A1 Title Page

Quality Assurance Project Plan

for

Lake Monitoring Network

Statistical, Reference and Regional Targeted Lakes

NJDEP QAPP Number:
FY25-31

Prepared by:
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Prepared for:
New Jersey Department of Environmental Protection (NJDEP)
Division of Water Monitoring, Standards and Pesticide Control (WMSPC)
Bureau of Freshwater & Biological Monitoring (BFBM)
Final
May 2025

Funding Source:
State Funding, Corporate Business Tax

Period of Applicability:
QAPP Approval Date – May 2025 through December 2029

A2 Approval Page

My signature below indicates my approval of the plan and my commitment to follow the procedures noted herein. I understand that changes to this plan shall not be made without approval/signature by all the signatories below.

NJDEP Project Manager	Johannus Franken Johannus Franken, Environmental Specialist 3 BFBM	05/21/25 Date
Program QA Specialist	Chris Kunz Chris Kunz, Bureau Chief BFBM	5/21/2025 Date
NJDEP QA Manager	Megan Rutkowski Megan Rutkowski, Research Scientist	5/22/2025 Date
Project Data Manager 1	Brian Taylor Brian Taylor, Section Chief BFBM	5/22/2025 Date
Project Data Manager 2	Carly Conticchio Carly Conticchio, Environmental Specialist 1 BFBM	05/22/25 Date

QAPP Approval Date*: 5/22/2025

*Any environmental information operations conducted prior to the OQA approval date may not be in compliance with the final approved version of the QAPP.

A3 Table of Contents, Document Format, and Document Control

E2	Appendices A: *List of Statewide Statistical Survey Lakes* B: *List of Potential Oversample Lakes* C: *List of Reference Lakes* D: *List of Regional Targeted Lakes* E: *Lake Sampling Parameters* F: *Laboratory Analysis* G: *Lake Field Data Sheet* H: *NJDOH CHEM 44 Organic and Inorganic Chemistry Sample Submittal* I: *Submerged Water Sampler Cleaning Method* J: *HAB Toxin Analysis Kit Instructions* K: *Laboratory Analysis Labels* L: *Standard Operating Procedures Partner (Fluorosense) Phycocyanin Meter Loan Program, 2024* M: *HAB Sample Lab Processing Procedure* N: Data Management Tables

A4 Project Purpose, Problem Definition, and Background

Title of Document	Date of Document	Pertinence to this QAPP
NJDEP's Quality Management Plan (QMP)	7/1/2020 – 6/30/2025	This QAPP was developed in accordance with the NJDEP's QMP.
NJDEP Field Sampling Procedures Manual (FSPM)	2024 Edition	This QAPP requires use of specific sampling equipment, collection and field data instruments, documentation and personnel protection.
NJDEP Surface Water Quality Standards, NJAC 7:9B (SWQS)	2023	This QAPP references the applicable Surface Water Quality Criteria for Project Action Levels.
2014 Integrated Water Quality Monitoring and Assessment Methods Document	2014	This QAPP adheres to the sampling frequency requirements outlined in this document.
NJDEP Lake Monitoring Network QAPPs	2005-2024	These QAPPs covered water quality monitoring of lakes statewide for each year listed.

Title of Document	Date of Document	Pertinence to this QAPP
NJDEP Aquatic Macrophyte Survey Program FY24-33	2024-2028	This QAPP requires use of the program sampling parameters.
NJDEP BFBM Lake Survey Design	2015	This QAPP requires use of the statistical lake survey design, commonly referred to as the statistical lake survey.

Background, Project Purpose and Problem Definition

Project Background

The NJDEP Lake Monitoring Network was designed to provide ambient water quality data necessary to assess the ecological health of the State's lentic water resources and the needs of the watershed management and water quality assessment {305(b)/303(d)} programs. This approach comports with the guidance provided in USEPA's publication, "Elements of a State Water Monitoring and Assessment Program," March 2003, which requires that states develop and implement long-term strategies that include monitoring of all state water body types, including lakes, and the needs of the watershed management and water quality assessment {305(b)/303(d)} programs.

