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State of Washington

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Freshwater Algae Control Program – Cyanobacteria Identification and Toxin Testing

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Programmatic Quality Assurance Project Plan

Freshwater Algae Control Program – Cyanobacteria Identification and Toxin Testing

October 2026

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2.0 Abstract

Cyanobacteria (commonly known as “blue-green algae”) pose an environmental and public health risk in water bodies across Washington state. In freshwater ecosystems, cyanobacteria can form open water blooms throughout the water column and surface accumulations. They can also form bottom-dwelling mats that may detach and float on the water surface or wash ashore. Excessive growth of cyanobacteria can disrupt ecosystem health and impair water quality. Some species of cyanobacteria can produce toxins (collectively called cyanotoxins) that are harmful to humans and animals.

Toxic cyanobacteria are a public health issue, and exposure can occur through water recreation and consumption of contaminated water or benthic mat material. Exposure to liver toxins (e.g., microcystins, cylindrospermopsins) and nervous system toxins (e.g., anatoxins, saxitoxins) may lead to adverse health outcomes for humans, pets, domestic animals, and wildlife. Toxin testing is the only way to confirm if cyanobacteria are producing toxins in a water body.

Ecology’s Freshwater Algae Control Program provides funding for statewide testing and partners with King County Environmental Laboratory for cyanobacteria identification and cyanotoxin analysis. This programmatic QAPP is an update of the 2010 Sampling and Analysis Plan submitted by King County. This document outlines the objectives of the testing program and the procedures to be followed to achieve those objectives.

3.0 Background

3.1 Introduction and problem statement

Commonly referred to as “blue-green algae,” cyanobacteria are actually photosynthetic bacteria and occur naturally in water bodies worldwide (Graham et al., 2008b). These plant-like organisms are typically single-celled, aquatic, and contain photosynthetic pigments (e.g., chlorophyll a). In freshwater ecosystems, cyanobacteria can form planktonic (open water) blooms throughout the water column and surface accumulations, or benthic (bottom-dwelling) mats that may detach and float on the water surface or wash ashore. Planktonic blooms tend to reduce water clarity in lakes and slow-flowing waters while benthic mats do not affect water clarity and can be found in rivers and lakes. These organisms prefer warmer waters with elevated nutrients (mainly nitrogen and phosphorus), increased light exposure, reduced water flow and mixing, and longer lake residence times with lower flushing rates (Graham et al., 2008b; Merel et al., 2013; USEPA, 2019b; Igwaran, et al. 2024).

Although many types of phytoplankton, such as filamentous green algae, are important to the biological productivity of freshwater systems, cyanobacteria have the potential to disrupt ecosystems and degrade water quality. Cyanobacteria can accumulate into blooms that decrease water clarity, reduce dissolved oxygen levels through decomposition, and alter food web dynamics (Igwaran, et al. 2024; USEPA, 2026). As mentioned above, cyanobacterial blooms can form and persist under optimal environmental conditions, with nutrient pollution and a warming climate contributing to a higher frequency, duration, and intensity of blooms (Merel et al., 2013; Griffith & Gobler 2020; Igwaran, et al. 2024; USEPA, 2026).

Cyanobacteria can also produce harmful metabolic byproducts, including taste-and-odor compounds (e.g., geosmin) and cyanotoxins. These toxins can lead to adverse health outcomes for humans and animals. The impacts of toxic cyanobacteria on human health, aquatic ecosystems, and risk management have been extensively studied (Chorus & Bartram, 1999; Merel et al., 2013; Chorus & Welker, 2021; Igwaran, et al. 2024). Cyanobacterial blooms and toxin exposure tend to occur throughout the summer and fall but can occur at any time of year. Additional information on cyanobacteria can be found on the United States Environmental Protection Agency (USEPA) “Harmful Algal Blooms (HABs) in Water Bodies” website (USEPA, 2026).

Studies by the Washington State Department of Ecology (Ecology) and others have shown that cyanotoxins can accumulate in the organs of fish (Johnson, 2010; Johnson et al., 2013; Hardy et al., 2015) and that cyanobacteria have persisted in the deeper sediments of lakes (Hobbs & Wong, 2019; Hobbs et al., 2022; Hobbs, 2024). Additional Ecology studies have investigated monitoring approaches for toxic blooms and factors influencing cyanobacteria and cyanotoxin production in lakes (Wong & Hobbs, 2020; Hobbs, 2023). Monitoring Washington lakes for toxic

cyanobacteria has relied on a collaborative approach between state and local agencies, including education and notification strategies to inform the public of toxic blooms (Trainer & Hardy, 2015; Hardy et al., 2016).

In 2005, the Washington State Legislature established the aquatic algae control account and freshwater aquatic algae control program under RCW 43.21A.667. Ecology administers the Freshwater Algae Control Program (FACP), which provides funding for public education, technical assistance, and pass-through grants to manage excessive freshwater algae (i.e., cyanobacteria; Ecology, n.d.-a). Since the only way to know if a cyanobacterial bloom is toxic is to have it tested, Ecology also established a cyanobacteria identification and toxin testing program in 2007 (Hamel, 2012). The purpose of the program is to help Washington residents, local health districts, and water body managers identify toxic cyanobacteria to inform water body use and management decisions. Ecology collaborates with the Washington State Department of Health (DOH) to oversee and advise local partners in the sampling of suspect blooms or mats for cyanotoxins. Samples may be collected from freshwater ponds, lakes, reservoirs, streams, and rivers by local health jurisdictions (LHJs), water body management entities, or state residents (collectively called program users).

Ecology and King County, represented by its Department of Natural Resources Environmental Lab (KCEL), have entered into an Interagency Agreement (IAA) in which King County provides water quality analysis services and Ecology pays for this work under the aquatic algae control account. KCEL performs cyanotoxin analysis and microscopic identification of cyanobacteria and sends lab results to Ecology, water body managers, and other program users. Phytoplankton and toxin results are available on the Washington State Toxic Algae database (Ecology, 2026a).

In 2010, a Sampling and Analysis Plan outlining the technical details for sample analysis under the FACP testing program was prepared by King County and DOH and submitted to Ecology. This document is provided in Appendix A. The purpose of this QAPP is to update the 2010 plan to ensure that collection and analysis of environmental samples will meet the goals and objectives of the cyanobacteria identification and toxin testing program.

3.2 Study area and surroundings

The testing program is relevant to freshwater bodies across Washington state.

3.2.1 History of study area

Because the study area encompasses all freshwater bodies in Washington, a historical account is not applicable.

3.2.2 Summary of previous studies and existing data

Existing data for Ecology's FACP testing program is available on the Washington State Toxic Algae database (Ecology, 2026a). Cyanotoxin and phytoplankton data are available for each

year starting in 2007. In addition, cyanotoxin data for various years between 1994 and 2014 are available on Ecology's Environmental Information Management (EIM) database. See Section 11.1.

3.2.3 Parameters of interest and potential sources

Cyanobacteria are photosynthetic prokaryotes and exhibit a diverse range of morphologies, physiologies, and habitats (USEPA, 2019b). They are widespread in the environment and can produce dermatoxic, hepatotoxic, neurotoxic, and cytotoxic compounds that are harmful to humans and animals (Lad et al., 2022). These toxins remain inside cyanobacteria cells (intracellular toxins) until they are released into the water following cell senescence or death (extracellular toxins)—the combined particulate (intracellular) and dissolved (extracellular) phases comprise the total toxins present (USEPA, 2019b).

Concentrations of cyanotoxins and taste-and-odor compounds in a water body vary based on a number of factors, including hydrology, morphology, geography, water chemistry, and biological interactions (Graham et al., 2008b). Graham et al. (2008b) also describe the temporal and spatial variability of cyanobacteria and their toxins in lakes and reservoirs. Exposure in recreational waters most often occurs in the summer months through direct ingestion of water, dermal contact, and inhalation of aerosolized water spray (Lad et al., 2022). The four most common freshwater cyanotoxins in Washington state are described in further detail below.

Microcystins

Microcystins are the most commonly tested for and detected cyanotoxin in Washington, with toxin results available on the Washington State Toxic Algae database since 2007 (Ecology, 2026a). Exposure to these toxins can occur through dermal contact, oral ingestion, and aerosol inhalation and have been associated with recreational waterborne disease outbreaks in Washington state (Hilborn et al., 2014). Microcystins are potent liver toxins and potential tumor promoters with over 300 different congeners identified (Lad et al., 2022). One of the most common congeners is Microcystin-LR (MC-LR), a stable, water-soluble compound that causes liver and other organ damage (USEPA, 2019b; Merel et al., 2013; Lad et al., 2022). The half-life of microcystins is typically four to 14 days in surface waters but may persist longer, depending on environmental factors (USEPA, 2019b). Importantly, microcystins can persist even after a toxic cyanobacterial bloom is no longer visible in a water body (USEPA, 2019b).

Cylindrospermopsin

While less common, cylindrospermopsin has been detected in Washington water bodies and toxin data has been reported on the Washington State Toxic Algae database since 2009

(Ecology, 2026a). It is a widely studied cyanotoxin due to its global distribution, aquatic bioaccumulation, and multi-organ toxicity (Yang et al., 2021). Cylindrospermopsin is highly water-soluble and mainly targets the liver; these toxins have also been found to be cytotoxic, genotoxic, immunotoxic, neurotoxic, endocrine disruptors, and possibly carcinogenic (Yang et al., 2021). Exposure may occur through accidental ingestion of contaminated water during recreational activities (e.g., swimming, boating), consumption of toxin-contaminated drinking water or food (e.g., fish, shellfish, algae supplements), or from bathing or showering (USEPA, 2019b; Yang et al., 2021). Cylindrospermopsin has had half-lives of 11 to 15 days and up to eight weeks reported in surface waters (USEPA, 2019b).

Anatoxin-a

Anatoxin-a is the second most commonly tested for and detected cyanotoxin in Washington. Data for this toxin has been reported on the Washington State Toxic Algae database since 2009 (Ecology, 2026a). A potent neurotoxin, it primarily targets nerve synapses and is often implicated in animal poisoning events at affected water bodies (Merel et al., 2013; DOH, 2025). In addition, anatoxin-a can reach dangerously high concentrations in floating benthic cyanobacterial mats (DOH, 2025). These benthic mats tend to be found in larger freshwater river systems but can also occur in lakes. The half-life of anatoxin-a has been reported to be less than 24 hours in eutrophic (nutrient-rich) lakes but about five days in a laboratory setting (DOH, 2025). Degradation can occur from sunlight exposure and at high water pH levels (DOH, 2025).

Saxitoxin

While also less common in Washington, detections of saxitoxin have been reported. Data is available on the Washington State Toxic Algae database dating back to 2009 (Ecology, 2026a). Saxitoxins damage nerve axons in the body's nervous system, which can induce nerve dysfunction, paralysis, and death due to respiratory failure (Merel et al., 2013). The half-lives of saxitoxins in natural water can vary from 9 to 28 days and some variants have been reported to persist for over 90 days (Merel et al., 2013; DOH, 2025). These neurotoxins are often associated with marine algae called dinoflagellates and can contaminate shellfish such as mussels, clams, and scallops and cause paralytic shellfish poisoning (DOH, 2025). In freshwater, saxitoxins can accumulate in zooplankton and freshwater bivalves (DOH, 2025).

Table 1 below summarizes common cyanotoxin groups, their primary target organs in mammals, and associated cyanobacteria genera.

Table 1. Common cyanotoxins, their primary target organs, and associated cyanobacteria.

Toxin Group	Primary Target Organ in Mammals	Cyanobacteria Genera Known to Produce Toxin
Microcystins	Liver	*Anabaenopsis, Aphanizomenon, Aphanocapsa, Dolichospermum* (*Anabaena*)*, Fischerella, Gloeotrichia, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Planktothrix, Pseudanabaena, Synechococcus, Synechocystis*
Cylindrospermopsin	Liver	*Aphanizomenon, Cylindrospermopsis, Dolichospermum* (*Anabaena*)*, Lyngbya, Raphidiopsis, Umezakia*
Anatoxin-a	Nerve Synapse	*Aphanizomenon, Cylindrospermum, Dolichospermum* (*Anabaena*)*, Microcystis, Oscillatoria, Planktothrix, Raphidiopsis*
Saxitoxin	Nerve Axon	*Aphanizomenon, Cylindrospermopsis, Dolichospermum* (*Anabaena*)*, Lyngbya, Oscillatoria, Planktothrix*

Information in this table is compiled from: Graham et al. (2008b), USEPA (2019), and DOH (2025). Note that this table does not represent a comprehensive list of all cyanobacteria genera that produce these four common toxins.

Cyanobacteria genera of most concern in United States recreational waters include *Microcystis*, *Dolichospermum* (*Anabaena*), and *Aphanizomenon*, based on past survey data (Hardy et al., 2021). These genera also constitute concerns in Washington state. In addition to identification of the most common planktonic cyanobacteria genera, recent studies have summarized common benthic-forming cyanobacteria genera as well, including *Aphanothece, Dolichospermum* (*Anabaena*)*, Lyngbya, Leptolyngbya, Microcoleus, Oscillatoria, Phormidium, Planktothrix, Schizothrix, Scytonema, Synechococcus,* and *Tychonema* (Quiblier et al., 2013; Patiño et al., 2023).

3.2.4 Regulatory criteria or standards

There are currently no cyanotoxin-specific criteria, but program results may be used to evaluate against numeric criteria that may be developed and approved in the future under WAC 173-201A.

The intent of this testing program is to provide quantitative results of freshwater cyanotoxin concentrations in recreational water samples, reported as micrograms per liter (µg/L), so that local managers can track water body toxins and make more informed management decisions to

protect public health and water resources. Water body and beach management decisions follow state and federal guidance.

In 2019, the USEPA published *Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin* establishing standards for microcystins and cylindrospermopsin (USEPA, 2019b). In 2021, DOH adopted USEPA's guidelines for microcystins and cylindrospermopsin and provided additional guidance values for anatoxin-a and saxitoxins, in their publication *Washington State Recreational Guidance for Microcystins, Anatoxin-a, Cylindrospermopsin and Saxitoxin* (DOH, 2025). Table 2 below summarizes USEPA and DOH's recreational guidance values for the four most common cyanotoxins in Washington.

Table 2. Cyanotoxin Recreational Guidance Values.

