QA Project Plan:

Cyanobacteria Monitoring on Lake Champlain
Version 2

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

April 30, 2015

EPA RFA#: 11030

Angela Shambaugh 5/8/15
Angela Shambaugh, Project Manager, VT DEC — Date

Andy Chevrefils, VT Dept. of Health — Date

Mike Winslow, Lake Champlain Committee — Date

Eric Howe 5/7/15
Eric Howe, Project Officer, LCBP — Date

Michael Jennings 5/7/15
Michael Jennings, Quality Assurance Program Manager, NEIWPCC — Date

Jeanne Voorhees, Project Officer, EPA — Date

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A - Project Management

A1 - QAPP Distribution List

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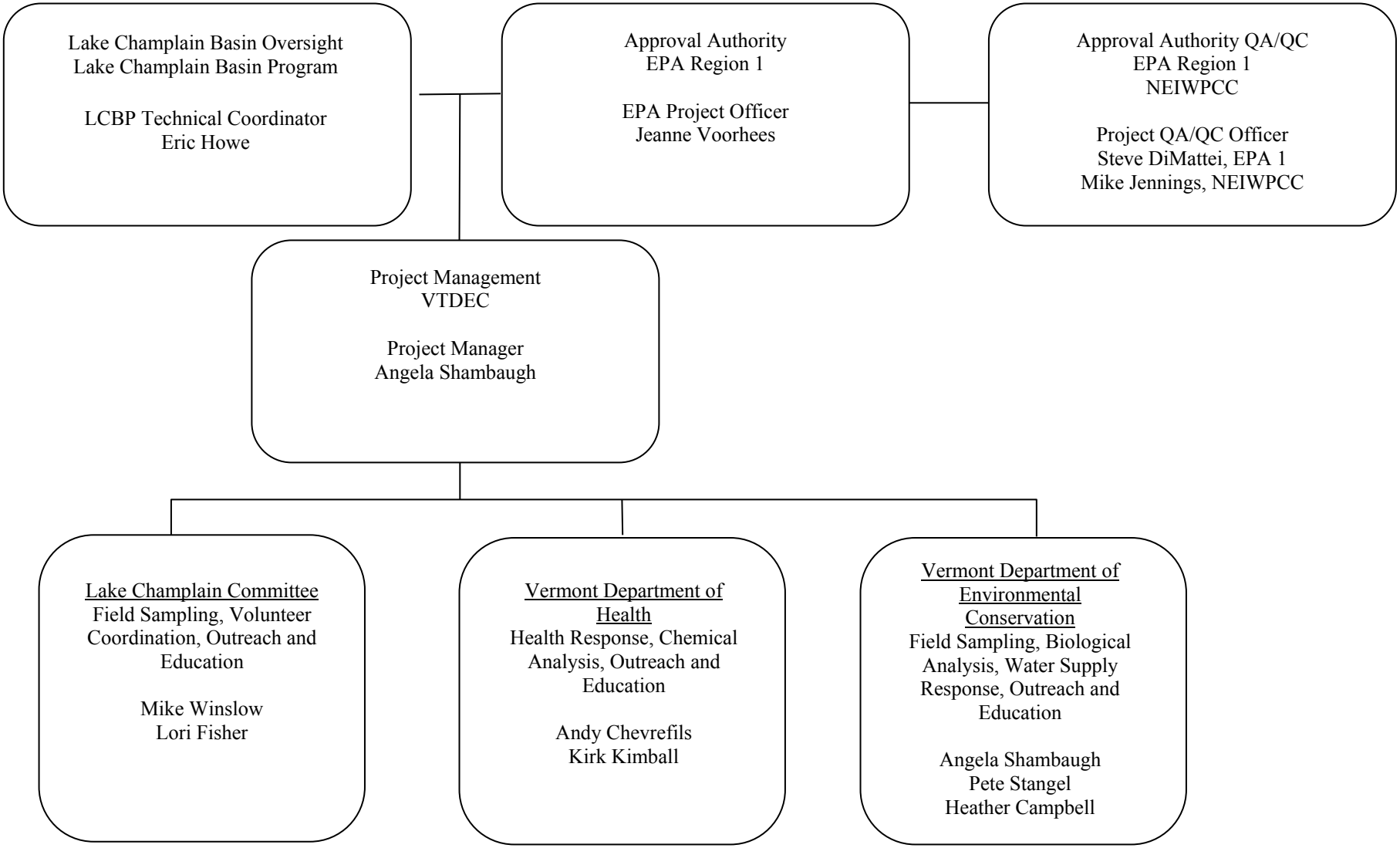
A2 - Project Organization



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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phytoplankton in fresh water and are present in Lake Champlain during much of the summer (Shambaugh et al. 2014, LTM unpublished data). Cell counts and associated toxins are below levels of concern in most of the lake each year (Shambaugh et al., 2014). 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).

Since 2002, the Lake Champlain Basin Program has funded an annual cyanobacteria monitoring program which utilizes cell density and toxin data to evaluate recreational conditions around the lake. Results are 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 has been well received locally and serves as a model at the regional and national level.

In 2012, oversight of the cyanobacteria monitoring program on Lake Champlain transferred from UVM to the State of Vermont and a visual assessment protocol for use by trained volunteers was added. As a result, the monitoring network was able 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. In 2015, 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, 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 2012). 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 the tiered alert protocol developed by UVM (Watzin et al., 2006). A weekly email will convey recent status and bloom observations to researchers, health and environmental

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officials, water supply managers and monitoring program participants who are responsible for cyanobacteria response at numerous private and public organizations around the lake. Through weekly condition updates posted 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. Additionally, the data will contribute to the knowledge and perspective about these organisms that has been gained since the program's inception in 2002. Project tasks and associated timelines are noted in Table 1.

Table 1. Timeline for project tasks. *as needed

Task	June 2015	July	Aug	Sep	Oct	Nov	Dec	Jan 2016	Feb	Mar	Apr	May
Field Collection	x	x	x	x	*							
Volunteer Monitoring	x	x	x	x								
Laboratory Analysis	x	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

As awareness of cyanobacteria and possible health concerns associated with exposure to these organisms has risen, the need for expanded expertise, technical assistance and communication around this issue has increased. Beginning in 2015, the role of the Project Manager will expand to include a formal facilitation, coordination and technical assistance role for the Basin Program and the State of Vermont beyond the basic monitoring and communication functions performed in previous years.