The Network was established in 2005 to provide water quality data necessary to assess the ecological health of the State's lentic water resource. The network began as a 5-year probabilistic (AKA statistical) survey designed by the U.S. EPA. Once all 5 years were established, the survey was repeated from 2010-2014. In 2015, the statistical survey was redesigned by BFBM to include a larger universe of lakes throughout the State. In addition, Reference and Regional Targeted networks were established for baseline and regional assessments.

Statewide Statistical Survey

The Statewide Statistical Survey will evaluate the trophic state of selected lakes and assess the ecological health of the State's lentic water resources. The data will be used to develop baseline, statewide status, and eventually trend information for New Jersey lakes.

Reference Lake Network

The Reference Lake Network will monitor lakes in 4 of 5 Omernik Level III ecoregions (Northern Piedmont not included) within the State. The data will document baseline information on minimally or non-impacted water quality, ecological integrity, and the trophic state of lakes within each ecoregion. Reference lakes will be monitored every year for the duration of the project and can be used to measure variations and trends resulting from climate change and land use. Data may also be compared to disturbed lakes within the same ecoregion to gauge the degree of

impairment. Reference data will also serve to inform criteria development, specifically nutrients, to determine criteria that are attainable and appropriate for the ecoregion, and adequate to control nutrient enrichment.

Targeted Regional Network

The Targeted Regional Network will monitor public lakes throughout the State. The Department has adopted a Regional Comprehensive Assessment Method for the Integrated Water Quality Monitoring and Assessment Report intended to produce a robust assessment of environmental conditions affecting water quality in a selected water region. This Regional Comprehensive Assessment will incorporate one of five water regions (Atlantic Coastal, Lower Delaware, Northwest, Raritan, and Northeast) during each Integrated Report cycle. The rotating region approach will result in a comprehensive assessment of the entire state every 10 years. This approach will encourage development of measures to restore, maintain and enhance water quality uses that maximize effectiveness and efficiency in achieving positive environmental outcomes that are tailored to the unique circumstances of each region. Lakes monitored for the Regional Comprehensive Assessment will adhere to the sampling frequency requirements as outlined in the 2014 Integrated Water Quality Monitoring and Assessment Methods Document. Targeted Regional lakes are selected for the Integrated Report cycle in each Water Region by BFBM and Bureau of Environmental Analysis, Restoration & Standards (BEARS).

Water quality data gathered during the project may be used for environmental decision making by BEARS during the enhancements to the NJDEP Surface Water Quality Standards (SWQS). This is outside the scope of this QAPP.

A5 Project Task Description

Table 1: Task / Deliverable Schedule			
Task/Deliverable	Responsible Individual	Anticipated Start Date	Anticipated End Date
Lake Monitoring Network QAPP Development Covers 5 years of the Statistical, Regional and Reference Lake Networks.	Johannus Franken	01/2025*	03/2025*
Statistical Lake water quality monitoring	Johannus Franken	03/2025*	11/2025*
Regional lake water quality monitoring	Johannus Franken	03/2025*	11/2025*
Reference lake water quality monitoring	Johannus Franken	07/2025*	08/2025*
Data QA/QC checks *Consists of water chemistry readings from handheld multiparameter water quality meter and laboratory analysis from NJDOH ECLS and BFBM. Data is formatted for submittal to the Water Quality Exchange and NJDEP Lake Mapper.*	Brian Taylor Carly Conticchio	12/2025*	02/2026*
Geospatial data *Consists of lakes (to be) monitored, in-lake monitoring stations, sediment coring locations and bathymetry and vegetation mapping.*	Johannus Franken Carly Conticchio Leigh Lager	12/2025*	02/2026*
Annual review of the QAPP *Review previous year's monitoring, data, issues. Review Reference lake suitability for objectives. Review/select Statistical Survey panels and oversample lake list. Review/select Targeted Regional lake list. Assess needs and amend QAPP, as necessary.*	Johannus Franken Chris Kunz Brian Taylor Carly Conticchio	12/2025*	02/2026*