Cyanotoxin	Washington State DOH Recreational Guidance Value[1]	USEPA Recreational Guidance Value[2]
Microcystins	8 µg/L[3]	8 µg/L
Cylindrospermopsin	15 µg/L	15 µg/L
Anatoxin-a	1 µg/L[4]	Not Applicable
Saxitoxin	75 µg/L	Not Applicable

[1]DOH *Washington State Recreational Guidance for Microcystins, Anatoxin-a, Cylindrospermopsin and Saxitoxin* (DOH, 2025)

[2]USEPA *Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin* (USEPA, 2019b)

[3]DOH recommends posting Tier I Warning signage for microcystin levels at or above 8 µg/L and posting Tier II Danger signage for microcystin levels above 2,000 µg/L

[4]DOH recommends posting Tier I Warning signage for anatoxin-a levels at or above 1 µg/L and posting Tier II Danger signage for anatoxin-a levels above 300 µg/L levels

µg/L = micrograms per liter

4.0 Project Description

4.1 Project goals

The mission of Ecology's Water Quality Program is to ensure that all aquatic life, and communities in the watershed, experience cool, clean water to refresh and sustain us in a changing climate. Ecology's FACP provides funding for statewide pass-through grant projects, cyanobacteria identification and toxin testing, and technical assistance on managing excessive freshwater algae (cyanobacteria).

The purpose of the cyanobacteria identification and toxin testing program is to help Washington residents, local health districts, and water body managers identify toxic cyanobacteria to make water body use and management decisions following DOH recreational guidance. This program provides information about Washington water bodies experiencing cyanobacterial blooms or mats, cyanobacteria genera of concern, cyanotoxin concentrations, the time of year when toxic blooms occur, and historical toxin results from compiled data. This data allows Ecology to focus technical and financial assistance on the most impacted water bodies (i.e., those experiencing recurrent, toxic blooms).

The goals of this program are as follows:

- Provide financial assistance for cyanotoxin and cyanobacteria laboratory analysis for residents, water body managers, and local health districts statewide per Ecology's contract with KCEL.
- Ensure quality assurance for sample data analyzed through the FACP testing program.
- Provide technical guidance to residents, water body managers, and local health districts on reporting cyanobacterial blooms and testing for cyanobacteria and cyanotoxins in freshwater ponds, lakes, reservoirs, streams, and rivers.

4.2 Project objectives

The objectives of this program include the following:

- Provide approximately $85,000 in funding each state fiscal year for water sample laboratory analysis, reporting, and database management. Coordinate with project partners and program users on sample collection, analysis, reporting, and interpretation of results.
- Coordinate with King County to ensure quality assurance of data and to maintain the Washington State Toxic Algae website and database. Monitor the occurrence of cyanobacterial blooms statewide and evaluate the results of the analyzed data.

- Provide technical assistance to program users through public engagement (e.g., pre-season and post-season workshops, conferences, public meetings, etc.) throughout the year. Send laboratory results and program updates to the public and subscribers of Ecology's FACP email distribution list on a weekly basis during the program testing season (Ecology, n.d.-b).

4.3 Information needed and sources

This program is being implemented to generate new environmental data (cyanobacteria identification and cyanotoxin concentrations) to be uploaded to the Washington State Toxic Algae database (Ecology, 2026a). Existing environmental data collected through this program will be reviewed to improve data quality and usability.

4.4 Tasks required

Program tasks include the following:

- Ecology will manage the current contract with KCEL and coordinate sample collection, analysis, and database management activities through this program. The contract may be amended or renewed for continued program implementation.
- Complete the scope of work outlined in the current contract between Ecology and KCEL, including:
    - Task 1 (Sample Analysis and Data Reporting): KCEL will provide sampling containers to program users, analyze freshwater samples for freshwater algae identification and toxins, and provide preliminary and final data reporting.
    - Task 2 (Database Management): Staff from the King County Department of Natural Resources and Parks (DNRP) / Science Section Lakes, Streams, Sound and Ground unit (LSSG) will provide database operation and management services for the Washington State Toxic Algae website, including weekly upload of KCEL Laboratory Information Management System (LIMS) data, troubleshooting the database, reviewing existing and incoming locations data to ensure accurate reporting, and implementing website and database improvements.
- Ecology will work with other Ecology staff, KCEL, and DOH on program process improvements on an as-needed basis and to update this planning document.
- Ecology will give presentations on program findings to Ecology's Water Quality Program, local and state partners, and the public.

4.5 Systematic planning process

This QAPP represents the systematic planning process for the program.

5.0 Organization and Schedule

5.1 Key individuals and their responsibilities

Table 3 shows the responsibilities of those who will be involved in this project.

Table 3. Organization of project staff and responsibilities

Staff	Title	Responsibilities
Joseph Teresi Ecology, WQP Email: jote461@ecy.wa.gov Phone: (360) 628-7516	Freshwater Algae Control Program Manager	Manages the contract, reviews the project scope and budget, completes the draft and final QAPP. Conducts QA review of data, analyzes and interprets data, and reviews data submission requirements into EIM. Reviews and approves the draft QAPP and the final QAPP.
Fran Sweeney King County Environmental Lab Email: francis.sweeney@kingcounty.gov Phone: (206) 477-7117	Aquatic Toxicology Unit Supervisor	Oversight of toxin analysis, QA/QC and data review.
Meghan Elkey King County Environmental Lab Email: meghan.elkey@kingcounty.gov Phone: (206) 477-7154	Laboratory Project Manager	Reviews the project scope and budget, tracks progress, reviews the draft QAPP, enters sample tracking information into lab database to facilitate data upload to website.
Rachael Gravon King County, DNRP/Science, LSSG Email: rachael.gravon@kingcounty.gov Phone: (206) 477-4845	Water Quality Planner/Limnologist	Manages the Washington State Toxic Algae website and database.
Christina Frans Ecology, EAP Email: cfra461@ecy.wa.gov Phone: (360) 995-2473	Ecology Quality Assurance Officer	Reviews and approves the draft QAPP and the final QAPP.

DNRP/Science: Department of Natural Resources and Parks / Science Section; EAP: Environmental Assessment Program; LSSG: Lakes, Streams, Sound and Ground unit; QA/QC: Quality Assurance/Quality Control; QAPP: Quality Assurance Project Plan; WQP: Water Quality Program

5.2 Special training and certifications

King County Environmental Lab is a well-established, Ecology-accredited laboratory (Accreditation Number G656-24b) and has many years of experience conducting cyanotoxin and phytoplankton analysis for this program.

5.3 Organization chart

See Table 3.

5.4 Proposed project schedule

Tables 4 through 6 list key activities, due dates, and lead staff for this project.

Table 4. Schedule for completing laboratory, reporting, and database work.

Task	Due Date	Lead Staff
Contract Item 1: Sample Analysis and Data Reporting per year	June 30, 2029	Fran Sweeney
Contract Item 2: Database Management per year	June 30, 2029	Rachael Gravon

The current interagency agreement between Ecology and KCEL includes a performance period from July 1, 2024, through June 30, 2029. Based on the current contract, KCEL will provide preliminary data results for cyanotoxin testing within three business days of sample receipt at the lab and will notify Ecology if data reporting will exceed three days. Results will be emailed as a PDF to the FACP manager, program user who conducted the sampling, and local health jurisdiction (if known). Laboratory staff will notify Ecology of any analytical anomalies, if any occurred, and potential bias or limitations of reported results. Ecology will also send preliminary data results to program users via Ecology's FACP email distribution list.

King County DNRP/Science LSSG staff will upload final laboratory data electronically to the Washington State Toxic Algae database within ten business days of sample receipt at the lab. Qualitative phytoplankton data will be uploaded to the database on a quarterly basis. In addition, KCEL will provide final data reports to Ecology on a monthly basis, as described in Section 11.2.

Ecology will coordinate with KCEL and DNRP/Science LSSG staff on general website and database updates and process improvements on an as-needed basis.

Table 5. Schedule for data entry.

Task	Due Date	Lead Staff
EIM data loaded[1]	TBD	FACP Manager
EIM QA	TBD	Ecology EIM staff
EIM complete	TBD	FACP Manager

[1]EIM Project ID: "ToxicAlgae20XX"
EIM: Environmental Information Management database
TBD: To be determined

Ecology will work with program partners to evaluate the quality and usability of program data for future submission into EIM. Data has not been uploaded to Ecology's EIM database since 2014. Cyanotoxin data collected prior to and during this testing program were submitted into EIM for years 1994, 1999, 2000, 2002, 2003, and 2005 through 2014 (Ecology, 2026b). See Section 11.1.

Table 6. Schedule for program data reviews and approvals.

Task	Due Date	Lead Staff
Review final data reports provided by KCEL	Monthly	Joseph Teresi
Review payment request and supporting documentation provided by KCEL	Biannually	Joseph Teresi

The Ecology FACP manager will review final data reports provided by KCEL on a monthly basis to verify and track submitted samples for analysis. Twice a year, the Ecology FACP manager will review payment documentation (i.e., invoice vouchers and supporting documents) prior to payment approval. Payment documentation provides a description of the work performed, the progress of the work, and related costs.

5.5 Budget and funding

Funding for the Ecology FACP comes from annual vessel registration fees (RCW 88.02.640). One dollar of each fee is deposited into the Aquatic Algae Control Account. The Legislature appropriates these funds to Ecology to manage the program (Hamel, 2012). Ecology and KCEL have entered into a performance-based agreement in which KCEL will provide water quality analysis services and Ecology will pay for this work under the Aquatic Algae Control Account. Tables 7 and 8 show the project budget and funding under this agreement and the laboratory budget details, respectively.

Table 7. Project budget and funding.

Item	Cost ($)
Contract Item 1: Sample Analysis and Data Reporting per year	$70,000
Contract Item 2: Database Management per year	$15,000
Annual Total	$85,000
Total Years	X 5 years
Total Project Cost 5 years	$425,000

Table 8. Laboratory budget details.

Parameter	Number of Samples	Number of QA Samples	Total Number of Samples	Cost Per Sample ($)[1]	Lab Subtotal ($)
Microcystins	Varies[2]	Varies[2]	Varies[2]	$64.00	Varies[2]
Anatoxin-a	Varies[2]	Varies[2]	Varies[2]	$107.00	Varies[2]
Saxitoxin	Varies[2]	Varies[2]	Varies[2]	$64.00	Varies[2]
Cylindrospermopsin	Varies[2]	Varies[2]	Varies[2]	$71.00	Varies[2]
Cyanobacteria ID	Varies[2]	Varies[2]	Varies[2]	$50.00	Varies[2]

[1]Costs are based on the current contract between Ecology and KCEL

[2]Number of samples, number of QA samples, total number of samples, and lab subtotals are variable due to the ad hoc nature of the state testing program.

6.0 Quality Objectives

6.1 Data quality objectives

The main data quality objective (DQO) for this project is to analyze cyanobacterial bloom samples collected in water bodies in Washington State for algal toxins (cyanotoxins). The analysis will use standard methods to obtain microcystin, anatoxin-a, saxitoxin, and cylindrospermopsin concentration data and qualitative phytoplankton identification data that meet measurement quality objectives (MQOs) that are described below.

6.2 Measurement quality objectives

Measurement quality objectives (MQOs) in this section establish the performance metrics and criteria for acceptance that provide the basis for evaluating data quality and usability.

6.2.1 Targets for precision, bias, and sensitivity

The MQOs for project results, expressed in terms of acceptable precision, bias, and sensitivity, are described in this section and summarized in Table 9 below.

Table 9. Measurement quality objectives for analysis of algal toxin samples

Parameter	Method Blank	Lab Duplicate (RPD)	Matrix Spike Duplicate (RPD)	ELISA Positive Control (% Recovery)	Matrix Spike (% Recovery)	Spike Blank (% Recovery)
Anatoxin-a	<MDL	NA	0-45	NA	50-150	50-150
Cylindrospermopsin	<MDL	NA	0-45	NA	50-150	50-150
Microcystin	<MDL	NA	0-45	70-130	50-150	60-140
Saxitoxin	<MDL	NA	0-45	70-130	60-140	60-140
Qualitative Phytoplankton Analysis	NA	70-100[1]	NA	NA	NA	NA

[1] The lab duplicate for Qualitative Phytoplankton analysis is not expressed in RPD. Two separate slides are prepared from the same sample and compared. The organisms found should match between 70 -100%. Sørenson's Index is used for the calculation.

MDL = method detection limit; NA = Not Applicable; RPD = Relative Percent Difference

6.2.1.1 Precision

Toxin Analysis:

Laboratory precision will be assessed using laboratory Matrix Spike and Matrix Spike Duplicate (MS/MSD) quality control samples. When both sample results are at, or exceed the MDL, the Relative Percent Difference (RPD) should not exceed criteria listed in Table 9. An RPD cannot be determined unless both values are at or above the MDL, because no values are reported if <MDL.

Qualitative Algae (Cyanobacteria) Identification:

Intra-operator precision will be evaluated using data from laboratory duplicates. The acceptance range for lab duplicates is 70-100%. The lab duplicate for Qualitative Phytoplankton analysis is not expressed in RPD. Two separate slides are prepared from the same sample and compared. The organisms found should match between 70 -100 %. Sørenson's Index is used for the calculation.

6.2.1.2 Bias

Bias is an indicator of the accuracy of analytical data. For this project, laboratory control samples or blank spikes, whichever are available, along with matrix spikes will be used to assess bias. Results should be within the lab's current control limits (see Table 9).

Bias will also be assessed by the evaluation of field blank and method blank data. Analytical results for method blanks should be less than the MDL.

The use of matrix spike recovery data will provide additional information regarding method performance on actual samples. The laboratory will use professional judgment regarding assessment of data quality and any subsequent action taken as a result of matrix spike recoveries. Note that unexpectedly high sample concentrations, as well as matrix properties, can interfere with MS/MSD recoveries.

6.2.1.3 Sensitivity

Sensitivity is measured through reporting limit performance (for example, in a regulatory setting, the method detection limit (MDL) is often used to describe sensitivity). MDLs and reporting detection limits (RDL) will be provided with each analytical data report. Sensitivity of water quality measurements is determined by the resolution of the instruments used (see Table 11).

6.2.2 Targets for comparability, representativeness, and completeness

6.2.2.1 Comparability

Analytical results may be used in comparisons to each other, among samples collected for this program, and with results of similar surveys reported in the past or future by other local agencies or in the peer-reviewed literature. The primary means to ensure the project meets these comparability requirements is through the use of Standard Operating Procedures (SOPs) and standard methods. Methods to be employed during the project are listed in Table 11. Copies of lab SOPs will be provided on request. There are currently no field sampling SOPs for cyanotoxins or cyanobacteria under this program, but general sampling instructions are provided by Ecology and KCEL.