The activities and deliverables pertinent to this new role will be developed more fully over the year, but will include expanding technical expertise and knowledge concerning cyanobacteria control options, taxonomy and monitoring methods; statewide consistency in monitoring and bloom response, increased technical assistance to the Drinking Water and Groundwater Protection Division and drinking water facilities around Vermont; and coordination at the regional level to develop consistency in monitoring and response activities in the Northeast. These new roles are outlined in Appendix A and deliverables would be part of the 2015 final report.

A5 – Quality Objectives and Criteria for Measurement Data

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

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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 E) or online reporting forms (Appendix D), 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.

Locations with long-term historical data include several monitored by UVM in St. Albans and Missisquoi Bays, the 15 sites associated with the Lake Champlain Long-term Water Quality and

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Biological Monitoring program, and several shoreline locations that have been monitored by dedicated citizen volunteers. More than 50 volunteers regularly assessed and reported conditions at locations around the lake during 2014 (Figure 1). We expect that many of these will return in 2015. 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. Visual assessments from Champlain and four inland lake sites, monitored as part of a climate change project underway at the VDH, will also be included in the weekly updates. A final list of 2015 field sites, including a map and a table with latitude and longitude, will be provided in the final project report to the LCBP.

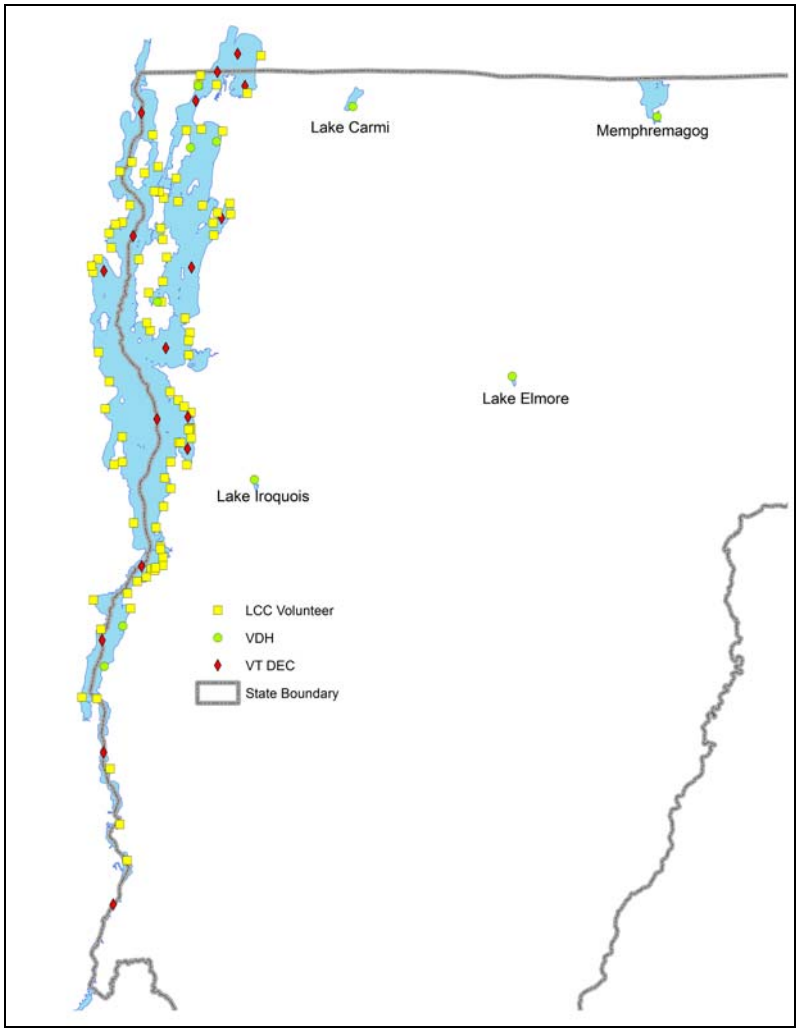

Figure 1. Anticipated monitoring coverage in 2015. Volunteer locations are subject to change.

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 A. Data collected by the partners

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will be provided to the VT DEC for inclusion in the weekly email and webpage updates. In 2015, staff associated with a VDH Climate Change project will also be contributing to the cyanobacteria monitoring efforts.

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 previous sampling date. Data and observations will be submitted to the Project Manager for inclusion in the weekly email and webpage updates. Any observations of significant scums or reports of scums will be shared with the stakeholders immediately.

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 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 within 72 hours of collection for the presence of potential toxin- producing cyanobacteria. 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 at those sites where potential toxin-producing taxa have been observed. Cyanobacteria in these samples will be enumerated within 72 hours. Detection of cyanobacteria at densities of 2000 cells per mL in the upper 3 m of the water column will trigger the progression to the Vigilance level.

Vigilance Level: Quantitative net samples for phytoplankton will be collected, mid-day, from locations where potential toxin-producing cyanobacteria reached densities of 2000 cells per mL in the upper 3 m of the water column during the previous visit. If water conditions at the site or previous samples suggest the onset of a bloom, an additional net plankton sample will be collected for possible toxin analysis. Toxin analysis would be initiated if cell densities exceed 4000 cells/mL. Phytoplankton samples will be enumerated within 48 hours. Potentially toxic cyanobacteria densities of more than 4000 cells per mL will trigger the progression to the Alert protocols for the next site visit.

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Table 2. Summary of project responsibilities by partner. *Jointly with Climate Change grant activities. **cylindrospermopsin will be routinely monitored for the first time in 2015

Task	Partners				
	Vermont DEC		**LCC**	VDH	
	Watershed Management Division	**DWGW Division**		**Laboratory**	**Radiological & Toxicological Program**
Field Collection	x		X (at selected sites)		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
Weekly Email updates	x				
Weekly Webpage updates					x
Field/ lab data QA/QC	x		x	x	
Project Coordination	x				
Maintenance of Central Database	x				
Annual Report	x				
Outreach	x	x	x		x

Alert Level 1 and 2: At these levels, 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. Water samples will be collected as close to mid-day as practical for determination

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of algal density and toxin concentration. Whole water samples will be collected at this point to reduce loss of colony fragments through the net. A subsample of filtered plankton will be screened by the VDH using enzyme-linked immunosorbant 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 or weeks). For this reason, alert level designations are based on the presence of microcystin rather than anatoxin.