**Recurring each year, ending 12/31*

A6 Information/Data Quality Objectives and Performance/Acceptance Criteria

Precision

Precision is the measure of agreement among repeated measurements of the same property under the same conditions. Precision will be assessed through the measurement of duplicate samples and through the calculation of relative percent difference (RPD) as follows:

$$RPD = \frac{|R1 - R2|}{\left(\frac{R1 + R2}{2}\right)} \times 100,$$

where

$R1$ is sample 1, and

$R2$ is sample 2.

For water quality parameter analysis, precision will be verified, and acceptance criteria applied, for each method as described in the certified environmental laboratory standard operating procedures (SOPs). An acceptance criteria limit of agreement within 20% relative percent difference is typical; however, SOPs shall be consulted for specifics. If agreement between analysis results is not attained, duplicate samples will need to be collected again.

The Lake Monitoring Network will collect approximately 250 water quality samples per year from multiple lake sampling stations. There are 4 visits to each of the statistical and targeted lakes. 5 lake sampling stations will be sampled in duplicate for lab analysis on an annual basis. The duplicate samples will be taken at one sampling station in one lake to represent the entire 1st, 2nd, 3rd and 4th visits to the Statistical/Targeted Regional Lake Networks and one sampling station in one lake for the single visit to Reference Lakes.

From FSPM:
The collection of duplicate samples provides for the evaluation of the laboratory's and field sampling team's performance by comparing analytical results of two samples from the same location. Duplicate samples, when required by the analytical method, are to be included for each matrix at a maximum rate of one for every twenty samples. If less than twenty samples are collected during a sampling episode, one duplicate should be performed. Duplicate requirements may be waived or expanded depending on the regulatory program or remedial phase. Keep in mind that various USEPA methods require a higher frequency of field duplicate samples. Therefore, the analytical methods should be reviewed to determine the appropriate number of field duplicates.

All analysis parameters will follow the receiving laboratory's OQA certification requirements.

All analyze immediately parameters will follow BFBM's OQA laboratory certification requirements.

Accuracy (Bias)

Accuracy is the measure of the overall agreement of a measurement to a known value. For water quality parameter analysis and laboratory analysis, accuracy will be verified for each method as described in the certified environmental laboratory SOPs.

All analysis parameters will follow the receiving laboratory's OQA certification requirements.

All analyze immediately parameters will follow BFBM's OQA laboratory certification requirements.

Representativeness

Statistical Survey Lakes

Beginning in 2023, 50 lakes, referred to as a Panel, will be sampled over 8 years. An alternating list of 12 and 13 lakes per year will be implemented to reach this goal. Each Panel of lakes will be monitored for 2 consecutive years (4 sampling events each year, 8 total) to provide sufficient data for inclusion in the Integrated Report. A total of 250 lakes were selected for sampling over a 40-year period. Individual Panels of 50 lakes will have sufficient statistical confidence to assess statewide status every 8 years. It is anticipated that statewide trends will be assessed after a statistically significant number of panels are completed.

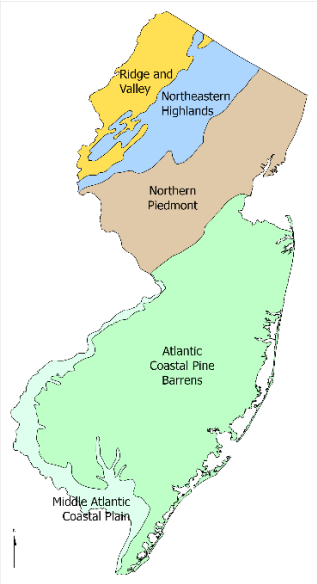
	Begin Year	End Year	Comment
Panel 7	2015	2016	Sampling complete
NLA	2017		Participated in 2017 National Lakes Assessment (NLA) in lieu of Statewide Survey.
Panel 8	2018	2022	Sampling complete. Lakes were partially sampled in 2018/2019 due to HAB intensive studies, not sampled in 2020 due to COVID-19 Pandemic and resumed modified routine schedule in 2021.
Panel 9	2023	2032	Sampling in progress
Panel 10	2033	2042	To be sampled
Panel 11	2043	2052	To be sampled