6.2.2.2 Representativeness

This program is primarily designed to evaluate the presence/absence of cyanobacterial toxins, and secondarily to estimate concentrations and geographic extent of the toxin distribution, should it be present. Phytoplankton population will be evaluated using the toxin sample. Representative samples will be obtained through the following practices:

- The use of generally accepted sampling procedures will allow for the collection of representative samples.
- Sub-sampling at KCEL will be conducted according to lab SOPs. These procedures are designed to obtain representative subsamples.

6.2.2.3 Completeness

Completeness will be evaluated by the following criteria:

- The number of usable data points compared to the projected data points as detailed in this programmatic QAPP.
- Compliance with the data quality criteria as presented in this section.
- Compliance with specified holding times.

The goal for the above criteria is to obtain 95% data completeness. However, where data is not complete, decisions regarding re-sampling and/or re-analysis will be made by a collaborative process involving both data users and data generators. These decisions will take into account the project data quality objectives as presented above.

6.3 Acceptance criteria for quality of existing data

Not applicable. This survey is primarily designed to evaluate the presence/absence of cyanobacterial toxins as blooms occur.

6.4 Model quality objectives

Not applicable.

7.0 Study Design

7.1 Study boundaries

Ecology's cyanobacteria identification and toxin testing provides coverage for "waters of the state," including those waters defined as "waters of the United States" in 40 CFR Subpart 122.2 within the geographic boundaries of Washington state, as well as those waters defined as "waters of the state" in RCW 90.48.020. Ecology ultimately determines which water bodies are sampled through this program on a case-by-case basis. Water bodies tested typically include publicly accessible freshwater ponds, lakes, reservoirs, streams, and rivers. Figure 1 below provides a map showing water bodies across Washington that have been tested through the FACP testing program.

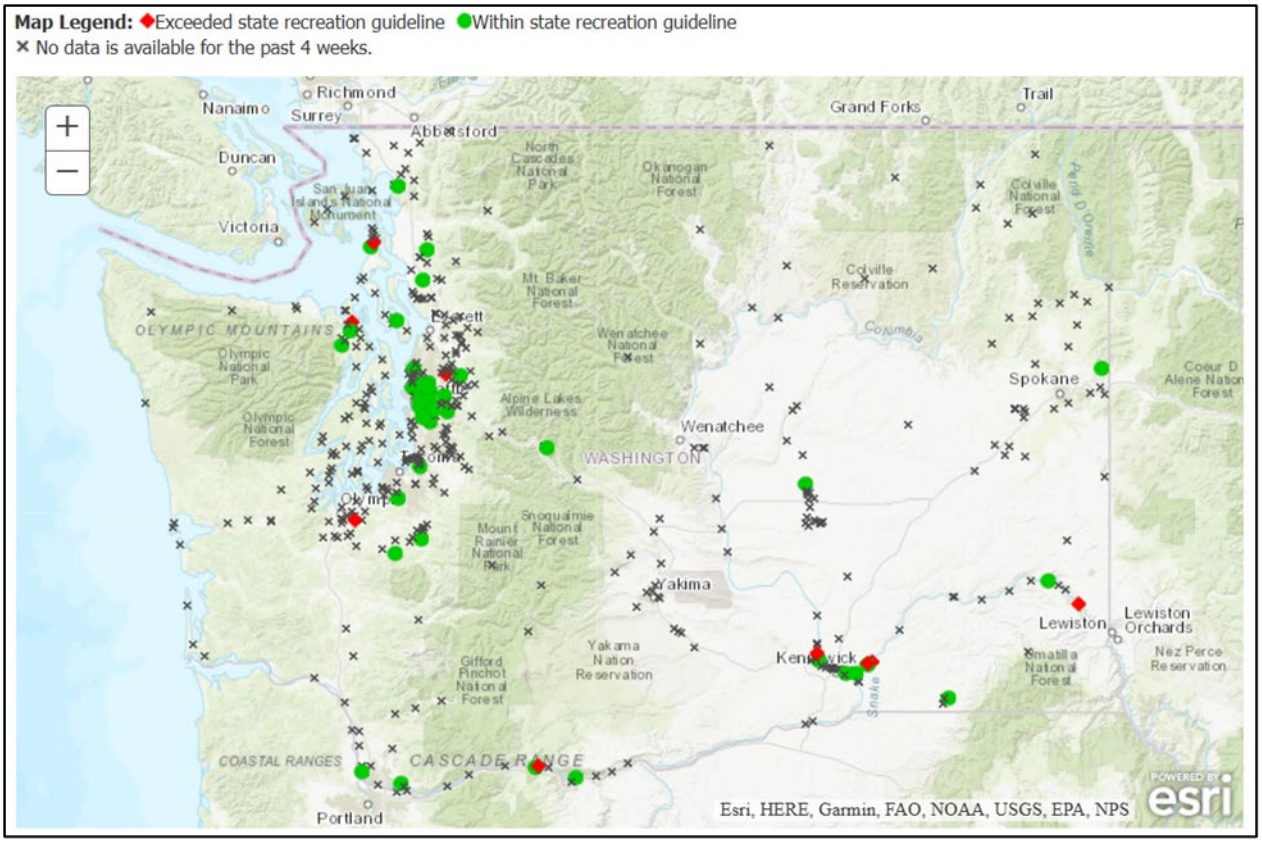


Figure 1. Water bodies tested across Washington state through the FACP testing program.

This map depicts water bodies tested between 2007 and October 23, 2025. The red diamond icons represent water bodies with sample results that exceeded state recreation guidelines for cyanotoxins. The green circle icons represent water bodies with sample results below state recreation guidelines for cyanotoxins. The gray "X" icons represent water bodies with no data available for the past four weeks from the time when the most recent sample was collected (Ecology, 2026a).

7.2 Field data collection

7.2.1 Sampling locations and frequency

An opportunistic (convenience) sampling approach is utilized for samples collected through the FACP testing program. Statewide program users may include state residents, LHJs, or water body management staff. Generally, a suspected cyanobacterial bloom or benthic mat is visually reported and verified, initiating sample collection. Reports of animal illness or death from suspected cyanotoxins at a water body may also prompt sample collection.

Ecology instructs program users to sample areas with surface scum for planktonic blooms or benthic mat materials for benthic blooms. Planktonic blooms tend to accumulate downwind, in shallow areas, along shorelines, or in sheltered coves while benthic mats may be attached to bottom substrates, detached and floating on the water surface, or washed up on shore. Program users (often LHJs) may determine sampling locations based on higher recreational use (e.g., public beaches, public boat launching ramps, accessible boat docks) or areas with reported scum that may have dissipated. Benthic mats are often sampled along water body shorelines or nearshore bottom substrates.

Sample timing is variable but generally occurs when cyanobacterial blooms exhibit peak growth from the summer to late fall. Suspect toxic blooms or mats may be sampled prior to peak water recreation periods such as holidays and special events (Memorial Day weekend, Juneteenth and its nearest weekend, Independence Day and its nearest weekend, Labor Day weekend, fishing tourneys, hydroplane races, triathlons, etc.). While cyanobacterial blooms tend to occur throughout the summer and fall, they can also occur at any time of year including the winter months.

If sample results indicate cyanotoxin levels above the state recreational guidelines, the program user may collect follow-up samples on a weekly basis until two consecutive weeks with no visible algae and toxins below the recreational guidance values, based on DOH guidance (DOH, 2025). Sampling frequency is also dependent on program budget, lab capacity, and water body-specific considerations. For example, some lakes exhibit elevated cyanotoxin levels each year and the water body manager may decide to close the area for the entire recreational season, or to sample on a less frequent basis (e.g., every two weeks).

7.2.2 Field parameters and laboratory analytes to be measured

Field parameters to be measured through this program are:

- General environmental conditions at time of sampling (weather, wind, air temperature)
- Lake use conditions (boaters, swimmers, and dogs present at the lake)

- Presence / absence of algal scum on water surface and its color

KCEL completes routine analysis of the parameters listed below but may expand laboratory analysis of cyanotoxins (e.g., toxin variants) for environmental and health investigations on an ad hoc basis. This may include pet or livestock illness or mortality events. Laboratory analytes to be measured include:

- Microcystins
    - Microcystin variants (MC-LA, -LF, -LR, -LY, -RR, -YR)
- Cylindrospermopsin
    - 7 Epi-Cylindrospermopsin
- Anatoxin-a
    - Homoanatoxin-a
    - Dihydroanatoxin-a
- Saxitoxin
- Cyanobacteria Microscopic Identification

7.3 Modeling and analysis design

Not applicable.

7.4 Assumptions underlying design

Ecology and KCEL advise program users to ensure sample integrity during sample collection and delivery to the lab. This includes using the correct sample container, meeting preservation and holding time requirements, and other considerations. Variations may compromise final data results and lead to qualified or rejected samples. This program makes the assumption that program users are following appropriate requirements for sample collection, preservation, and storage. See Sections 8.2 and 8.3 for more information.

7.5 Possible challenges and contingencies

Ecology relies on program users to report and document suspect cyanobacterial blooms and mats, and to collect and ship samples to KCEL for cyanobacteria identification and toxin testing. This ad hoc sampling approach, while providing timely information, does not represent a comprehensive or systematic investigation of cyanobacterial blooms that occur in Washington in any one year. Sampling efforts are often decreased in the late fall and winter due to lower

recreational use on water bodies, reduced LHJ capacity, or program budget constraints even though blooms can still occur (Hamel, 2012).

Although Ecology provides guidance to program users on water sample collection, sample integrity may be compromised, resulting in the lab qualifying or rejecting results. Due to this opportunistic sampling approach and reliance on program users for sampling activities, it is not appropriate to conduct inferential statistical analysis on toxin data and draw conclusions when comparing different water bodies. Nevertheless, LHJs rely on the FACP testing program for actionable information about toxic cyanobacteria that enables them to protect human and animal health in Washington water bodies. Ecology also uses toxin data to help set funding priorities for control and prevention activities (Hamel, 2012).

Ecology also relies on KCEL for lab analysis, data reporting, and website updates. High sample volume in the peak season (generally summer to late fall) may constrain the lab's capacity, especially when there are staffing or equipment issues. High analyte concentrations can also result in follow-up dilution analyses, which increases lab time and materials expended.

7.5.1 Logistical problems

The following logistical issues may arise:

- Cyanobacterial blooms are difficult to predict and optimal sample timing may not be performed by the program user due to capacity issues, lab closure, etc. Prompt communication is required to coordinate sample collection and processing.
- Access to private property (e.g., boat launches, docks) may inhibit the ability of program users to collect bloom samples from the water body. Contacting property owners ahead of time is often necessary.
- Staff turnover and sampling material availability may impact program users. Prompt communication is required to coordinate sample collection and processing.

7.5.2 Practical constraints

The following practical constraints may arise:

- Problems with connecting and collaborating with local and state agencies, resident volunteers, or KCEL. To alleviate this, Ecology and KCEL will notify each other ahead of time regarding staff leave, holiday lab closure, or other service impacts. Ecology and DOH will maintain an updated LHJ and water body manager cyanobacteria contacts list that is posted to the Washington State Toxic Algae website and meet regularly to discuss program improvements and public outreach.

- Higher sampling demand in any given year may impact the program budget. This may result in the Ecology FACP manager making cost-saving measures (e.g., restricting some analytes measured by the lab, allowing follow-up sampling on a less frequent basis).
- A laboratory accreditation waiver form was completed for the phytoplankton analysis described in this programmatic QAPP.

7.5.3 Schedule limitations

Schedule limitations may include:

- The timing of sample collection, shipping, receiving, analysis, and reporting is critical for this program. The lab accepts samples Monday through Friday, but it is preferred to submit samples earlier in the week if possible. Samples may be shipped to the lab for next-day morning arrival but should never be shipped on a Friday. Additional coordination is needed if samples are submitted to the lab in person. Since the lab provides toxin results within three business days of sample receipt, it is important for program users to consider the timing of receiving toxin results for any subsequent water body use and management decisions.
- Lab reporting deadlines may be missed based on extenuating circumstances.
- Budget impacts may affect peak season and off-season sampling.

8.0 Field Procedures

8.1 Invasive species evaluation

Environmental ethics and Washington law prohibit the transportation of certain organisms, including fish, shellfish, wildlife, plants, and plant parts (RCW 77.15, RCW 77.135, WAC 16-752). This includes aquatic noxious weeds (RCW 17.10).

Field procedures should include visual inspections and following appropriate cleaning and decontamination practices for all boats, equipment, and gear to minimize the spread of invasive species. Ecology has developed protocols for minimizing the risk of spreading any organisms, especially aquatic invasive species, within or between water bodies or other field sites as a result of fieldwork, reconnaissance activities, or other operations (Glisson, 2024). The Washington Department of Fish and Wildlife (WDFW) also provides invasive species protocols that are often adopted by local, state, and federal agencies, Tribes, and non-profit organizations (WDFW, 2022).

8.2 Measurement and sampling procedures

General Procedure

Instructions on how to report and test suspected toxic cyanobacteria are provided on the Washington State Toxic Algae website (Ecology, 2026a). Program users typically contact their local health department, water body manager, DOH, or Ecology directly to verify a cyanobacterial bloom or benthic mat and initiate sampling. All sample requests must be approved by Ecology. A cyanobacteria contacts list is provided on the state website and provides contact information for LHJs and water body managers. Resampling of water bodies follows this same procedure.

Once the program user is approved by Ecology, KCEL will provide a sampling kit (if requested), including sample collection materials and a field sampling information form with a chain of custody. Program users must also generate a sample tracking number using the automated sample number generator on the state website. After collection, samples are shipped with the completed sampling form overnight to the lab if they cannot be delivered same day. While Ecology pays for laboratory analysis, the cost of collection and delivery of samples is the responsibility of the agency or individual conducting the sampling.

Sampling activities should follow guidance from local health jurisdictions or state authorities and collection and sample handling should follow any procedures established by the method or laboratory. Samples should be collected using appropriate personal protective equipment (PPE), which may include protective gloves of appropriate length, boots or hip waders, eye protection, or other applicable PPE. Samplers are encouraged to minimize potential for contact

with cyanobacteria-impacted water and should rinse any exposed areas with soap and water, potentially following an established health and safety plan. Care should also be taken to minimize the spread of invasive species during field collection, including visual inspections and following appropriate cleaning and decontamination practices for all boats, equipment, and gear.