The presence of microcystin and densities of potential toxin-producing cyanobacteria above 4000 cells/ml are designated as Alert Level 1 conditions. Per VDH guidelines established in 2005, ambient toxin concentrations ≥ 6 µg per L microcystin represent a potential threat to human health and will trigger progression to Alert Level 2. By relying on the results of the ELISA for action, we may miss anatoxin-producing blooms, but technology offers no rapid screening method for anatoxin at this time. To date, anatoxin concentrations have not exceeded 0.2 µg per L in Lake Champlain samples submitted for analysis, well below Vermont recreational guidelines. If anatoxin-producing species predominate in a bloom, this information will factor into the appropriate communication to public health officials. Cylindrospermopsin analysis has been in added in 2015, in anticipation of EPA drinking water health advisories scheduled to be released in May 2015. At this time, VDH has not released state guidance values for this toxin in deference to those pending EPA values. Prior to the start of monitoring for 2015, guidance values and response levels for cylindrospermopsin and microcystin will be communicated to stakeholders.

Visible scum or highly discolored water present at tiered alert sites will also trigger progression to Alert Level 2, regardless of microcystin concentration. 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.

Collection of whole water samples for toxins and algal enumeration will occur at the next visit to Alert Level 2 sites. The VDH will evaluate recreational activity levels and possible drinking water intakes in the vicinity, 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 B and C.

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 2012). The protocol is outlined in Appendix D. 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). In 2014, subcategory 1d ("Little BGA present - recreation not impaired ") was added to remind volunteers that small amounts of cyanobacteria should be reported here.

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Monitors will visit their designated shoreline location each week, beginning in June 15th and ending mid-September 2015. Using the photographs and identification triggers noted in Appendix D, monitors will complete a field form. Some monitors may provide photographic documentation. Field forms and any photographs will be provided to the Volunteer Coordinator. Each week, following review by the LCC coordinator, completed assessments will be collated and sent to VT DEC by noon Thursday for inclusion in the weekly email and webpage update. 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.

Qualitative check samples and the VDH Climate Change Grant

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.

Staff associated with the VDH Climate Change grant project will visit six Champlain sites and four inland lake sites 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.

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. Plankton samples for toxin analysis will be filtered in the field by the VTDEC. Filters will be sealed in aluminum foil packets, placed on ice, and transported to the VDH laboratory for further processing. Unfiltered whole water samples collected 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 and filter package. Label information will include sample date, description (e.g. tow or whole water sample), 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. Examples of the logs can be found in Appendix E.

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	South Lake	43.71483	--73.383	x		biweekly	x	x
	LTM 04	South Lake	43.95166	-73.40783	x		biweekly	x	x
	LTM07	Main Lake - south	44.126	-73.41283	x		biweekly	x	x
	LTM09	Main Lake - south	44.24216	-73.32916	x		biweekly	x	x
	LTM16	Main Lake - central	44.42583	-73.232	x		biweekly	x	x
	LTM19	Main Lake - central	44.471	-73.29916	x		biweekly	x	x
	LTM21	Main Lake - central	44.47483	-73.23166	x		biweekly	x	x
	LTM25	Malletts Bay	44.582	-73.28116	x		biweekly	x	x
	LTM33	Main Lake - north	44.70116	-73.41816	x		biweekly	x	x
	LTM34	Inland Sea	44.70816	-73.22683	x		biweekly	x	x
	LTM36	Main Lake - north	44.75616	-73.355	x		biweekly	x	x
	LTM40	St. Albans Bay	44.78533	-73.16216	x		biweekly	x	x
	LTM46	Main Lake - north	44.94833	-73.34	x		biweekly	x	x
	LTM50	Missisquoi Bay	45.01333	73.17383	x		biweekly	x	x
	LTM51	Missisquoi Bay	45.04166	-73.12966	x		biweekly	x	x
	Highgate Springs	Missisquoi Bay	44.99176	-73.11338	x		biweekly	x	x
Shoreline	St. Albans Boat launch	St. Albans	44.79424	-73.17227	x		biweekly	x	x
LCC Quanti-tative Sites - shoreline	North Beach	Main Lake - central	44.492	-73.23983		x	weekly	x	x
	Red Rock Beach	Main Lake - central	44.44274	-73.22443		x	weekly	x	x
	Highgate Springs-Shipyard	Missisquoi Bay	44.97966	-73.10769		x	weekly	x	x
	North Hero State Park	Inland Sea	44.92078	-73.2402		x	weekly	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
Volunteer Sites - shoreline	Not finalized for 2015	lakewide	-	-		X	weekly		
VDH Climate Change Grant - shoreline	Arnold Bay, VT	Main Lake - south	44.14938	-73.36733		x	weekly	X	X
	Alburgh Springs, VT	Missisquoi Bay	44.99217	-73.21742		x	weekly	X	X
	Maquam Shore Rd, Swanton VT	Inland Sea	44.90378	-73.16709		x	weekly	X	X
	Stephensen Point Fish and Wildlife Access, North Hero VT	Inland Sea	44.89484	-73.23253		x	weekly	X	X
	Keeler Bay, South Hero VT	Inland Sea	44.65133	-73.30205		x	weekly	X	X
	Tri-town Rd, West Addison VT	Main Lake - south	44.08445	-73.4074		x	weekly	x	x
	Lake Carmi	State park beach	44.96143	-72.87498		x	weekly	x	x
	Lake Memphremagog	Prouty beach	44.94657	-72.20821		x	weekly	x	x
	Lake Iroquois	Hinesburg Town Beach	44.37906	-73.08587		x	weekly	x	x
	Lake Elmore	State park beach	44.54146	-72.52813		x	weekly	x	x

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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 C.

Toxin Analysis
Once potential toxin-producing cyanobacteria are documented in a sample, the sample may be analyzed for the presence of toxins. Analyses may be performed on whole water samples or concentrated phytoplankton.

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. Filtered cell material for all toxin testing will be lysed by freezing prior to analysis. A copy of the ELISA test kit instructions and the analytical procedures for LC/MS/MS are located in Appendix C. 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

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information about variability in cell density. Duplicate analyses are not considered priority and may be completed after the 48 hour target window has passed.