Statistical lakes were selected using USEPA probabilistic (AKA statistical) site selection methodology {Generalized Random Tessellation Stratified (GRTS) survey design for a point resource with reverse hierarchical ordering (RHO), see Lake Survey Design}. Potential sites included all lakes (public and private) on DEP Geographic Information System (GIS) Coverage "NJ National Hydrography Dataset," greater than or equal to 5 acres, minimum one meter deep, including potable water supply reservoirs and unnamed lakes. This list of lakes is commonly referred to as the "universe" of NJ lakes.

All potential lakes will be visited by BFBM staff to ascertain that the waterbodies are suitable for sampling per the design criteria (see Appendix A, List of Statewide Statistical Survey Lakes). Actual sampling sites in each lake will be determined during the first on-water site visit and will be selected based on actual morphometry, as determined by this on-site visit. At that time, each in-lake station will be recorded with a handheld Global Navigation Satellite System (GNSS, formerly known as Global Positioning System or GPS) and stored on the DEP GIS system. This will allow printing of aerial photography maps showing actual sample sites. If a lake does not meet design criteria such as inadequate depth, or if access or safety issues exist, the lake will be eliminated. A replacement will be selected, in order, from the list of potential oversample lakes (see Appendix B, List of Potential Oversample Lakes). Digital photographs will also be taken showing an overview of the lake, outlet, and drainage pipes.

Reference Lakes

Reference lakes will be monitored to document baseline, status, and trend information on minimally or non-impacted water quality, ecological integrity, and the trophic state of lakes within each ecoregion. Seven (7) statewide reference lakes representing 4 of 5 Omernik Level III ecoregions (Northern Piedmont not represented) will be sampled once annually in the growing season to demonstrate the status established in previous samplings at these lakes. Sites will be sampled once each year, during the growing season, during the last two weeks of July and first two weeks of August.



These lakes are intended to be minimally disturbed by human activity and preferably in an area protected from human-induced changes. The lakes were selected using the following criteria:

Manmade or natural lakes:

- ≥5 acres in surface area
- 1-meter depth minimum
- Surrounding land use <20% Urban + Agricultural
- < 2% impervious cover
- No discharges into lake
- No regulated discharges upstream of lake inlet

Shoreline Characteristics (if available):

- Trees/Shrub > 75%
- Lawns/grasses < 25%
- Bare ground < 5%
- Shoreline anthropogenic disturbance (shoreline modifications/development) < 5%

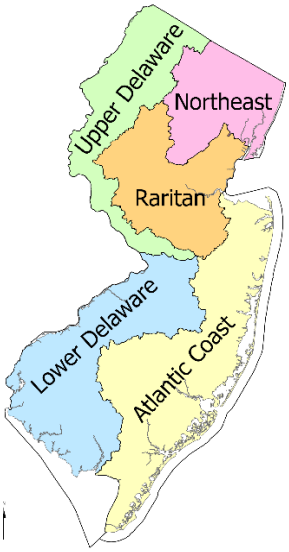
Most of the lake shorelines and watersheds are protected by NJDEP State Parks and Forests-, Fish and Wildlife-, and/or conservation non-profit-managed lands so they have low potential for future disturbance. See Appendix C, List of Reference Lakes.

Multiple sampling events were performed annually at all reference lakes from 2015 through 2019. Mount Misery Lake was sampled in 2006 and 2011. Deer Park Pond was also sampled in 2005 and 2010 but eliminated as a reference lake in 2023 due to multiple access issues and possible dam removal.