For sample collection, sampling containers should be held just under the water surface, allowing water to fill the bottle and air to bubble out. Samplers should fill the sample container completely with only minimal headspace. Samples must be chilled immediately after collection and during transport to the shipping location. Chilling should be done on wet ice to 4±2°C prior to shipping. Following chilling, samples must be shipped overnight with frozen ice packs for 10AM arrival at KCEL. Samples received outside holding and/or temperature requirements may be rejected or analyzed and qualified with an "SH" (sample handling) flag in the final data. With prior coordination, samples may also be delivered directly at KCEL outside of the Sample Login area during the hours of 9AM-4PM Monday through Friday. Samples should be shipped earlier in the week if possible and never shipped on a Friday as the laboratory is closed on weekends. Confirmation should be given to KCEL or Ecology when the sample has shipped.

KCEL shipping address:

Attn: Sample Login
King County Environmental Laboratory
322 West Ewing Street
Seattle, WA 98119
206-477-7117

It is preferable that the laboratory receives chilled samples instead of frozen samples since frozen samples have the potential to break glass containers during shipping. For extenuating circumstances, samples may be shipped frozen to the lab for toxin analysis; however, samples should never be shipped frozen for qualitative phytoplankton analysis as this will negatively impact sample results.

Additional Sampling Procedure Guidance

As of the date of this programmatic QAPP, Ecology and DOH do not provide published field sampling SOPs for cyanotoxins or cyanobacteria. However, general sampling instructions are provided by Ecology and KCEL.

The USEPA's HAB Methods webpage describes current methods used to sample, monitor, detect, and identify cyanobacteria and cyanotoxins in drinking and ambient freshwaters (USEPA, 2025). In addition, sample collection information is provided in USEPA's analytical methods for cyanotoxins in drinking water and ambient water, including information in the protocols on sample collection, preservation, and storage requirements.

See Section 7.2.1 for more information on sampling strategies. The following resources may also be used for additional guidance on monitoring and field sample collection:

- USEPA 2019 publication: *Recommendations for Cyanobacteria and Cyanotoxin Monitoring in Recreational Waters, Revision 1* (USEPA, 2019a).
- United States Geological Survey (USGS) 2008 publication: *Chapter A7. Section 7.5. Cyanobacteria in Lakes And Reservoirs: Toxin And Taste-and-Odor Sampling Guidelines* (Graham et al., 2008a).
- USGS 2008 publication: *Guidelines for Design and Sampling for Cyanobacterial Toxin and Taste-and-Odor Studies in Lakes and Reservoirs* (Graham et al., 2008b).
- Interstate Technology and Regulatory Council (ITRC) technical guidance on monitoring considerations and approaches for harmful cyanobacterial blooms: *Strategies for Preventing and Managing Harmful Cyanobacterial Blooms* (HCB-1; ITRC, 2021).
- ITRC technical guidance on monitoring considerations and approaches for benthic harmful cyanobacterial blooms: *Strategies for Preventing and Managing Benthic Harmful Cyanobacterial Blooms* (HCB-2; ITRC, 2022).
- Oregon Health Authority provides a 2021 guidance document for lake managers who intend to monitor water bodies when potentially harmful cyanobacterial blooms are detected: *Sampling Guidelines Cyanobacteria Blooms in Recreational Waters* (Oregon Health Authority, n.d.).
- Under California's State Water Resources Control Board, the Water Quality Monitoring Council's Surface Water Ambient Monitoring Program (SWAMP) provides SOPs on their California Freshwater Harmful Algal Bloom Field Guide website, including water sample collection for cyanotoxins in water and benthic cyanobacteria (State of California, n.d.).

8.3 Containers, preservation methods, holding times

Table 10 summarizes requirements for sample containers and quantities, storage and preservation, and holding times for all analyses in this program.

Table 10. Sample containers, preservation, and holding times.

Parameter	Minimum Quantity Required	Container	Storage Conditions Prior to Preservation	Preservation Holding Time	Preservation Technique	Preparation Holding Time	Analysis Holding Time
Anatoxin-a	One 250 mL bottle sufficient for all analyses[1]	250 mL Amber wide-mouth glass	Cool to ≤ 6 °C	48 hours	Freeze at -20 °C	28 days if frozen	48 hours
Cylindrospermopsin			Cool to ≤ 6 °C	48 hours	Freeze at -20 °C	7 days	48 hours
Microcystin			Cool to ≤ 6 °C	48 hours	Freeze at -20 °C	7 days if frozen	48 hours
Saxitoxin			Cool to ≤ 6 °C	48 hours	At the lab split into 40 mL Amber VOA Vial add diluent at 1:10. Cool to 4 °C	7 days (14 days if frozen)	7 days (14 days if frozen)
Qualitative Phytoplankton Analysis			NA	ASAP	Place a minimum of 50 mL aliquot in 60 mL glass bottle and deliver to Microbiology immediately. Receipt in Microbiology Lab: transfer 5 mL into a 10 mL centrifuge tube, store at 2-8°C. Add 1% Lugol's	NA	30 days

Parameter	Minimum Quantity Required	Container	Storage Conditions Prior to Preservation	Preservation Holding Time	Preservation Technique	Preparation Holding Time	Analysis Holding Time
					preservative to remaining sample and store at 2-8°C until processed.		

NA = Not Applicable; mL = milliliters; VOA = Volatile Organic Analysis

[1]the 250 mL minimum quantity is also appropriate for benthic mat samples

8.4 Equipment decontamination

KCEL provides sampling materials to program users that are shipped back to the lab. KCEL follows their laboratory decontamination protocols.

Field procedures should include visual inspections and following appropriate cleaning and decontamination practices for all boats, equipment, and gear to minimize the spread of invasive species. Ecology has developed protocols for minimizing the risk of spreading any organisms, especially aquatic invasive species, within or between water bodies or other field sites as a result of fieldwork, reconnaissance activities, or other operations (Glisson, 2024). The Washington Department of Fish and Wildlife (WDFW) also provides invasive species protocols that are often adopted by local, state, and federal agencies, Tribes, and non-profit organizations (WDFW, 2022).

8.5 Sample ID

The cyanobacteria identification and toxin testing program uses an automated sample number generator to establish unique sample IDs. Instructions on how to generate a sample number are provided on the Washington State Toxic Algae website (Ecology, 2026a). KCEL utilizes these sample IDs for database tracking, sample result dissemination, and contract invoicing.

For qualitative cyanobacteria identification only, KCEL follows their qualitative Cyanobacteria Only method to provide identification of the most common toxin-producing organisms. Organisms are either Present (P) or Not Found (NF). Sample biomass is not reported, and organisms are identified to the Genus level only. In the Washington State Toxic Algae database, a blank space may be used instead of NF.

KCEL completes qualitative identification of cyanobacteria using various publications and reference materials (Bold & Wynne, 1978; Canter-Lund & Lund, 1996; Guiry & Guiry, 2023; Mathews, 2016; Prescott, 1982; Prescott, 1978; Rosen, 2015; Wehr, 2015).

Prior to 2019, KCEL followed their Qualitative Phytoplankton method to provide identification (to the genus level only) of all organisms seen in a sample, including diatoms and non-toxic green algae. The qualifiers (P) for present, (D) for dominant, and (S) for subdominant were used. The D and S qualifiers were based on the biovolume of the organisms seen in the sample.

8.6 Chain of custody

Chain of custody documentation is included on program sampling forms that are shipped to program users along with sample collection materials. The Algae Sampling Data Supplemental Information Form (including chain of custody) is provided in Appendix B.

8.7 Field log requirements

Field data are recorded in the sampling forms KCEL and Ecology provide for program users. The following information is recorded on the sampling forms:

- Sample date and time of day
- Water body name and county
- Ecology tracking number (generated online)
- Sample location description, latitude, and longitude
- Contact information of sampler (including name, affiliation, phone number, and email address)
- Environmental conditions at time of sampling (e.g., weather, wind, air temperature)
- Lake use conditions (e.g., boaters, swimmers, dogs present at the lake)
- Posting decision of water body manager (e.g., Warning, Danger)
- Presence / absence of algal scum on water surface and its color
- Presence / absence of a benthic mat in the collected sample
- Confirmation on whether bloom photos were collected
- General notes/observations
- Indication if sample is being collected in response to human or animal illness
- Requested analyses and chain of custody
- Confirmation that the chain of custody was signed, the proper sampling container was used, and whether the sample was shipped overnight with a frozen ice pack
- Sample temperature upon lab receipt

The Algae Sampling Data Supplemental Information Form (including chain of custody) is provided in Appendix B.

8.8 Other activities

Communication between Ecology, KCEL, DOH, local agencies, and program users is critical for this testing program. Program users must contact Ecology regarding approval for all water body testing. Ecology also relies on program users to report and document water body blooms and benthic mats. Ecology coordinates with KCEL, DOH, and local agencies on approved sampling activities and sampling logistics. As described in Section 12.3, KCEL reports laboratory results to Ecology and program users and King County DNRP/Science LSSG staff upload data to the Washington State Toxic Algae database. Ecology shares results with program users via an email

distribution list and the Washington State Toxic Algae website map is updated to show if water bodies are above or below the state cyanotoxin recreational guidance values. Following reporting of results, Ecology and DOH may provide assistance to local agencies on decision making, following DOH guidance. In Washington state, local jurisdictions have the authority to post advisories on water bodies within their districts (RCW 70.05.070).

9.0 Laboratory Procedures

9.1 Lab procedures table

Table 11 lists analytical methods, method detection limits, and reporting detection limits that will be used for ambient water algal toxin analysis.

Table 11. Measurement methods (laboratory).

Analyte	Analytical Method	KCEL SOP #	Method Detection Limit	Reporting Detection Limit
Anatoxin-a[1]	LC/MS/MS	4066	0.01 ug/L	0.05 ug/L
Cylindrospermopsin[2]	LC/MS/MS	4070	0.025 ug/L	0.125 ug/L
Microcystins[3]	ELISA	4065	0.3 ug/L	0.6 ug/L
Saxitoxin[4]	ELISA	4062	0.02 ug/L	0.08 ug/L
Qualitative Phytoplankton Analysis[4]	As per SOP	5057	NA	NA

ELISA = Enzyme-Linked Immunosorbent Assay; LC/MS/MS= Liquid Chromatography-Tandem Mass Spectrometry; NA = Not Applicable; SOP = Standard Operating Procedure; ug/L = micrograms per liter

[1]Anatoxin-a is analyzed by LC/MS/MS following solid phase extraction (SPE).

[2]Cylindrospermopsin is analyzed by direct injection LC/MS/MS using methods modified from EPA Method 545 for ambient waters.

[3]Total microcystins are analyzed by Abraxis ADDA ELISA and EPA Method 546 ambient water procedures.

[4]There is no EPA reference method for saxitoxin or qualitative phytoplankton analysis.

9.2 Sample preparation method(s)

The sample preparation procedures, freeze/thaw 3x plus 0.45 µm filtration, and Solid Phase Extraction (SPE) are applied to determine the *Total* concentration of toxin present in a sample (e.g. dissolved in water and present within algal cells). The total value of each toxin is reported in ug/L.

For qualitative phytoplankton analysis, samples are preserved and concentrated in the original collection vessel for 12 - 24 hours prior to charging a viewing chamber. After sedimentation, siphon off approximately 25 mL of sample. Do not disturb the concentrate at the bottom of the bottle during siphoning. If the sample contains an algal or benthic mat, the mat is separated (using tweezers). Pick the sample out into thin strips prior to charging the viewing chamber.

Algal mats may also be processed for a short time in a blender to break the mats apart prior to charging the viewing chamber. Samples with a high density of algal cells such as scums collected during bloom events may be diluted prior to charging the viewing chamber. A one-to-one mix of sample and Reverse Osmosis (RO) filtered water may be used to dilute a concentrated sample.

9.3 Special method requirements

KCEL Microcystins ADDA ELISA Sample Dilution Procedure

KCEL's testing program supports the two-tiered decision making process outlined in their 2025 recreational guidance (DOH, 2025).

There are 2 screening values for microcystins:

- The recreational guidance value is ≥ 8 ug/L.
- The danger level is ≥ 2,000 ug/L **or** local health department posts danger sign at its discretion.

The microcystin ADDA ELISA will require sample dilution to support decision making at both the 8 ug/L and 2,000 ug/L criteria. The calibration curve is 0.30 to 10.0 ug/L and inclusive of the recreational guidance value of 8 ug/L. If a result exceeds the calibration curve range of 10 ug/L on initial analysis, KCEL will follow Section 12.2 of EPA Method 546 for concentrations up to the danger level of ≥ 2,000 ug/L (USEPA, 2016).

If a result exceeds the range of the calibration curve, dilute the sample with laboratory water. Based on the estimated concentration, select a dilution factor that results in a diluted sample concentration near the EC50 of the calibration curve. The concentration in the diluted sample must fall between the MDL and the highest calibration standard. Analyze the diluted sample in a subsequent Analysis Batch. Incorporate the dilution factor into the final concentration calculations.

KCEL will report the sample result within the calibration range of the lowest dilution. Samples requiring multiple dilutions are analyzed over two or more days with a delay in reporting.

Preparation of Stomach Contents in Cases of Reported Pet Illness or Death

KCEL occasionally receives stomach contents for analysis in cases of reported pet illness or death. The stomach contents should be placed in a sealed, leak-proof container (clean specimen cup of plastic or glass) and labelled with the client name, animal name, and sample type. The primary container should be placed with some absorbent material into a secondary container such as a sealed Ziploc or other plastic bag. The secondary container, ice pack, and additional absorbent material (if needed) should be placed into a shipping box.

It is preferable to receive as much sample material as can be collected. A minimum of 5 grams is recommended. Ideally, stomach contents are sent chilled; however, stomach contents are typically received frozen. The sample is thawed and at least 2 grams are transferred into a glass scintillation vial with a known volume of methanol/water. The sample is vortexed thoroughly, sonicated, and centrifuged. The supernatant is transferred to a new tube, dried down and resuspended in solvent compatible with LC/MS/MS analysis. Note that stomach content consistency can vary greatly, and more intensive processing/cleanup may be required for some samples.

Sampling and shipping should follow the general requirements of Section 8.2. Ecology and DOH will coordinate sampling approval and reporting of stomach contents.

There are no standard methods for stomach content analysis of cyanotoxins in animal stomach contents. Methodologies and analytical guidance rely on Bouteiller et al. (2022) and Sundaravadivelu et al. (2022).

Generally, biospecimen samples must be acquired within 48 hours from animal symptom onset. Procedures for collection and analysis of additional biospecimen types must be coordinated with Ecology, DOH, and KCEL.