Plankton Counts – 10% of plankton counts will be replicated over the course of the summer. Replicate analyses are not considered priority and may be completed after the 48 hour target window has passed. 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 manufacturer's recommendations. Blanks and standards are included in each run. Anatoxin analysis follows protocols developed by the VDH laboratory. Each anatoxin run includes standards, blanks and spikes. Preparation and analysis procedures for toxin samples are located in Appendix C.

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, 2008). 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

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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 will be 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 submitted to and kept by the respective Laboratory Supervisors. The log will document any maintenance and service of the equipment. A log entry will include the following information:

- Name of person maintaining 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 problems
- List of follow-up activities after maintenance
- Date the next maintenance will be needed

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.

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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 tape 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 access through the VDH's Environmental Public Health Tracking portal - https://webmail.vdh.state.vt.us/IAS/querytool?Topic=Water

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.

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E – Budget

All permanent staff at the VT DEC and the VDH conduct program activities as part of their normal job duties. The CDC Climate Grant provides support for a summer field technician at VDH and a portion of the analytical costs.

Category	LCC	VDH	VT DEC	Total
Personnel	29,000			**29,000**

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Appendix A – Task Allocation by Partner

A1 - 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 central collection point for these data, which they will collate 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 Burlington, 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
    - 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
  - Collate and provide volunteer reports to the VT DEC each week for inclusion in weekly email update

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- Provide a summary of the volunteer network operation for inclusion in the annual report
- Maintain an electronic database of volunteer reports and photographs

A2 - 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
    - Update the current conditions section of the webpage following receipt of the weekly email update, typically by close of business each Friday.
  - 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 respond to drinking water concerns
  - Respond to public inquiries about algae and health
- Laboratory
  - Conduct microcystin analyses
  - Conduct cylindrospermopsin analyses
  - 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 VT DEC for weekly email updates by Thursday noon each week
    - Provide information to VT DEC for annual program summary
    - Maintain an electronic database of results

A3 - 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 respond to drinking water concerns, i.e. public notification language, additional sampling.

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- Education and Outreach
    - Participate in LCC-organized workshops for water suppliers
    - Provide outreach and general guidance to public water suppliers
    - Post informational material on the DWGWPD websiteRespond to inquiries about water supply concerns

A4 - VT DEC, Watershed Management Division (WsMD)

The Watershed Management Division will be responsible for overall project management, field collections at mid-lake stations, 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 and process phytoplankton and toxin samples following the tiered alert protocol mid-lake open water sites and the St. Albans Boat launch
    - 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 weekly email update
    - 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 on Fridays.
- 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

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        - 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

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- Provide data and information in support of a regional assessment of historical and current bloom frequency

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Appendix B – VT DEC 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 B1 lists sampling equipment for phytoplankton and toxin collections.

B.1. Equipment and Preservatives
1. 75 mL glass sample vials - algae
2. Lugols solution
3. squirt bottles
4. plankton net – 63 um mesh
5. field filtration set-up and forceps
6. Whatman 934-AH glass fiber filters
7. aluminum foil
8. sample labels
9. coolers and ice
10. marking pens and pencils
11. field collection sheets/field notebook
12. VDH sample bottles - Kit BGA-1, microcystin, and Kit ANA-1, anatoxin

B1 - Phytoplankton Collection

B.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

B.1.2. Whole Water Collection

- Collect a water sample by carefully placing a bucket 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.

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- 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

B.2 Toxin Sample Collection

B.2.1. Field Filtered Surface Water for the VDH Laboratory

- Collect surface water as noted in B.2.2
- Filter a well-mixed aliquot onto a glass fiber filter.
- Repeat with additional aliquot until filter contains visible algae
- Fold filter in half and seal into a foil envelope
- Label envelope with station, date and volume filtered
- Place on ice and transport to the VDH laboratory.

B.2.2. Surface Water for the VDH Laboratory

- Collect a surface water sample as outlined in B.2.2.
- 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.

B.3. Field Sampling Parameters

Table B2. Field preservation and processing procedures for water quality samples.

Parameter	Field Processing	Preservation	Container	Holding Times
Live Phytoplankton	a,b	A	1	2
Preserved Phytoplankton	a,b	B	1	4
ELISA samples	a,c	A	2,3	3
LC/MS/MS samples	a,c	A	2,4	1,5

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Processing: a - whole water
b – net plankton
c - filter

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

Containers: 1 - 75 mL glass test tube
2 – Whatman 934-AH glass fiber filter
3 – 40ml glass vials, septum-top (VDH toxin analysis)
4 – 80 ml polyethylene bottle (VDH toxin analysis)

Holding times: 1 - 24 hours
2 – 48 hours
3 - 36 hours
4 - 6 months
5 – 8 hours (filters only)

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

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Appendix C – Laboratory Protocols

C.1. Plankton for identification and enumeration

C.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 15 minutes
- record the volume of the aliquot (most chambers use 1 mL) and the volume of the concentrated tow samples

C.1.2. Qualitative Samples

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

C.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.

C.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

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- 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.

C. 2. Toxin Concentration – VDH Laboratory

C.2.1. Plankton sample

- Use acid cleaned glass filtration equipment and 934-AH filters
- Shake sample gently to mix and measure out an aliquot using an acid cleaned graduated cylinder
- Filter the aliquot onto the filter using a vacuum pump at 5 inches Hg or less
- Rinse sides of funnel with a small amount of clean (Type I) water
- If sufficient plankton are present, remove the filter and place in a clean 15 mL glass centrifuge with snap cap.
- If the filter does not have a distinct green layer of plankton on the surface, filter an additional aliquot. Continue filtering as needed.
- Record volume filtered on processing log
- Add 8 mL of 50% methanol to each vial
- Freeze samples at -80°C for a minimum of 24 hrs.

C.3 Anatoxin Analysis – Vermont Department of Health

C.3.1 Preparation of Filtered Plankton Samples

- Place filter into labeled large glass test tube.
- Add 3ml each of methanol and acetonitrile and vortex for 30 seconds.
- Centrifuge at 4000 rpm for 10 minutes
- Transfer liquid to a clean 15 ml centrifuge tube.
- Add an additional 3ml each of methanol and acetonitrile to the original test tube. Repeat vortex and centrifuge steps. Add this liquid to the corresponding centrifuge tube.
- Evaporate the centrifuge tube to dryness using a TurboVap.
- Reconstitute with MilliQ-grade water and vortex for 30 seconds.
- Transfer liquid into 10ml labeled volumetric flask and bring up to volume.

