water, and rate the algae intensity. The rating scale runs from one (a, b, c, or d) to three, with one being clear water with little to no blue-green algae present and three being a blue-green algae bloom in progress.

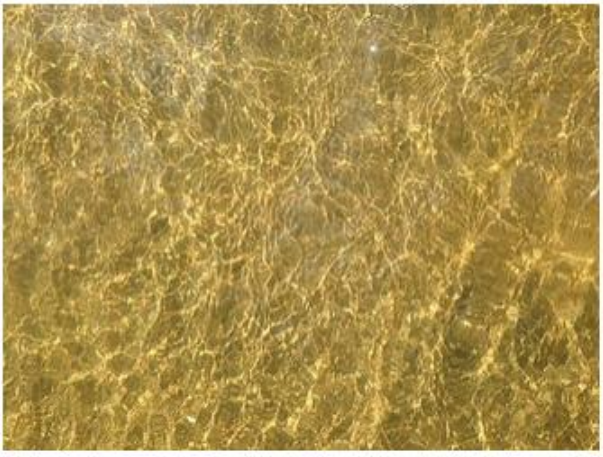
For category 2 and 3 conditions, three digital photographs should be submitted via the online form. Remember to avoid direct contact if the bloom is well developed.

Category 1a: Little to no blue-green algae 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 - Little to no blue-green algae present - brown and turbid

Brown turbid low visibility through water column

Brown and cloudy does not indicate presence of blue-green algae

Category 1c - Little to no blue-green algae present - other material

Other material that doesn't count as blue-green algae 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 blue-green algae

From a distance duckweed can look like algae

Stringy algae attached to the bottom are not blue-greens

Duckweed up close

Category 1d - Little blue-green algae present - enjoyment of water not impaired

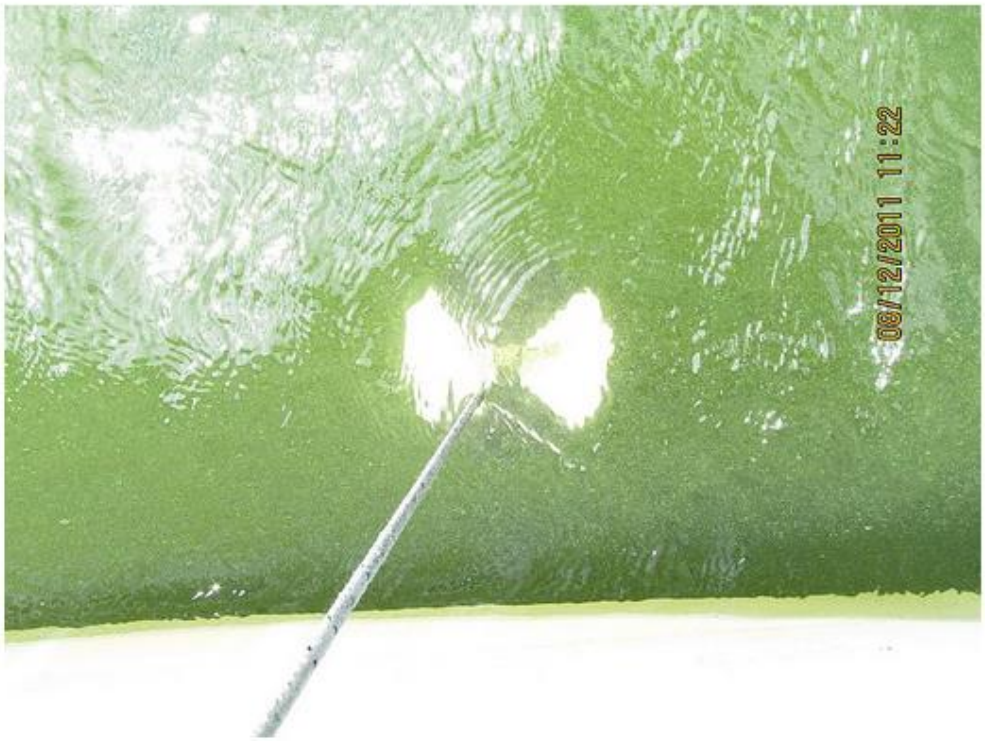
Green floating balls may be visible, but only on close inspection and in densities so low that they do not impair recreational enjoyment of the water. There are no surface or near shore accumulations of blue-green algae.

Water appears perfectly clear

But close inspection shows some blue-green algae present

Category 2: Blue-green algae present, but at less than 'bloom' levels - enjoyment of water slightly impaired

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 algae accumulation at shoreline.



Some algae in water but not a uniform layer

Possible narrow band of algae at shoreline

Category 3: Blue-green algae bloom in progress - enjoyment of water substantially impaired

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 algae at the surface - not stringy

Thick surface scum present

Open water surface green to turquoise

Instructions for Photographing Algae Blooms

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.

2. Photograph both a close-up and a broad view.

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

When collecting a water sample to photograph take care to avoid exposure to blue-green algae. Wear gloves, don't wade or immerse yourself in the water and wash any exposed portions of your body immediately after collecting the sample. It is okay not to collect a physical sample for photography if you are uncomfortable doing so.

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 using the year, month, day-photographer's name-location-photo type.

Example file name: 2014-07-15_MWinslow_DonaldsonPt_Closeup

Appendix F – Forms

F1 - VT DEC Field Form

VT DEC Cyanobacteria Monitoring Project – 2016

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.