9.4 Laboratories Accredited for Methods

King County Environmental Laboratory is accredited by the Department of Ecology for anatoxin-a, cylindrospermopsin, microcystin, and saxitoxin. Accreditation is not offered for the qualitative phytoplankton analytical method; therefore, a laboratory accreditation waiver form has been completed for use in this program (Appendix C).

10.0 Quality Control Procedures

Laboratory analytical quality control (QC) procedures involve the use of four basic types of QC samples. QC samples are analyzed within a batch of client samples to provide an indication of the performance of the entire analytical system. QC samples go through all sample preparation, clean up, measurement, and data reduction steps in the procedure. In some cases, the laboratory may perform additional tests that check only one part of the analytical system.

10.1 Table of field and laboratory quality control

Table 12 presents the quality control samples, types, and frequency associated with each parameter.

Table 12. Quality control samples, types, and frequency.

Parameter	Spike Blank	Laboratory Method Blanks	Analytical Duplicates	Laboratory Matrix Spikes	Laboratory Matrix Spike Duplicates	ELISA Positive Control
Anatoxin-a	1 for every 20 samples[1]	1 for every 20 samples	NA	1 for every 20 samples	1 for every 20 samples	NA
Cylindrospermopsin	1 for every 20 samples	1 for every 20 samples	NA	1 for every 20 samples	1 for every 20 samples	NA
Microcystin	1 for every 20 samples	1 for every 20 samples	NA	1 for every 20 samples	1 for every 20 samples	1 for every 20 samples
Saxitoxin	1 for every 20 samples	1 for every 20 samples	NA	1 for every 20 samples	1 for every 20 samples	1 for every 20 samples
Qualitative Phytoplankton Analysis	NA	NA	1 for every 20 samples	NA	NA	NA

ELISA = Enzyme-Linked Immunosorbent Assay; NA = Not Applicable

[1]20 samples or less typically represent an analytical batch when samples are prepared and analyzed the same day using the same reagents and equipment, and by the same procedure.

Each type of QC sample listed above will have MQOs associated with it (Section 6.2) that will be used to evaluate the quality and usability of the results.

There may be instances where algal toxin data is reported without the full suite QC data from Table 12. This may be due to an expedited analysis request, testing capacity, or supply chain issues during peak season.

10.2 Corrective action processes

KCEL standard operating practice is to detect and correct analytical difficulties during sample analysis. Should the lab have difficulty in meeting the data quality objectives outlined in this programmatic QAPP, the lab will work with the data user to develop and implement corrective action and to document the problem and resolution using KCEL's Data Anomaly Form (DAF).

11.0 Data Management Procedures

11.1 Data recording and reporting requirements

Several EIM Study IDs exist for this program. Cyanotoxin data collected prior to and during this testing program was submitted into EIM for years 1994, 1999, 2000, 2002, 2003, and 2005 through 2014 (Ecology, 2026b). Table 13 below summarizes program data submitted to Ecology's EIM database.

Table 13. Summary of FACP Study IDs Entered into EIM.

Study ID	Field Collection Date Range
ToxicAlgae1994	08/01/1994 - 09/08/1994
ToxicAlgae1999	08/01/1999 - 08/01/1999
ToxicAlgae2000	07/01/2000 - 07/01/2000
ToxicAlgae2002	04/23/2002 - 04/23/2002
ToxicAlgae2003	09/04/2003 - 09/04/2003
ToxicAlgae2005	06/23/2005 - 09/14/2005
ToxicAlgae2006	10/04/2006 - 10/04/2006
ToxicAlgae2007	05/22/2007 - 12/12/2007
ToxicAlgae2008	03/12/2008 - 12/16/2008
ToxicAlgae2009	02/11/2009 - 12/29/2009
ToxicAlgae2010	01/06/2010 - 12/16/2010
ToxicAlgae2011	01/01/2011 - 12/21/2011
ToxicAlgae2012	01/10/2012 - 06/04/2012
ToxicAlgae2013	06/04/2013 - 12/03/2013
ToxicAlgae2014	01/08/2014 - 05/12/2014

EIM = Environmental Information Management database

Final toxin data are also uploaded to the Washington State Toxic Algae database (Ecology, 2026a) by King County DNRP/Science LSSG staff. Batch uploads, from LIMs download, are completed quarterly throughout the year.

11.2 Laboratory data package requirements

Data reduction, review, and reporting will be performed using KCEL's LIMS and associated SOPs. KCEL's Preliminary Data Report format will be used to expedite reporting following peer review. Preliminary data reports will be followed by final data reports monthly. Data reports will include sample location, collection date and time, parameter name, measured concentration, units, method detection limit (MDL) and reporting detection limits (RDL), if available, and qualifiers SH - Sample Handling (samples received outside holding and/or temperature requirements), P – Presence (cyanobacteria genera is present), NF - Not Found (cyanobacteria genera was not found), and TA - Text Available (additional narrative information is available in the LIMS text field.). Prior to 2019, D - Dominant and S - Subdominant were used, which were based on the biovolume of the organisms seen in the sample.

11.3 Electronic transfer requirements

Data will be provided to data recipients in an electronic format as outlined in 11.2.

11.4 EIM/STORET data upload procedures

Ecology will work with program partners to evaluate the quality and usability of program data for future submission into EIM. Future data entered into EIM will follow a formal data review process where data are reviewed by the FACP program manager, the data submitter, and an independent reviewer. Program data will not be uploaded to USEPA's STOrage and RETrieval Dashboard (STORET).

11.5 Model information management

Not applicable.

11.6 Process Overview: Loading Data to nwtoxicalgae.org

Data for the Washington State Toxic Algae website is received by the DNRP/Science LSSG regularly from KCEL and processed through a standardized workflow. Preliminary toxin data are saved to the designated SharePoint workspace and batch-processed using an R script that formats and validates the data. The resulting output file is uploaded to the Washington State Toxic Algae website Data Loader, where any site-code issues or unmatched locations are

resolved before final submission. Preliminary data are uploaded to the Washington State Toxic Algae database as soon as possible after they are received.

Individual samples or corrections can also be entered manually through the site's administrative dashboard. On a monthly basis, finalized toxin results are exported from LIMS, cleaned to remove non-analytical fields, checked for correct site identifiers, and uploaded in batch to the website.

When new sampling locations are encountered, they are added to the Washington State Toxic Algae site list with the appropriate lake name, county, Water Resource Inventory Area (WRIA), and coordinates. Any corrections to site codes or new site entries are communicated back to KCEL so LIMS can be updated accordingly.

12.0 Audits and Reports

12.1 Field, laboratory, and other audits

No audits are planned for the field work described in this program.

The Ecology Environmental Laboratory Accreditation Program evaluates a laboratory's quality system, staff, facilities and equipment, test methods, records, and reports. It also establishes that the laboratory is capable of providing accurate, defensible data. All assessments are available from Ecology upon request, including internal performance and audits from Ecology's Manchester Environmental Laboratory (MEL).

Labs using analytical methods for phytoplankton analysis are not currently accredited by Ecology. KCEL submitted a Laboratory Accreditation Waiver Form to Ecology's FACP manager to request to waive required use of an accredited lab. This form has been approved by the acting QA Officer and is provided in Appendix C. The form identifies the QC samples that will be analyzed to help demonstrate performance of the method (e.g., acceptable bias and precision).

Ecology's FACP manager will review contract invoices for environmental samples analyzed and database management completed through this program. Invoices are typically reviewed and approved on a biannual basis.

12.2 Responsible personnel

Ecology's Laboratory Accreditation Unit is responsible for auditing analytical laboratories.

Ecology's FACP manager will be responsible for additional project audits.

12.3 Frequency and distribution of reports

As described in Section 5.4, KCEL will provide preliminary data reports to Ecology within three business days of sample receipt at the lab and will notify Ecology if data reporting will exceed three days. Results will be emailed as a PDF to the FACP manager, program user who conducted the sampling, and local health jurisdiction (if known). Laboratory staff will notify Ecology of any analytical anomalies, if any occurred, and potential bias or limitations of reported results. Ecology will also send preliminary data results to program users via Ecology's FACP email distribution list (Ecology, n.d.-b).

King County DNRP/Science LSSG staff will upload final laboratory data electronically to the Washington State Toxic Algae database within ten business days of sample receipt at the lab. Qualitative phytoplankton data will be uploaded to the database on a quarterly basis. In addition, KCEL will provide final data reports to Ecology on a monthly basis, as described in

Section 11.2. Delays in reporting may occur due to sample dilutions, KCEL service interruptions, or other extenuating circumstances.

It is important to note that, for saxitoxin and cylindrospermopsin analysis and data reporting, these less common algal toxins may be held for batching as surveillance and reported within 10 business days of sample receipt at the lab. This is due to these analytes using the same instruments and lab staff as the two more common toxins (microcystins and anatoxin-a).

Environmental data generated by this programmatic QAPP may be included in future Ecology publications completed by the Ecology FACP manager.

12.4 Responsibility for reports

KCEL will be responsible for submitting preliminary and final laboratory data reports to Ecology. Ecology's FACP manager will be responsible for Ecology publications that use environmental data outlined in this programmatic QAPP.

13.0 Data Verification

13.1 Field data verification, requirements, and responsibilities

During sample collection, program users complete sampling forms provided by KCEL and Ecology. The Ecology FACP manager provides guidance to program users on how to complete these forms, which include general observations and environmental conditions (e.g., algae scum color, weather conditions). These forms also ask for confirmation that the chain of custody was signed, the proper sampling container was used, and whether the sample was shipped overnight with a frozen ice pack. See Section 8.7 for all field log requirements.

Program users submit sampling forms to KCEL, who then provide this data to King County DNRP/Science LSSG to transfer into the Washington State Toxic Algae database. King County and Ecology review this data for obvious errors or inconsistencies and may follow up with the program user for clarification. However, more thorough field data verification may not be completed due to limited staff capacity.

13.2 Laboratory data verification

Data reported by the lab must pass a review process before final results are available to the client. A “Peer Review” process is used where a second analyst or individual proficient at the method reviews the data set. The reviewer will complete a data review checklist which will document the completeness of the data package and if any QC failures exist. The Laboratory Project Manager will coordinate this data review.

Once data review is complete and all data quality issues have been resolved or corrected, the status of the data in LIMS will be changed to “Approved”. Once a data set has been approved, it is “Moved” or “Posted” or transferred to the portion of the LIMS database known as the Environmental Data System (EDS) where all historical LIMS data are maintained. Signatures or initials of the lab lead and reviewer(s) indicate formal approval of hardcopy data or reports (non-LIMS), typically on the review checklist. A copy of this approved checklist should be stored with the final electronic data package.

13.3 Validation requirements, if necessary

Not applicable.

13.4 Model quality assessment

Not applicable.

14.0 Data Quality (Usability) Assessment

14.1 Process for determining project objectives were met

The Ecology FACP manager will determine if the project data are useable by assessing whether the data met the MQOs (outlined in Table 9) and discussing the data with KCEL. Based on this assessment, the data will be accepted, accepted with appropriate qualifications, or rejected and considered for re-analysis.

It is expected that the Ecology FACP manager and KCEL will be in regular contact throughout the project.

14.2 Treatment of non-detects

Laboratory analysis of toxin samples may result in concentrations below the limit of detection. The MDL is the minimum concentration that the analyte can be reliably detected by a specific analytical method and distinguished from the blank. The RDL is the minimum concentration that the analyte can be reliably measured and reported by the laboratory. The RDL represents the lowest concentration where the analyte has been shown to meet specified precision and accuracy limits. Depending upon the program or DQOs, the LIMS RDL reflects the Practical Quantitation Limit (PQL), Minimum Level (ML), Quantitation Limit (QL), or Lower Limit of Quantitation (LLOQ).

Methods for data analysis including toxin concentrations below the MDL or RDL will be determined by Ecology's FACP manager. Regression on-order statistics (ROS) or Kaplan-Meier may be appropriate for summary statistics, distributional analysis, and other analyses.

14.3 Data analysis and presentation methods

Environmental data results from this program will be primarily uploaded to the Washington State Toxic Algae database (Ecology, 2026a). Descriptive statistical analysis and data visualization will be completed using Microsoft Office suite or statistical programming software (e.g., R) to characterize cyanotoxin and cyanobacteria results in a water body or collection of water bodies. Data collected through this program will also be used by LHJs and other water body managers to determine management decisions. Due to the ad hoc nature of this testing program, inferential statistical analysis will not be completed (see Section 14.4).

14.4 Sampling design evaluation

The FACP testing program relies on opportunistic (convenience) sampling and will not yield meaningful statistical comparisons between water bodies. See Section 7.2.1. As a result of this sampling approach, sample collection is not randomized and can lead to sampling bias and

limited generalizability. Based on program budget as well as logistical and practical constraints, statistical power is also limited.

14.5 Documentation of assessment

Future Ecology publications and reports may present the findings and interpretations of environmental data generated by this programmatic QAPP to the extent possible.

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16.0 Appendices

Appendix A. 2010 Sampling and Analysis Plan for the Department of Ecology Toxic Algae Program

Appendix B. Algae Sampling Data Supplemental Information Form

Appendix C. Laboratory Accreditation Waiver Form

Appendix D. Glossaries, Acronyms, and Abbreviations

Appendix A. 2010 Sampling and Analysis Plan for the Department of Ecology Toxic Algae Program

This appendix is not an Ecology publication, and has been included as supporting evidence for the QAPP.

SAMPLING AND ANALYSIS PLAN FOR THE DEPARTMENT OF ECOLOGY TOXIC ALGAE PROGRAM

SUBMITTED TO:

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SUBMITTED BY:

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Water and Land Resources Division
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June 2010

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PROJECT NUMBER:	421520 – 300
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1 INTRODUCTION

Toxic cyanobacteria have been increasingly detected in western Washington lakes since the first documented toxic episode in American Lake in 1989 (Jacoby *et al*. 1994, Jacoby and Kann 2007). State health officials are concerned that the rate of occurrence appears to be increasing over time, leading to the possibility of increased human and animal exposure to cyanotoxins. Toxic cyanobacteria blooms are emerging as a national and international issue (Ecology 2007).

Mass accumulations or "blooms" of cyanobacteria in freshwater ecosystems are caused in part by nutrient enrichment. Cyanobacteria blooms form surface scums, decreased water column transparency, dissolved oxygen depletion and unpalatable drinking water due to taste and odors. Some cyanobacteria also produce toxic compounds (cyanotoxins) that have caused livestock, wildlife and pet fatalities worldwide (reviewed by Carmichael 1994; Chorus 2001). Although many cyanobacteria blooms are not toxic, a bloom that is not toxic one day may become toxic during the same growing season.