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C.3.2 Preparation of Water Samples

- Prepare a Buchner funnel by placing a Millipore 40µm glass fiber filter over the frit. Cover the filter paper with deactivated glass wool to a depth of approximately 0.5 inches. Pour sample through filter and apply vacuum for 5 minutes. This step is necessary only if a large amount of algae is present in the water sample.
- Transfer 10ml of water (filtered or unfiltered) into a labeled 10ml volumetric flask.

C.3.3 Concentration of Prepared Samples

- Condition each Supelco LC-WCX 3 mL 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.
- Reconstitute with 200 µL of 15% acetonitrile containing 0.05% trifluoracetic acid.
- Vortex and analyze on the LC/MS/MS

C.4 Microcystin by ELISA

C.4.1 Processing Concentrated Plankton for Microcystin by ELISA

- Freeze concentrated plankton for a minimum of 24 hours,
- Allow vial to remain at room temperature until thawed. Shake vigorously and then re-freeze. Repeat this cycle for a total of 3 thawing periods
- Thaw and shake vigorously
- Centrifuge for 10 minutes at 3000 rpm to pelletize the filter residue.
- Dilute a portion of the supernatant 1:10 in organic-free RO water before preparing sample for analysis by ELISA following the manufacturer's protocols

C.4.2 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.

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C.4.3 Instructions for Microcystin by ELISA using Envirologix test kits

ENVIROLOGIX™

QuantiPlate™ Kit for Microcystins

Highlights:

- *Quantitative laboratory detection of Microcystin toxin in surface water*
- *Detects from 0.16 to 2.5 ppb*
- *High Sensitivity Option for potable water samples (see Appendix) detects from 0.05 to 0.83 ppb*

Contents of Kit:

- *12 strips of 8 antibody-coated wells each, in plate frame*
- *1 vial of Negative Control*
- *1 vial of 0.16 ppb Microcystin LR Calibrator*
- *1 vial of 0.6 ppb Microcystin LR Calibrator*
- *1 vial of 2.5 ppb Microcystin LR Calibrator*
- *1 bottle of Assay Diluent*
- *1 bottle of Microcystin-enzyme Conjugate*
- *1 packet of Wash Solution salts*
- *1 bottle of Substrate*
- *1 bottle of Stop Solution*

Precision

	Recovery (%CV)	OD (%CV)
Intra-Assay	**n=7**	
0.25 ppb	3.9%	1.6%
1.0 ppb	5.8%	5.3%
Inter-Assay	**n=11**	
0.25 ppb	6.0%	n/a
1.0 ppb	3.6%	n/a

Catalog Number EP 022

Intended Use

The EnviroLogix QuantiPlate Kit for Microcystins is designed for the quantitative laboratory detection of Microcystin toxin in surface water samples, with an assay quantitation range from 0.16 to 2.5 parts per billion (ppb). (See the Appendix at the end of this package insert describing an alternate assay protocol, suitable for use with colorless and/or potable water samples, with a quantitation range from 0.05 to 0.83 ppb.)

How the Test Works

This QuantiPlate Kit for Microcystins is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA).

In the test, Microcystin toxin in the sample competes with enzyme (horseradish peroxidase)-labeled Microcystin for a limited number of antibody binding sites on the inside surface of the test wells.

After a simple wash step, the outcome of the competition is visualized with a color development step. As with all competitive immunoassays, sample concentration is inversely proportional to color development.

Darker color = Lower concentration
Lighter color = Higher concentration

Limit of Detection

The Limit of Detection (LOD) of this Kit is 0.147 ppb. The LOD was determined by interpolation at 81.3% B_0* from a standard curve. 81.3% B_0 was determined to be 3 standard deviations from the mean of a population of negative water samples.

*100% B_0 equals the maximum amount of Microcystin-enzyme conjugate that is bound by the antibody in the absence of any Microcystin in the sample (i.e. negative control). %B_0 = (OD of Sample or Calibrator/OD of Negative Control) x 100.

Limit of Quantification

The Limit of Quantification (LOQ) of this Kit was validated at 0.175 ppb (quantification between the 0.160 ppb lowest calibrator and 0.175 ppb may be reliable, but has not been validated). The LOQ was determined by fortifying a population of negative water samples at 0.175 ppb. The mean recovery was 108% with a coefficient of variation (CV) [(standard deviation/mean) x 100] of 13.6%.

Precision

Microcystin-fortified control solutions were repetitively analyzed both within a single assay, and in different assays on different days. The data is expressed as %CV for both the recovered concentration and for absorbance (OD).

Fortification and Recovery

Six surface water samples were fortified with Microcystin to a concentration of 1.0 ppb. The average recovery was 111%, with a CV of 3.6%.

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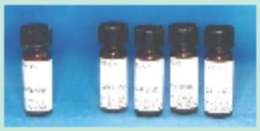
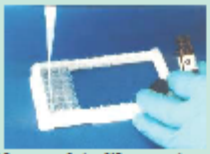
Cross-Reactivity

Compound	50% B_0	LOD 81.3% B_0
Microcystin LR	0.50	0.15
Microcystin LA	0.81	0.24
Microcystin RR	0.92	0.27
Microcystin YR	1.42	0.44
Nodularin	0.73	0.21

Remove unneeded strips



Select Calibrators and Control



Add controls/calibrators/sample

Cross-Reactivity

This Kit does not distinguish between the Microcystin toxin variants, but detects their presence to differing degrees. The accompanying table shows the value for 50% B_0 and the value for the 81.3% B_0 limit of detection for four Microcystin toxin variants and nodularin toxin. Concentration is in ppb. Humic acid did not interfere in the assay up to a concentration of 100 ppm.

Materials Needed

- disposable tip adjustable air-displacement pipette which will measure 20 µL, 100 µL and 125 µL
- marking pen (indelible)
- tape or Parafilm®
- timer (30 minutes)
- distilled water for preparing Wash Solution
- glassware for storing Wash Solution
- wash bottle for washing strips with Wash Solution
- microtiter plate reader or strip reader
- microtiter plate washer (optional)
- twelve-channel pipette that will measure 20 µL, 100 µL and 125 µL (optional)
- racked dilution tubes for loading samples into the plate with a 12-channel pipette (optional)
- orbital plate shaker (optional)

Preparation of Solutions

Wash Buffer:
To make 1 L, add the contents of one packet of phosphate-buffered saline - Tween 20, pH 7.4 (**Wash Solution salts**) to 1 L of distilled water. Mix thoroughly to dissolve the salts. This can be stored at room temperature.