Table 1. General features of cyanotoxins (modified from Chorus and Bartram 1999).

Toxin Group	Primary Target organ in mammals	Cyanobacterial genera
Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*
Nodularian	Liver	*Nodularia*
Anatoxin-a	Nerve Synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Anatoxin-a (S)	Nerve Synapse	*Anabaena*
Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsins	Liver	*Cylindrospermopsis, Aphanizomenon*
Lyngbyatoxin-a	Skin, G.I. Tract	*Lyngbya*
Saxitoxins	Nerve Axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharide	Potential irritant; affects any exposed tissue	ALL

Cyanotoxins include a broad, diverse range of chemicals and mechanisms of toxicity (Carmichael 1994; Sivonen and Jones 1999). Major classes of cyanotoxins include the cyclic peptides, which are primarily hepatotoxins (microcystins and nodularins); alkaloids and an organophosphate, which are strong neurotoxins (anatoxin-a, anatoxin-a (S), and saxitoxins); a cyclic guanide alkaloid, which inhibits protein synthesis (cylindrospermopsin); lipopolysaccharides, which have pyrogenic properties; and dermatoxic alkaloids (aplysiatoxins and lyngbyatoxins) (Table 1) (Chorus 2001). This SAP will focus on the analysis of three toxins: microcystin, anatoxin-a, and saxitoxin.

Microcystins are the most commonly tested and detected cyanotoxins in Washington and have been found at water concentrations from <1 to 4,810 µg/L (Jacoby and Kann 2007). Microcystins have seven amino acids and are known as hepatotoxins (produced by species of *Microcystis, Planktothrix,* and *Anabaena*). These hepatotoxins are of concern due to their prevalence and potential to harm animals and humans. Hepatotoxins damage liver tissues, and at high doses can cause liver failure and death (Carmichael 1994). Nodularins have five amino acids (produced by *Nodularin spumigena*). The mechanism of toxicity involves inhibition of the specific protein phosphatase enzymes possessed by all eukaryotic cells. In addition, microcystins are suspected tumor-promoters and teratogens (Falconer 1998). These toxins have been associated with elevated rates of primary liver cancer in people drinking waters with high densities of cyanobacteria (Yu 1989).

While microcystins appear to be more common than neurotoxins, neurotoxins are notoriously potent and rapid-acting poisons that have caused severe animal poisonings in North America, Europe and Australia (WHO 2003, Botana 2007). The neurotoxin anatoxin-a is an alkaloid with high toxicity. It acts as a post-synaptic, depolarizing, neuromuscular blocking agent. Depending upon the size of the animal and amount of the toxin present, illness or death may occur within minutes to a few hours after exposure. Signs of anatoxin-a poisoning are staggering, paralysis, muscle twitching, gasping, and convulsions – all potentially leading to death. Anatoxin-a can be produced by some species of cyanobacteria including *Anabaena, Aphanizomenon, Cylindrospermum, Planktothrix* (*Oscillatoria), and Microcystis* spp.. Determination of anatoxin-a in natural waters has been challenging due to its typically low concentration and/or rapid degradation to nontoxic degradation products (Botana 2007).

Saxitoxin is another neurotoxin produced by blue-green algae and is a carbamate alkaloid sodium channel blocker. Saxitoxins have been observed in *Aphanizomenon, Anabaena, Lyngbya, Cylindrospermopsis,* and *Planktothrix.* Twenty variants have been identified (Huisman *et al*.). Animals with saxitoxin/neosaxitoxin toxicosis may exhibit weakness, staggering, loss of muscle coordination, difficulty in swallowing, labored respiration, complete muscle paralysis, and death. Humans may exhibit tingling around the mouth and fingertips, as well as slurred speech. Nearly all systemic effects of saxitoxin are due to its effect on nerve axon membranes. Saxitoxins from *Anabaena circinalis* have been observed to bioaccumulate in an Australian mussel to levels exceeding human health international guidelines in as few as seven days (Shumway et al. 1995).

Saxitoxins and anatoxin-a(s) are among the most neurotoxic substances known (WHO 2003). They do not occur in lakes and rivers as frequently as microcystins. After ingestion of a sub-lethal dose of neurotoxins produced by cyanobacteria, recovery appears to be complete. Chronic effects have not been observed. Neurotoxins have the potential to be lethal by causing suffocation, specifically through paralysis by exposure to saxitoxins. No human deaths from exposure to neurotoxins associated with recreational use of water are known.

Saxitoxins undergo a series of slow chemical hydrolysis reactions in the dark at room temperature. The half-life for breakdown reactions are in the order of 1 – 10 weeks, with more than 3 months required for greater than 90% breakdown. In some circumstances, the degradation of a bloom will increase the toxicity level for a period of up to three weeks before toxicity abates in the following 2-3 months. This is the result of the more toxic properties of some breakdown products than the intact toxin. Boiling an extract of *Anabaena* may also increase toxicity. No detailed studies have been carried out on saxitoxin breakdown in sunlight with or without pigments (Chorus and Bartram 1999).

Highest published concentrations from cyanobacterial bloom samples measured by HPLC are 3,400 ug/g dw saxitoxin (Australia), 7,300 ug/g dw microcystins (China, Portugal); anatoxin-a 4,400 ug/g dw (Finland) (Chorus and Bartram (1999).

1.1 Project Background

Cyanobacterial toxins produced by harmful algal blooms (HABs) are a worldwide public health threat. Health effects from cyanobacteria exposure in recreational waters are diverse and include skin rashes and lesions, vomiting, gastroenteritis, conjunctivitis, headaches, and eye, ear and throat irritations (Pilotta *et al.* 1997, Chorus *et al.* 2000, Codd *et al.* 2005). While many of these reactions have been documented, information on cyanobacterial species, cell densities, and toxins present during exposure is typically lacking (Chorus 2005, Codd *et al.* 2005). Attempts to characterize human health hazards during primary contact activities have been based on limited human case histories, reported animal poisonings, and human epidemiological data (Pilotto *et al.* 1997).

Toxic cyanobacterial blooms have been documented in numerous Washington State lakes during the past 30 years (Jacoby and Kann 2007). State health officials are concerned that the rate of occurrence appears to be increasing over time, which will lead to the possibility of increased human and animal exposure to cyanotoxins. The first documented health effects of cyanotoxins in Washington State occurred in the 1970s and 1980s, causing the deaths of several dogs. More recently, animal deaths have been associated with the detection of anatoxin-a in several western Washington lakes (*e.g.,* American Lake, Pierce County, December 1989 and 2007; Kitsap Lake, Kitsap County, October 2001; and Anderson Lake, Jefferson County, June 2006). Over the past decade, several anecdotal reports have been received of human illness, such as gastrointestinal symptoms and rashes, following recreational exposure to blooms. A particularly toxic bloom of *Microcystis* occurred in Lake Sammamish in 1997 and was believed to have caused illnesses of children swimming in the lake and the death of a dog (Johnston and Jacoby 2004).

Following the aforementioned animal deaths, Washington's legislature funded the Freshwater Algae Control Program, implemented by Washington State Department of Ecology (Ecology, DOE). As part of this program, local jurisdictions and citizens can submit bloom samples for state-funded analysis of toxin concentrations and phytoplankton population review. The remainder of this document outlines the technical details for the analysis of these samples.

1.2 Regulatory Status of Cyanotoxin Criteria and Guidelines

In 2005, the Washington State Legislature established funding for a Freshwater Algae Control Program (RCW 43.21A.667) through the Department of Ecology (Ecology, DOE) to assist local governments in the management of freshwater algae problems. As part of this program Ecology partnered with the Washington Department of Health (WaDOH) to develop recreational guidelines values for cyanotoxins. WaDOH has recommended a three-tiered approach using the recreational guidance values of 6.0 µg/L microcystins and 1 µg/L anatoxin-a for managing Washington Lakes (WaDOH, May 2008). More information about this three-tiered management approach can be found at the Washington State Department of Health webpage: http://www.doh.wa.gov/ehp/algae/guidelines.htm.

2 Project Design

2.1 Laboratory Organization

Table 2.1 – Laboratory Project Team Members

Name	Title	Affiliation	Responsibility
Fran Sweeney	Laboratory Project Manager/Supervisor	KCEL– Aquatic Toxiciology	Coordination of laboratory activities, lab QA/QC and data reporting.
Colin Elliott	Quality Assurance Officer	KCEL	Overall laboratory QA/QC.
Eric Thompson	Supervisor	KCEL - Microbiology	Microbiology QA/QC
Jim Buckley	Scientist III	KCEL– Aquatic Toxiciology	Primary Scientist – Anatoxin-a
Julie Alaimo	Scientist II	KCEL– Aquatic Toxiciology	Primary Scientist – Microcystins
Gary Yoshida	Scientist II	KCEL– Aquatic Toxiciology	Primary Scientist – Saxitoxin
Debbie Turner	Scientist II	KCEL– Microbiology	Primary Scientist – Phyto ID

2.2 Approach

The Department of Ecology Toxic Algae Monitoring Program is a statewide program that responds to bloom events. The exact sampling locations and water bodies varies from year to year depending on regional bloom conditions across the state. Samples are typically collected by the local health department, but may be accepted from other

government agencies, concerned citizens and volunteer lake stewards. The program uses focused sampling efforts to collect blooms or accumulations of cyanobacteria. A bloom will be defined as a visually observable accumulation of phytoplankton in the water column or as a surface accumulation.

Samples are collected and shipped to the King County Environmental Lab (KCEL) for analyses. Based on the phytoplankton population and historical knowledge of the lake, samples may be analyzed for Microcystin, Anatoxin-a, Saxitoxin, and/or qualitative algae identificaton. Initial bloom response typically consists of microcystin analysis and algae identification. Turnaround times are:

- Microcystins – 3 business days from sample receipt for final data. Verbal results may be available sooner.
- Anatoxin-a – preliminary data typically available within 5 business days, and final data within 7.
- Saxitoxin – case by case, typically within 3 days with advanced notice.
- Qualitative algae identification – 3 business days from sample receipt.

2.3 Sampling Procedures

The following sampling procedures are based on methods of Carmichael (2001), Chorus (2001), Johnston and Jacoby (2002).

2.3.1 Sample Container and Preservation Requirements

Note that these are the ideal collection containers for the toxicity studies. Due to the nature of the program, all samples received will be analyzed regardless of the type of container. However, grossly compromised samples (broken bottles, not shipped on ice, not shipped overnight etc.) will be rejected and the sampler notified that they will need to resample. Samples received in containers different from those listed in the following table will be flagged with an SH data qualifier.

Table 2.3 - Sample Container & Preservation Requirements (liquid/water matrix)

Parameter	Container	Preservation	Hold time
Phyto ID	1x 250- ml Plastic Amber Wide Mouth (AWM). *Alternatively 1x 1L Plastic Amber for ID, Microcystins and Anatoxin.*	Lugol's solution added to sample upon arrive in Laboratory Store at room temperature in the dark Overnight ship or hand deliver in dark cooler w/icepacks.	365 days
Microcystins ELISA	250- ml Glass, AWM *Alternatively 1x 1L Plastic Amber for ID, Microcystins and Anatoxin.*	Store 4°C, Dark Overnight ship or hand deliver in dark cooler w/icepacks.	24 to 48 hours to freeze, 14 days for analysis
Anatoxin-a HPLC	1x 1– L Plastic Amber bottle *Alternatively 1x 1L Plastic Amber for ID, Microcystins and Anatoxin.*	Store 4°C, Dark Overnight ship or hand deliver in dark cooler w/icepacks	48 hours for prep 28 days for analysis
Saxitoxin ELISA	Amber VOA vial	At time of collection, 18ml of sample into 2ml of preservative. Contact lab for pre-preserved vials. Store - 4°C, Dark Overnight ship or hand deliver in dark cooler w/icepacks	7 days with preservation, up to 14 days if frozen for analysis

2.3.2 Water sample collection and storage procedure to test for toxins:

- Fill the bottles by dipping the bottle mouth-down into the water, collecting the visible accumulation of algae scum. Use caution and appropriate personal protective equipment (PPE) as blooms can contain high levels of toxins. Label the bottles if not pre-labeled – include date, time, location and sample tracking number.
- Place the sample bottles for Microcystins and Anatoxin-a directly in a cooler with ice packs (no preservative required).
- *If saxitoxin is of concern, contact the Lab for a pre-preserved sample container.* The sample container will have the Sample Diluent added prior to being taken into the field and it will be necessary to add a specific volume of sample in the ratio of 1:10 (diluent:sample). 2 mL of sample diluent will be added to a 40 mL sample container and in the field 18 mL of sample must be pipetted (alternately, a graduated cylinder

can be used for thick cellular material) to the sample vial. The sample container is then inverted several times for mixing then placed in a cooler with ice packs.

- Phytoplankton samples will be collected in 250 mL and preserved with 1.75 mL of Lugol's solution upon arrival at the lab.

3 Laboratory Analysis

Samples will be analyzed using the procedures and detection limits listed in the table below.

Table 3 - Project Analysis Summary

Parameter	Reference	Method Detection Limit	Reporting Detection Limit
Microcystins by ELISA	Envirologix, 2006 Envirologix, 2008 KCEL SOP 440v2	0.05 µg/L	0.05 µg/L
Saxitoxin by ELISA	Abraxis, 2010 KCEL SOP 462v0	0.02 ug/L	0.02 ug/L
Anatoxin-a by HPLC	KCEL SOP 457v0	1.85 ug/L/ CF* Typically <0.05 ug/L	Same value as the Method Detection Limit
Qualitative Algae Identification	KCEL SOP 557v0	NA	NA

* CF = concentration factor

3.1 Microcystins and Saxitioxin by ELISA

3.1.1 Cell Lysing Procedure

The objective of the cell lysing is to generate a sample in which all toxin (extra- and intracellular) has been converted into a form that can be measured by ELISA, thus providing a close approximation of the total concentration in the ambient sample. The resulting concentration should be representative of a recreational exposure in which a swimmer ingests ambient water and cells as a combined dose. All samples collected for this study will be analyzed subsequent to a lysing step, and results will be reported as *Total Microcystins and/or Total Saxitoxin*.

Each sample will be prepared for analysis by the following lysing process:

- 5-mL aliquots will be frozen for a minimum of 12 hours.
- Thawed at room temperature and then immediately sonicated (ultrasonic disruption) using the Misonex Sonicator.
- Samples will be filtered through a 0.45 μm filter prior to analysis.