How to Run the Assay

- Read all of these instructions before running the kit.
- Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed strips and reagents at room temperature - do not remove strips from bag with desiccant until they have warmed up).
- Organize all samples, reagents and pipettes so that steps 1 and 2 can be performed in 10 minutes or less.
- If more than three strips are to be run at one time, the 10 minutes is likely to be exceeded, and the use of a multi-channel pipette is recommended (see "Note" below).
- If three or fewer strips are to be run, use a disposable-tip air-displacement pipette and a clean pipette tip to add each Calibrator and sample to the wells. Assay Diluent, Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip on the end of the Combitip for these three reagents.
- If fewer than all twelve strips are used, reseal the unneeded strips and the desiccant in the plastic bag provided.
- Use the well identification markings on the plate frame to guide you when adding the samples and reagents. Two strips may be used to run the Negative Control (NC), three Calibrators (C1-C3) and four samples, in duplicate. More samples require more strips. For an example plate layout see Figure 1.

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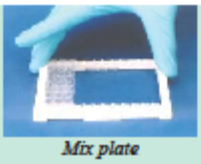
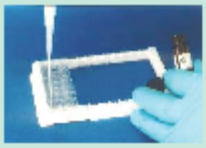


Mix plate

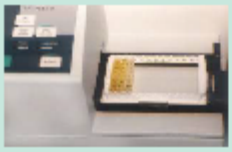
Incubate

Bottle Wash method

Strip Plate Wash option



Complete protocol and add Stop Solution



Read plates in a Plate Reader within 30 minutes of the addition of Stop Solution

1. Rapidly add **125 µL** of **Microcystin Assay Diluent** to each well that will be used, preferably with a repeating or multi-channel pipetter.
2. Immediately add **20 µL** of **Negative Control** (NC), **20 µL** of each **Calibrator** (C1-C3) and **20 µL** of each **sample** (S1-S8) to their respective wells, as shown at left. (Follow this same order of addition for all reagents.) **Do not add Microcystin-enzyme Conjugate in this step.**
3. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!

NOTE: In order to minimize setup time it is recommended that a multi-channel pipette be used in steps 1, 2, 5, 8 and 10 when more than 3 strips are used.

4. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 30 minutes. If an orbital shaker is available shake at 200 rpm.
5. Add **100 µL** of **Microcystin-enzyme Conjugate** to each well. Do not empty the well contents or wash the strips at this time.
6. Thoroughly mix the contents of the wells as in step 3. Cover the wells with tape or Parafilm and incubate at ambient temperature for 30 minutes. Use orbital shaker if available.
7. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the wells completely with **Wash Solution**, then shake to empty. Repeat this wash step four times. Slap the plate on a paper towel to remove as much Wash Solution as possible. Alternatively, use a microtiter plate washer with **Wash Solution** for the wash step.
8. Add **100 µL** of **Substrate** to each well.
9. Thoroughly mix the contents of the wells, as in step 3. Cover the wells with new tape or Parafilm and incubate for 30 minutes at ambient temperature. Use orbital shaker if available.

Caution: Stop Solution is 1.0 N Hydrochloric acid. Handle carefully.

10. Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

NOTE: Read the plate within 30 minutes of the addition of Stop Solution.

How to Interpret the Results

Spectrophotometric Measurement

1. Set the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)
2. If the plate reader does not auto-zero on air, zero the instrument against 200 µL water in a blank well. Measure and record the optical density (OD) of each well's contents. Alternatively, measure and record the OD in every well, then subtract the OD of the water blank from each of the readings.
3. A semi-log curve fit should be used for the standard curve if the microtiter plate reader you are using has data reduction capabilities. If not, calculate the results manually as described in the next section.

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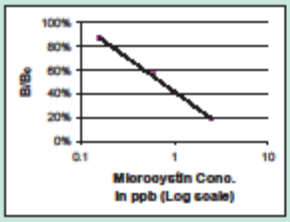
Illustrative standard curve



Precautions and Notes

- *Store all components at 4°-8°C (39°-46°F) when not in use.*
- *Do not expose components to temperatures greater than 37°C (99°F) or less than 2°C (36°F).*
- *Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.*
- *Do not use kit components after the expiration date.*
- *Do not use reagents or test well strips from one QuantiPlate Kit with reagents or test well strips from a different QuantiPlate Kit.*
- *Do not expose Substrate to sunlight during pipetting or while incubating in the test wells.*
- *Do not dilute or adulterate test reagents or use samples not called for in the test procedure.*
- *As with all tests, it is recommended that results be confirmed by an alternate method when necessary.*
- *Observe any applicable regulations when disposing of samples and kit reagents.*
- *Microcystin LR in aqueous solution will stick to plastics such as polypropylene. Collect and process samples in glass containers. Clear samples free of organic material can be stored refrigerated for up to two weeks before analysis.*

How to Calculate the Quantitative Results

1. After reading the wells, average the OD of each set of calibrators and samples, and calculate the $\%B_0$ as follows:

$$\%B_0 = \frac{\text{average OD of Calibrator or sample}}{\text{average OD of Negative Control}} \times 100$$

The $\%B_0$ calculation is used to equalize different runs of an assay. While the raw OD values of Negative Controls, Calibrators, and samples may differ from run to run, the $\%B_0$ relationship of calibrators and samples to the Negative Control should remain fairly constant.

The CV for each pair of Calibrator and sample OD values should not exceed 15%.

2. Graph the $\%B_0$ of each Calibrator against its Microcystin concentration on a semi-log scale (see Illustrative Standard Curve, left).
3. Determine the Microcystin concentration of each sample by finding its $\%B_0$ value and the corresponding concentration level on the graph.
4. Interpolation of sample concentration is only possible if the $\%B_0$ of the sample falls within the range of $\%B_0$'s of the Calibrators.

If the $\%B_0$ of a sample is higher than that of the lowest Calibrator, the sample must be reported as less than 0.16 ppb.

If the $\%B_0$ of a sample is lower than that of the highest Calibrator, the sample must be reported as greater than 2.5 ppb. If a concentration must be determined for these high level samples, dilute the sample 1:8 in distilled water. Run this dilution in a repeat of the immunoassay. If the result now falls within the range of the $\%B_0$'s of the Calibrators, you must then multiply the concentration measured in the diluted sample by a factor of 8.

Figure 1a. Example of a typical plate setup. (1 x 8 strips)

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC										
B	C1	C1										
C	C2	C2										
D	C3	C3										
E	S1	S1										
F	S2	S2										
G	S3	S3										
H	S4	S4										

Figure 2a. Illustrative quantitative calculations

Well contents	OD	Average OD	%CV	$\%B_0$	Microcystin Concentration (ppb)
Negative Control	1.398 1.347	1.373	2.628	100	NA
0.16 ppb Calibrator	1.184 1.177	1.181	0.419	86	NA
0.6 ppb Calibrator	0.773 0.776	0.775	0.274	56.4	NA
2.5 ppb Calibrator	0.246 0.250	0.248	1.14	18.1	NA
Sample	0.573 0.567	0.570	0.744	41.5	1.01

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

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This Limited Warranty states the entire obligation of EnviroLogix with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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APPENDIX

Instructions for Assay Protocol with Increased Sensitivity

The following assay protocol will produce an assay with calibrator values of 0.05, 0.20 and 0.83 ppb. This protocol is suitable only for colorless and/or potable water samples; surface waters containing visible organic matter will likely cause interference in the assay

NOTE: All of the precautions and notes discussed under **HOW TO RUN THE KIT** apply to this assay format.

In addition to the items listed above, these additional items will be needed for this assay protocol:

- disposable tip adjustable air-displacement pipette which will measure 50 and 200 µL
- glass test tubes in which to dilute the calibrators

Dilution of Calibrators

Dilute the Negative Control and the 3 Calibrators 1:3 in distilled water by adding 100 µL of calibrators supplied with this kit to 200 µL of distilled water. Label these dilutions Negative Control, 0.05, 0.20 and 0. 83 ppb. Mix thoroughly.

Assay Protocol

1. Rapidly add **50 µL** of **Microcystin Assay Diluent** to each well that will be used, preferably with a repeating or multi-channel pipetter.
2. Immediately add **50 µL** of **Negative Control** (NC), **50 µL** of each diluted **Calibrator** (C1-C3) and **50 µL** of each **sample** (S1-S8) to their respective wells, as shown in Figure 1. (Follow this same order of addition for all reagents.) **Do not add Microcystin-enzyme Conjugate in this step.**
3. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!

NOTE: In order to minimize setup time it is recommended that a multi-channel pipette be used in steps 1, 2, 5, 8 and 10 when more than 3 strips are used.

4. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 30 minutes. If an orbital shaker is available shake at 200 rpm.
5. Carefully remove tape or Parafilm and then add **100 µL** of **Microcystin-enzyme Conjugate** to each well. Do not empty the well contents or wash the strips at this time.

HIGH SENSITIVITY PROTOCOL

Limit of Detection

The Limit of Detection (LOD) of the High Sensitivity Protocol for this Kit is 0.03 ppb. The LOD was determined by interpolation at 90.9% B_0 from a standard curve. 90.9% B_0 was determined to be 3 standard deviations from the mean of a population of negative water samples.

Limit of Quantification

The Limit of Quantification (LOQ) of this Kit's High Sensitivity Protocol was validated at 0.06 ppb (quantification between the 0.05 ppb lowest calibrator and 0.06 ppb may be reliable, but has not been validated). The LOQ was determined by fortifying a population of negative water samples at 0.06 ppb. The mean recovery was 88% with a coefficient of variation (CV) [(standard deviation/mean) x 100] of 9.3%.

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6. Thoroughly mix the contents of the wells as in step 3. Cover the wells with tape or Parafilm and incubate at ambient temperature for 30 minutes. Use orbital shaker if available.

7. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the wells completely with **Wash Solution**, then shake to empty. Repeat this wash step four times. Slap the plate on a paper towel to remove as much **Wash Solution** as possible. Alternatively, use a microtiter plate washer with **Wash Solution** for the wash step.

8. Add **100 µL** of **Substrate** to each well.

9. Thoroughly mix the contents of the wells, as in step 3. Cover the wells with new tape or Parafilm and incubate for 30 minutes at ambient temperature. Use orbital shaker if available.

Caution: Stop Solution is 1.0 N Hydrochloric acid. Handle carefully.

10. Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

NOTE: Read the plate within 30 minutes of the addition of Stop Solution.

Assignment of Calibrator Values

In this assay format, assign the low, middle and high calibrators microcystin concentrations of 0.05 ppb, 0.2 ppb and 0.83 ppb, respectively.

For Interpretation and Calculation of Results see the sections on **How to Interpret the Results** and **How to Calculate the Results** above. The information contained in those sections is applicable to this more sensitive assay format, with the exception that the calibrator values are different.

*original document obtained on line at http://www.envirologix.com/library/ep022insert.pdf, 4/3/13. There have been no revisions.

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C.5 Cylindrospermopsin by ELISA

C.5.1 Processing Concentrated Plankton for Cylindrospermopsin by ELISA

- Freeze concentrated plankton for a minimum of 24 hours,
- Allow vial to remain at room temperature until thawed. Shake vigorously and then re-freeze. Repeat this cycle for a total of 3 thawing periods
- Thaw and shake vigorously
- Centrifuge for 10 minutes at 3000 rpm to pelletize the filter residue.
- Dilute a portion of the supernatant 1:10 in organic-free RO water before preparing sample for analysis by ELISA following the manufacturer's protocols

C.5.2 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

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

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

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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.

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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.

Provided with kits purchased in 2015.

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Appendix D – Visual Assessment.

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

D.1. On-line reporting form

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

Reporting Blue-Green Algae on Lake Champlain

Please use this form to report on water quality conditions with regard to algae on Lake Champlain.

Blue-green algae blooms can be easily confused with other natural phenomena. Please consult our guide to Recognizing Blue Green Algae in Lake Champlain before reporting a bloom. If there is a bloom, avoid direct contact (*see Vermont Department of Health link*).

Also, our guide to categories of algae bloom intensity and our instructions for photographing algae blooms will be helpful in filling out the form below.

Algae Report Form

Type of report	○ Regular weekly ○ Supplemental
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 the 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	Browse… No file selected.
Photo - water surface broad view	Browse… No file selected.
Photo - water sample in clear container	Browse… No file selected.