3.1.2 Microcystins by ELISA

The Envirologix ELISA test kit uses polyclonal antibodies that bind either microcystins or a microcystin-enzyme conjugate. Microcystins in the sample compete with the microcystin-enzyme conjugate for a limited number of antibody binding sites. The same number of antibody binding sites are available on every test well, and each test well receives the same number of microcystin-enzyme conjugate molecules. A sample that contains a low concentration of microcystins allows the antibody to bind many microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of microcystins allows fewer microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution. The plate kit does not differentiate between microcystin toxin variants but detects the presence of Microcystin –LR, LA, RR, YR and Nodularin to differing degrees (Table 3.1.2). The microcystin high-sensitivity assay has an operational range of 0.05 to 0.83 ug/L. Samples with values less than 0.05 will be reported as less than MDL, samples above 0.83 will be diluted and re-run to give a reportable value from within the standard curve.

Table 3.1.2 Microcystin cross reactivity

Toxin Variant	Sensitivity/Cross Reactivity [1,2]
Microcystin - LR	100 %
Microcystin - LA	62 %
Microcystin - RR	54 %
Microcystin - YR	35 %
Nodularin	68 %

[1] For example, Nodularin is detected at 68 % of the actual dose.
[2] Source Larrivee pers comm. 2010.

3.1.3 Saxitoxin ELISA

The test is a direct competitive ELISA based on the recognition of saxitoxin by specific antibodies. Saxitoxin, when present in a sample, and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the Saxitoxin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. The saxitoxin ELISA recognizes saxitoxin and other PSP toxins with varying degrees (Table 3.1.3). The saxitoxin assay has an operational range of 0.02 to 0.4 ug/L. Samples with

values less than 0.02 will be reported as less than MDL, samples above 0.4 will be diluted and re-run to give a reportable value from within the standard curve.

Table 3.1.3 –Saxitoxin cross- reactivity	
Toxin Variant	Sensitivity/Cross Reactivity [1]
STX	100%
Decarbamoyl STX	29 %
GTX 2 and 3	23 %
GTX 5B	23 %
Sulfo GTX 1 and 2	2.0 %
Decarbomyl GTX 2 and 3	1.4 %
Neosaxitoxin	1.3 %
Decarbomyl Neo STX	0.6 %
GTX 1 and 4	<0.02 %

[1] Abraxis, 2010

3.2 Anatoxin-a

The neurotoxin anatoxin-a is an alkaloid with high toxicity (LD_{50} i.p. mouse 200 µg/kg). It acts as a post-synaptic, depolarizing, neuromuscular blocking agent. There is evidence that anatoxin-a can be produced by some species of *Anabaena*, *Aphanizomenon*, *Cylindrospermum* and *Oscillatoria*.

The highly sensitive method adapted at KCEL uses fluorimetric derivatization with HPLC. Following extraction with methanol, anatoxin-a is converted into a fluorescent derivative using 4-fluoro-7-nitro-2,1,3-benzoxadiazole; the fluorescent compound is then separated and detected by HPLC. Increased sensitivity is accomplished by previously concentrating the sample and the extract.

3.2.1. Sample Handling and Preparation

Anatoxin-a degrades readily, especially in sunlight and at high pH. At KCEL the bottles are immediately transferred to a 4 °C chamber.

Cells are lysed by digestion in 0.05 M acetic acid at 60 °C. The digestate is neutralized with NaOH, filtered to 0.7 µm and treated by Weak Cation Exchange Solid Phase Extraction (SPE). The SPE column elutriate is evaporated under nitrogen and held at -20 °C until analyzed.

Samples and standards are derivatized with 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). The reaction is terminated at 60 minutes, and samples are filtered and transferred to autosampler vials.

3.2.1 Chromatography

HPLC analysis is performed with an Agilent 1200 series system using a Zorbax C_{18} column, 45% acetonitrile-phosphate buffer as the mobile phase and fluorimetric detection at 470 nm / 530 nm. Run time is 37 min per injection.

Anatoxin-a analytical standards are purchased commercially, diluted and run along with samples to create a 4-point calibration curve. Methyl pipecolinate is added to all samples and standards and used as a reference standard.

3.2.2 Method Detection Limits

Because samples are concentrated prior to chromatography each sample carries a sample-specific concentration factor (CF) and therefore the final MDL is also sample-specific. The concentration factor is based on the volume of water extracted by SPE, the volume of extract dried, and the final volume of the derivatized extract.

MDL, SPE method: The current Calculated MDL is 1.85 μg/L
The final MDL for each SPE sample is:

Final MDL = (Calculated MDL) / CF = (1.85 μg/L)/CF

For example, the final sample MDL for a 40 mL sample prepared as above and derivatized to a final volume of 400 μL is: Final MDL = (1.85 μg/L) / (40/0.4) = 0.0185 μg/L.

4 Laboratory Microscopic Analyses of Phytoplankton Sample

The qualitative phytoplankton method is utilized for the identification and ranking of phytoplankton taxa found in fresh water samples.

4.1 Sample Preparation:

Wet Mount: A one ml aliquot of an unpreserved sample may be scanned, upon sample receipt in the microbiology lab, for the presence of *Anabaena sp.*, *Aphanizomenon sp.*, *Microcystis sp.*, and filamentous Cyanobacteria.

Preservation: Approximately one ml of Lugol's iodine solution is added to the remaining sample to preserve the phytoplankton.

Concentration: The preserved sample is refrigerated overnight, allowing the phytoplankton to settle and concentrate on the bottom of the bottle.

4.2 Analytical Procedure:

Microscopic Survey: After an overnight sedimentation period, all but approximately five mL of sample is siphoned off without disturbing the concentrated sample on the bottom of the bottle.

A Sedgewick-Rafter slide is charged with one ml of concentrated sample. The charged slide is allowed to settle for a minimum of 15 minutes.

The charged chamber is scanned for dominant and subdominant (most common organisms) taxa. This is done by reading parallel transects of the slide, left to right, top to bottom, until the whole slide has been scanned and the data recorded. The dominant, subdominant status of the organisms are determined by number and approximate biomass. The presence of all cyanobacteria should be noted and identified to genus level, when possible. The presence of other phytoplankton maybe reported and identified to genus level. Identified organisms are reported with one of the following qualifiers: D- dominant, S- subdominant or P- present.

5 DATA QUALITY OBJECTIVES

The procedures and practices described in this study-specific SAP are designed to generate data of sufficient quality to support decision making. Critical elements of laboratory data quality objectives are discussed in this section. Procedures to attain these data quality objectives are discussed throughout this document. In particular, Section 7.0, Quality Control Procedures, addresses many of the procedures necessary to obtain data that meet these data quality objectives.

5.1 Laboratory Precision

Toxicity Studies: Laboratory precision will be assessed using laboratory Matrix Spike and Matrix Spike Dup (MS/MSD) QC samples. When both sample results are at or exceed the MDL the RPD (relative percent difference) should be less than 25 % for ELISA and 45 % for anatoxin-a. An RPD cannot be determined unless both values are at or above the MDL since no values are reported if <MDL. Note that the MDL and the Reporting Detection Limit (RDL) are the same for the ELISA.

Qualitative Algae Identification: Inter-operator precision will be evaluated using data from laboratory duplicates. An interim minimum acceptance value of </=40% difference is in place until sufficient data has been collected to determine the most appropriate value. Major differences between two analysts will require the sample be retested.

5.2 Bias

Bias is an indicator of the accuracy of analytical data. For this project, laboratory control samples or blank spikes, whichever are available, along with matrix spikes will be used to assess bias. Results should be within the lab's current control limits (see table 6.2).

Bias will also be assessed by the evaluation of field blank and method blank data. Analytical results for method blanks should be less than the MDL.

The use of matrix spike recovery data will provide additional information regarding method performance on actual samples. The laboratory will use professional judgment regarding assessment of data quality and any subsequent action taken as a result of matrix spike recoveries. Note that unexpectedly high sample concentrations can interfere with MS/MSD recoveries.

5.3 Representativeness

This survey is primarily designed to evaluate the presence/absence of cyanobacterial toxicity, and secondarily to estimate concentrations and geographic extent of the toxin distribution, should it be present. Samples for evaluation of phytoplankton population will be collected. Representative samples will be obtained through the following practices:

- The use of generally accepted sampling procedures will allow for the collection of representative samples.
- Sub-sampling within the KCEL will be conducted according to lab standard operating procedures. These procedures are designed to obtain representative subsamples.

5.4 Comparability

Data comparability will be obtained through the use of standard sampling procedures and analytical methods. Additionally, adherence to the procedures and QC approach contained in this SAP will provide for comparable data throughout the duration of this project. Before making changes to sample collection, storage or analysis procedures, each must be evaluated to verify that comparability will not be compromised.

5.5 Completeness

Completeness will be evaluated by the following criteria:

- The number of usable data points compared to the projected data points as detailed in this SAP.
- Compliance with the data quality criteria as presented in this section.
- Compliance with specified holding times.

The goal for the above criteria is to obtain 100% data completeness. However, where data are not complete, decisions regarding re-sampling and/or re-analysis will be made by a collaborative process involving both data users and data generators. These decisions will take into account the project data quality objectives as presented above.

6 QUALITY CONTROL PROCEDURES

6.1 Laboratory Quality Control Procedures

The KCEL is accredited for toxin analysis by the Washington State Department of Ecology. As a requirement of this accreditation, the lab is audited by the Washington State Department of Ecology.

6.1.1 Quality control samples

For samples analyzed at the KCEL, the frequency of quality control samples to be performed for this project is shown in the following table. All listed types of QC samples shown below may not be available for all lab analyses and certain QC may be dropped from an instrument run in order to expedite bloom/public health samples.

Table 6.1.1 - Laboratory Quality Control Samples

Type of Quality Control Sample	Description	Frequency
Method Blank	An aliquot of clean reference matrix carried through the analytical process and used as an indicator of contamination.	1 per sample batch. Maximum sample batch size equals 20 samples or one 96-well ELISA plate.
Positive Control ELISA	A standardized spike of the primary ELISA toxin, typically the mid-level standard.	1 per sample batch. Maximum sample batch size equals 20 or one 96-well ELISA plate.
Matrix Spike/ Matrix Spike Duplicate (MS / MSD)	A MS is a second sample aliquot fortified with a known concentration of target analyte(s), and processed through the entire analytical procedure; used as an indicator of sample matrix effect on the recovery of target analyte(s). A MSD is a third sample aliquot fortified with a known concentration of target analyte(s), and processed through the entire analytical procedure; used as an indicator of sample matrix effect on the recovery of target analyte(s) as well as method precision. MS / MSD used with methods where samples typically show no detectable responses, thus do not provide useful information of batch precision.	1 per sample batch. Maximum sample batch size equals 20 or one 96-well ELISA plate. MS/MSD may be dropped for bloom samples due to interferences from high concentrations of toxins and necessary dilutions.
Spike Blank (SB)	Known concentration of target analyte(s) introduced to clean reference matrix, processed through the entire analytical procedure and used as an indicator of method performance.	1 per sample batch. Maximum sample batch size equals 20 samples. or one 96-well ELISA plate.
Lab duplicate	lab duplicate is a second aliquot of a sample, processed concurrently and in an	1 per sample batch. Maximum sample batch size equals 20

	identical manner with the original sample. Analysis of the lab duplicate is used as an indicator of method precision and laboratory subsampling procedures. The lab duplicate can also be used to provide information regarding the homogeneity of the sample matrix.	samples. or one 96-well ELISA plate.

6.2 QC Control Limits

Current control limits for the above QC samples are summarized in Table 6.2.

Table 6.2 – Laboratory CQ Limits

Parameter	**Method Blank**	**Spike Blank**	**Lab Dup**	**Positive Control**	**Matrix Spike**	**Matrix Spike Dup**
Condition	**<MDL**	**% Rec**	**RPD**	**% Rec**	**% Rec**	**RPD**
M-LR	**<MDL**	**80-160**	**0-25**	**70-140**	**80-160**	**0-25**
STX	**<MDL**	**70-130**	**0-25**	**70-130**	**70-130**	**0-25**
ATX	**<MDL**	**80-120**	**0-45**	**NA**	**80-120**	**0-45**

7 DATA REDUCTION, REVIEW, AND REPORTING

7.1 Toxin and algae data

Data reduction, review and reporting will be performed using KCEL's LIMS and associated standard operating procedures. Final laboratory data will be provided to data recipients in KCEL's EXCEL-based "Comprehensive Report" format. When necessary, KCEL's "Preliminary Data Report" format will be used to expedite reporting. Preliminary data reports will be followed by final data reports. Data reports will include sample location, collection date and time, parameter name, measured concentration, units, method detection limit (MDL) and reporting detection limits (RDL), if available, and qualifiers Presence (P), Subdominant(S) and Dominant (D).

7.2 Corrective Action

KCEL standard operating practice is to detect and correct analytical difficulties during sample analysis. Should the lab have difficulty in meeting the data quality objectives outlined in this SAP, the lab will work with the data user to develop and implement corrective action and to document the problem and resolution using KCEL's data anomaly form (DAF).

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Appendix B. Algae Sampling Data Supplemental Information Form

Access to this form is provided at the following Ecology form publication link:
https://apps.ecology.wa.gov/publications/SummaryPages/ECY070295.html

Appendix C. Laboratory Accreditation Waiver Form

Department of Ecology

Request to Waive Required Use of Accredited Lab

Use form ECY 070-152a for a waiver for Microbial Source Tracking (MST)

Reference: Executive Policy 22-02

Date:	3/7/2025	**Program:**	WQP – Freshwater Algae Control Program
Name of Requester:	Eric Thompson	**Work Phone:**	206-477-7165

Project Name: Freshwater Algae Control Program Interagency Agreement No. C2500006

Laboratory name: King County Environmental Lab

Activity Tracker number: N/A

Test(s) for which a waiver is requested:
(Identify analyte/matrix/method; for example, Dieldrin by HR-GC/MS)

Analysis Type	Matrix	Analyte	Analysis Method
Plankton Analysis	Freshwater	Phytoplankton	identification and ranking of phytoplankton taxa

Justification for Request:
(Describe some of the possible reasons for requesting to waive the requirement to use an accredited lab are listed. Click to check as many boxes as apply and explain in the narrative.)

☐ Data quality goal does not warrant accreditation [1]
☒ There is no lab accredited to do the test(s) [2]
☒ EAP Lab Accreditation Section cannot accredit for test(s) [3]
☐ There is no accredited lab in a reasonable distance [4]
☐ No lab can be accredited in time to meet the need [4]

[1] Screening data, for example may not necessitate use of an accredited lab.
[2] Check the Lab Accreditation website or Contact the EAP Lab Accreditation Unit, or both.
[3] Contact the EAP Lab Accreditation Unit
[4] Check the Lab Accreditation website or Contact the EAP Lab Accreditation Unit, or both.

☒ Other (explain in narrative)

Narrative (Explain the above checked boxes)**:**

The Department of Ecology's Freshwater Algae Control Program includes a contract for analytical laboratory support of the state's cyanotoxin testing program. This includes analysis for phytoplankton in freshwater samples. There is no lab accredited for phytoplankton analysis at this time. King County Environmental Lab is a well-established Ecology-accredited laboratory and has many years of experience conducting phytoplankton analysis for this program.

Associated Quality Control Tests:

Exemption from the requirement to use an accredited lab does not relieve the data user from the requirement to assure lab data meets quality objectives. Reviewing quality control (QC) test results, routinely performed as part of the lab accreditation process, becomes an especially important issue for the user of data coming from a non-accredited lab. Click all boxes to indicate the QC tests the lab will be required to report along with the environmental data. Refer to this description of QC tests, if needed, to determine the significance of each test.

☐ Check standards	☒ Duplicates (laboratory)
☐ Method Blanks	☐ Surrogates
☐ Matrix Spikes	☐ Laboratory Splits
☐ Matrix Spike Duplicates	☐ Standard/Certified Reference Materials

*Note: there are no QC tests associated with phytoplankton analysis.

When completed, save the request and submit it to the Agency's QA Officer by e-mail.

Approval Signatures:

_____

Ecology Quality Assurance Officer Date

_____

Environmental Assessment Program Manager or Ecology Deputy Director Date

To request materials in a format for the visually impaired, visit https://ecology.wa.gov/accessibility, call at Ecology at 360-407-6764, Relay Service 711, or TTY 877-833-6341.

Appendix D. Glossaries, Acronyms, and Abbreviations

Glossary of General Terms

Ambient: Background or away from point sources of contamination. Surrounding environmental condition.

Designated uses: Those uses specified in Chapter 173-201A WAC (Water Quality Standards for Surface Waters of the State of Washington) for each water body or segment, regardless of whether or not the uses are currently attained.

Dissolved oxygen (DO): A measure of the amount of oxygen dissolved in water.

Eutrophic: Nutrient rich and high in productivity resulting from human activities such as fertilizer runoff and leaky septic systems.

Nutrient: Substance such as carbon, nitrogen, and phosphorus used by organisms to live and grow. Too many nutrients in the water can promote algal blooms and rob the water of oxygen vital to aquatic organisms.

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

Pollution: Contamination or other alteration of the physical, chemical, or biological properties of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will, or are likely to, create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

Primary contact recreation: Activities where a person would have direct contact with water to the point of complete submergence including, but not limited to, skin diving, swimming, and water skiing.

Sediment: Soil and organic matter that is covered with water (for example, river or lake bottom).

Surface waters of the state: Lakes, rivers, ponds, streams, inland waters, salt waters, wetlands and all other surface waters and water courses within the jurisdiction of Washington State.

Turbidity: A measure of water clarity. High levels of turbidity can have a negative impact on aquatic life.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

Acronyms and Abbreviations

cyanoHAB	cyanobacterial Harmful Algal Bloom
DAF	Data Anomaly Form
DNRP/Science	King County Department of Natural Resources and Parks / Science Section
DO	(see Glossary above)
DOH	Washington State Department of Health
DQO	Data Quality Objective
e.g.	For example
EAP	Environmental Assessment Program
Ecology	Washington State Department of Ecology
EDS	Environmental Data System
EIM	Environmental Information Management database
et al.	And others
FACP	Freshwater Algae Control Program
GIS	Geographic Information System software
GPS	Global Positioning System
HAB	Harmful Algal Bloom
i.e.	In other words
IAA	Interagency Agreement
ITRC	Interstate Technology and Regulatory Council
KCEL	King County Environmental Lab
LHJ	Local Health Jurisdiction
LIMS	Laboratory Information Management System
LLOQ	Lower Limit of Quantitation
LSSG	King County Lakes, Streams, Sound and Ground unit
MC-LR	Microcystin-LR variant
MDL	Maximum Detection Limit
MEL	Manchester Environmental Laboratory
ML	Minimum Level
MQO	Measurement Quality Objective
MS/MSD	Matrix Spike and Matrix Spike Duplicate
NF	Not Found
P	Present
PDF	Portable Document Format
PPE	Personal Protective Equipment

PQL	Practical Quantitation Limit
QA	Quality Assurance
QC	Quality Control
QL	Quantitation Limit
RCW	Revised Code of Washington
RDL	Reporting Detection Limit
RO	Reverse Osmosis
ROS	Regression on-order statistics
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SOP	Standard Operating Procedures
SPE	Solid Phase Extraction
SWAMP	State of California Surface Water Ambient Monitoring Program
USEPA	United State Environmental Protection Agency
USGS	United States Geological Survey
WAC	Washington Administrative Code
WDFW	Washington Department of Fish and Wildlife
WQA	Water Quality Assessment
WQP	Water Quality Program
WRIA	Water Resource Inventory Area

Units of Measurement

°C	degrees centigrade
G	gram, a unit of mass
Kg	kilograms, a unit of mass equal to 1,000 grams
kg/d	kilograms per day
km	kilometer, a unit of length equal to 1,000 meters
m	meter
mm	millimeter
mg	milligram
mg/d	milligrams per day
mg/kg	milligrams per kilogram (parts per million)
mg/L	milligrams per liter (parts per million)
mL	milliliter
ng/g	nanograms per gram (parts per billion)
ng/kg	nanograms per kilogram (parts per trillion)
ng/L	nanograms per liter (parts per trillion)
s.u.	standard units
µg/g	micrograms per gram (parts per million)

µg/kg	micrograms per kilogram (parts per billion)
µg/L	micrograms per liter (parts per billion)
µm	micrometer

Quality Assurance Glossary

Accreditation: A certification process for laboratories, designed to evaluate and document a lab's ability to perform analytical methods and produce acceptable data. For Ecology, it is "Formal recognition by (Ecology)…that an environmental laboratory is capable of producing accurate analytical data." [WAC 173-50-040] (Kammin, 2010)

Accuracy: The degree to which a measured value agrees with the true value of the measured property. USEPA recommends that this term not be used, and that the terms *precision* and *bias* be used to convey the information associated with the term *accuracy* (USGS, 1998).

Analyte: An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined. The definition can be expanded to include organisms, e.g., fecal coliform, Klebsiella (Kammin, 2010).

Bias: The difference between the sample mean and the true value. Bias usually describes a systematic difference reproducible over time and is characteristic of both the measurement system and the analyte(s) being measured. Bias is a commonly used data quality indicator (DQI) (Kammin, 2010; Ecology, 2004).

Blank: A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or inadvertent introduction of analyte during various stages of the sampling and analytical process (USGS, 1998).

Calibration: The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured (Ecology, 2004).

Check standard: A substance or reference material obtained from a source independent from the source of the calibration standard; used to assess bias for an analytical method. This is an obsolete term, and its use is highly discouraged. See Calibration Verification Standards, Lab Control Samples (LCS), Certified Reference Materials (CRM), and/or spiked blanks. These are all check standards but should be referred to by their actual designator, e.g., CRM, LCS (Kammin, 2010; Ecology, 2004).

Comparability: The degree to which different methods, data sets and/or decisions agree or can be represented as similar; a data quality indicator (USEPA, 1997).

Completeness: The amount of valid data obtained from a project compared to the planned amount. Usually expressed as a percentage. A data quality indicator (USEPA, 1997).

Continuing Calibration Verification Standard (CCV): A quality control (QC) sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run (Kammin, 2010).

Control chart: A graphical representation of quality control results demonstrating the performance of an aspect of a measurement system (Kammin, 2010; Ecology 2004).

Control limits: Statistical warning and action limits calculated based on control charts. Warning limits are generally set at +/- 2 standard deviations from the mean, action limits at +/- 3 standard deviations from the mean (Kammin, 2010).

Data integrity: A qualitative DQI that evaluates the extent to which a data set contains data that is misrepresented, falsified, or deliberately misleading (Kammin, 2010).

Data quality indicators (DQI): Commonly used measures of acceptability for environmental data. The principal DQIs are precision, bias, representativeness, comparability, completeness, sensitivity, and integrity (USEPA, 2006).

Data quality objectives (DQO): Qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions (USEPA, 2006).

Data set: A grouping of samples organized by date, time, analyte, etc. (Kammin, 2010).

Data validation: An analyte-specific and sample-specific process that extends the evaluation of data beyond data verification to determine the usability of a specific data set. It involves a detailed examination of the data package, using both professional judgment and objective criteria, to determine whether the MQOs for precision, bias, and sensitivity have been met. It may also include an assessment of completeness, representativeness, comparability, and integrity, as these criteria relate to the usability of the data set. Ecology considers four key criteria to determine if data validation has actually occurred. These are:

- Use of raw or instrument data for evaluation.
- Use of third-party assessors.
- Data set is complex.
- Use of EPA Functional Guidelines or equivalent for review.

Examples of data types commonly validated would be:

- Gas Chromatography (GC).
- Gas Chromatography-Mass Spectrometry (GC-MS).
- Inductively Coupled Plasma (ICP).

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:

- No qualifier – data are usable for intended purposes.
- J (or a J variant) – data are estimated, may be usable, may be biased high or low.
- REJ – data are rejected, cannot be used for intended purposes.
  (Kammin, 2010; Ecology, 2004).

Data verification: Examination of a data set for errors or omissions, and assessment of the Data Quality Indicators related to that data set for compliance with acceptance criteria (MQOs). Verification is a detailed quality review of a data set (Ecology, 2004).

Detection limit (limit of detection)**:** The concentration or amount of an analyte which can be determined to a specified level of certainty to be greater than zero (Ecology, 2004).

Duplicate samples: Two samples taken from and representative of the same population, and carried through and steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis (USEPA, 1997).

Field blank: A blank used to obtain information on contamination introduced during sample collection, storage, and transport (Ecology, 2004).

Initial Calibration Verification Standard (ICV): A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples (Kammin, 2010).

Laboratory Control Sample (LCS): A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and analytical methods employed for regular samples (USEPA, 1997).

Matrix spike: A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias due to interference or matrix effects (Ecology, 2004).

Measurement Quality Objectives (MQOs)**:** Performance or acceptance criteria for individual data quality indicators, usually including precision, bias, sensitivity, completeness, comparability, and representativeness (USEPA, 2006).

Measurement result: A value obtained by performing the procedure described in a method (Ecology, 2004).

Method: A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed (EPA, 1997).

Method blank: A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank will contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples (Ecology, 2004; Kammin, 2010).

Method Detection Limit (MDL): This definition for detection was first formally advanced in 40CFR 136, October 26, 1984 edition. MDL is defined there as the minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99% probability of being identified, and reported to be greater than zero (Federal Register 2025).

Based on EPA's revision 2 (USEPA, 2016), the revised MDL definition is "The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results."

Percent Relative Standard Deviation (%RSD): A statistic used to evaluate precision in environmental analysis. It is determined in the following manner:

$$\%RSD = (100 * s)/x$$

where s is the sample standard deviation and x is the mean of results from more than two replicate samples (Kammin, 2010).

Parameter: A specified characteristic of a population or sample. Also, an analyte or grouping of analytes. Benzene and nitrate + nitrite are all parameters (Kammin, 2010; Ecology, 2004).

Population: The hypothetical set of all possible observations of the type being investigated (Ecology, 2004).

Precision: The extent of random variability among replicate measurements of the same property; a data quality indicator (USGS, 1998).

Quality assurance (QA): A set of activities designed to establish and document the reliability and usability of measurement data (Kammin, 2010).

Quality Assurance Project Plan (QAPP): A document that describes the objectives of a project, and the processes and activities necessary to develop data that will support those objectives (Kammin, 2010; Ecology, 2004).

Quality control (QC): The routine application of measurement and statistical procedures to assess the accuracy of measurement data (Ecology, 2004).

Relative Percent Difference (RPD): RPD is commonly used to evaluate precision. The following formula is used:

[Abs(a-b)/((a + b)/2)] * 100

where "Abs()" is absolute value and a and b are results for the two replicate samples. RPD can be used only with 2 values. Percent Relative Standard Deviation is (%RSD) is used if there are results for more than 2 replicate samples (Ecology, 2004).

Replicate samples: Two or more samples taken from the environment at the same time and place, using the same protocols. Replicates are used to estimate the random variability of the material sampled (USGS, 1998).

Representativeness: The degree to which a sample reflects the population from which it is taken; a data quality indicator (USGS, 1998).

Sample (field): A portion of a population (environmental entity) that is measured and assumed to represent the entire population (USGS, 1998).

Sample (statistical): A finite part or subset of a statistical population (USEPA, 1997).

Sensitivity: In general, denotes the rate at which the analytical response (e.g., absorbance, volume, meter reading) varies with the concentration of the parameter being determined. In a specialized sense, it has the same meaning as the detection limit (Ecology, 2004).

Spiked blank: A specified amount of reagent blank fortified with a known mass of the target analyte(s); usually used to assess the recovery efficiency of the method (USEPA, 1997).

Spiked sample: A sample prepared by adding a known mass of target analyte(s) to a specified amount of matrix sample for which an independent estimate of target analyte(s) concentration is available. Spiked samples can be used to determine the effect of the matrix on a method's recovery efficiency (USEPA, 1997).

Split sample: A discrete sample subdivided into portions, usually duplicates (Kammin, 2010).

Standard Operating Procedure (SOP): A document which describes in detail a reproducible and repeatable organized activity (Kammin, 2010).

Surrogate: For environmental chemistry, a surrogate is a substance with properties similar to those of the target analyte(s). Surrogates are unlikely to be native to environmental samples. They are added to environmental samples for quality control purposes, to track extraction efficiency and/or measure analyte recovery. Deuterated organic compounds are examples of surrogates commonly used in organic compound analysis (Kammin, 2010).

Systematic planning: A stepwise process which develops a clear description of the goals and objectives of a project, and produces decisions on the type, quantity, and quality of data that

will be needed to meet those goals and objectives. The DQO process is a specialized type of systematic planning (USEPA, 2006).

References for QA Glossary

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