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Photo - water sample in clear container	Browse... No file selected.
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 (add details below)
Other details	
Water temperature	
Water Surface	○ Calm ○ Rolling ○ White-caps
Name	
Email	
Address	
Telephone	

Submit Form

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D.2. Determining Algae Bloom Intensity

- http://www.lakechamplaincommittee.org/get-involved/volunteers/bga-monitors/algaebloomintensity/

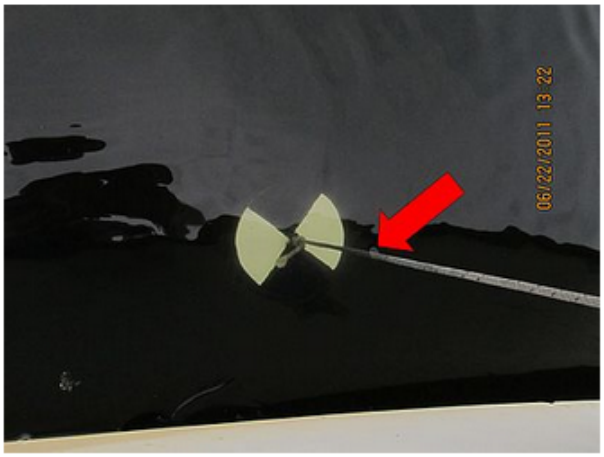
General Instructions

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.

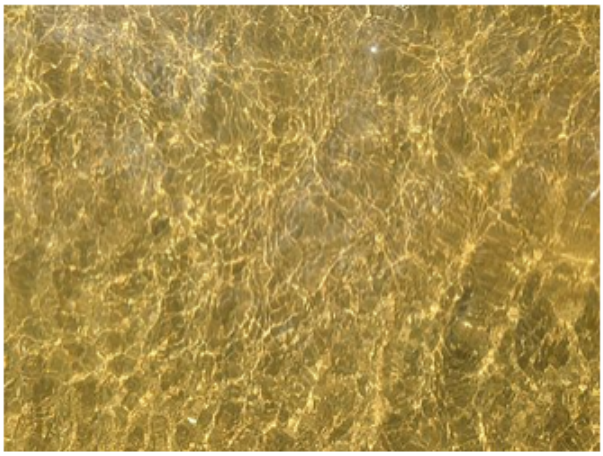
For category 2 and 3 conditions, three digital photographs should be submitted via the online form. Remember to avoid direct contact if the bloom is well developed.

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

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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

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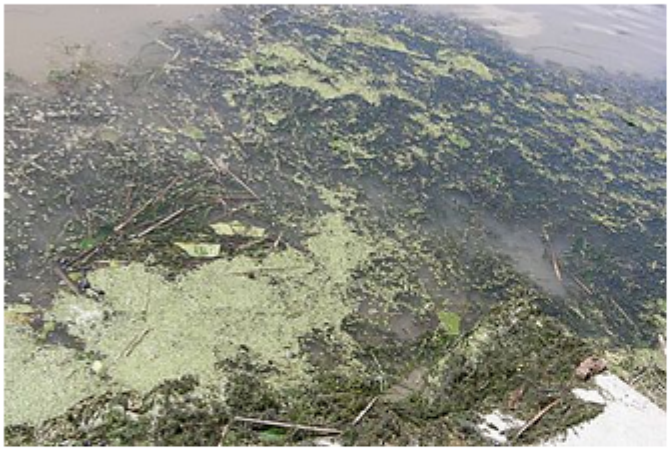
Category 1c - Little to no blue-green algae present - other material

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

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Stringy algae attached to the bottom are not blue-greens

Duckweed up close

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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

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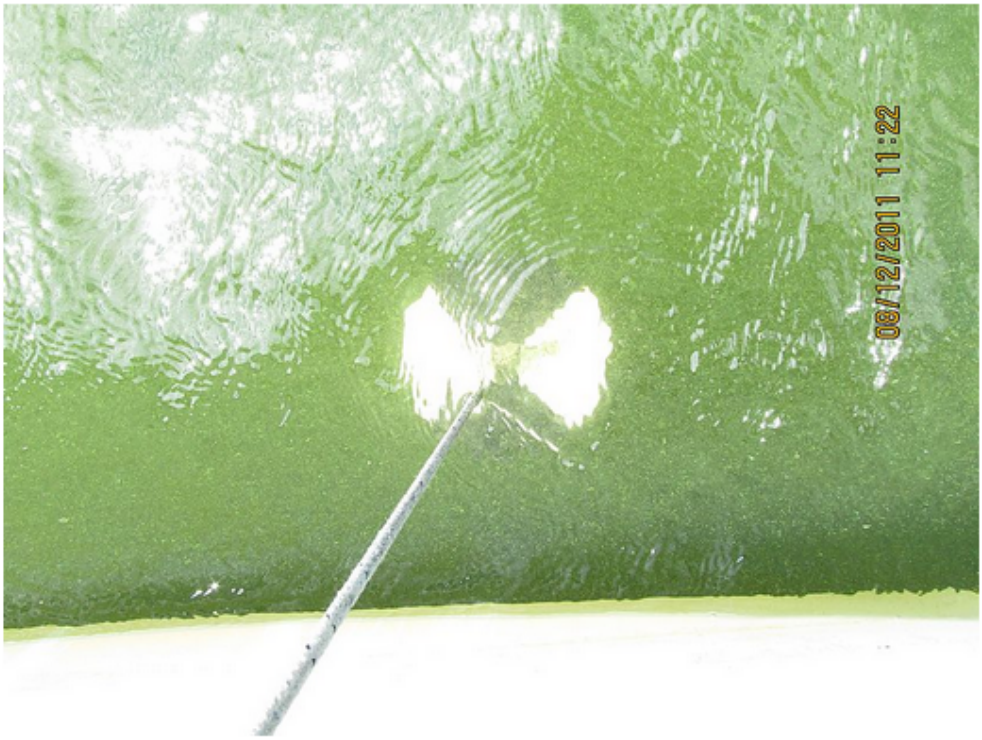
Category 2: Blue-green algae present, but at less than 'bloom' levels - enjoyment of water slightly impaired

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

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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

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Thick surface scum present

Open water surface green to turquoise

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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.

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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

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Appendix E – Forms

E.1. VT DEC Field Form

VT DEC Cyanobacteria Monitoring Project – 2015

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	

E2.

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.