In addition, Hands Millpond, Mashipacong Pond and Mount Misery Lake have continuous monitoring arrays deployed in the water column and along the shoreline for the Lake Regional Monitoring Network (Lake RMN). Water column arrays will have loggers to monitor dissolved oxygen, temperature, and water level while shoreline loggers will record temperature and pressure. This effort is a partnership with United State Environmental Protection Agency (U.S. EPA) and covered under the NJDEP Lake RMN QAPP (approved in April 2021).

Targeted Regional Lakes

Targeted regional lakes will be monitored to produce an assessment of environmental conditions affecting water quality in each of the five NJ water regions. Sites are expected to be sampled for 2 years, 4 times per year, March through November (total of 8 sampling events).



Ten (10) targeted regional lakes will be sampled 8 times over 2 years (4 times annually during each year). Targeted regional lakes are selected from the "universe" of lakes (see NJDEP BFBM Lake Survey Design). The "universe" is filtered for lakes >10 acres located on public and open space parcels and then chosen by BFBM using best professional judgement. Once lakes are selected, BFBM will consult with BEARS to ensure the lakes represent the respective Water Region to align with the current Integrated Report assessment cycle. (See Appendix D, List of Regional Targeted Lakes).

See Appendix E, Lake Sampling Parameters, for a table of the sampling frequency and associated parameters for each lake type.

Comparability

Comparability is defined as the measure of confidence that one data set can be compared to another and/or that ensures data sets can be combined for decision making. As noted above, the Lake Monitoring Network builds off the project from the previous year. The same equipment will be used for sampling, and the same analytical methods will be used for laboratory analysis, so results are expected to be comparable to past data sets.

Completeness

We have established a goal 100% for the parameters being measured and strive to complete the project in its entirety. If the study is not completed in its entirety, the amount of valid data will be reviewed at the conclusion of the project and the project manager and project will decide whether the acceptable data can be used.

Sensitivity

The method detection limits, and reporting levels associated with the analyses performed during this project, were provided by the NJDOH (ID 11036) and NJDEP BFBM (ID 11896) and are included in Appendix F, Laboratory Analysis.

A7 Distribution List

Table 3: Distribution List

Name	Organization	Title	E-mail Address
Johannus Franken	NJDEP BFBM	Project Manager	Johannus.franken@dep.nj.gov
Chris Kunz	NJDEP BFBM	Project QA Manager	Chris.kunz@dep.nj.gov
Megan Rutkowski	NJDEP – Office of Quality Assurance	NJDEP QA Manager	Megan.Rutkowski@dep.nj.gov
Brian Taylor	NJDEP BFBM	Project Data Manager 1	Brian.k.taylor@dep.nj.gov
Carly Conticchio	NJDEP BFBM	Project Data Manager 2	Carly.conticchio@dep.nj.gov

John Abatemarco	NJDEP BFBM	Field personnel	John.abatemarco@dep.nj.gov
Kelly Krolic	NJDEP BFBM	Field personnel	Kelly.krolik@dep.nj.gov
Frank Klapinski	NJDEP BEARS	Environmental Scientist	Frank.klapinski@dep.nj.gov
Doug Haltmeier	NJDOH	Lab Manager	Douglas.haltmeier@doh.nj.gov

A8 Project Organization

Roles and Responsibilities of Key Project Personnel

Name	Organization	Project Role	Project Duties
Johannus Franken	NJDEP BFBM	Project Manager	QAPP maintenance and distribution, field activity management including QA/QC, laboratory coordination, data manager coordination, GPS/GIS coordination
Chris Kunz	NJDEP BFBM	Project QAM*	Planning, documenting, coordinating, and assessing effectiveness of the QAPP
Megan Rutkowski	NJDEP – Office of Quality Assurance	NJDEP QAM**	QAPP review and approval
Brian Taylor	NJDEP BFBM	Project Data Managers 1	Database management, data entry, Water Quality Exchange submissions
Carly Conticchio	NJDEP BFBM	Project Data Manager 2	Data management, data entry, Water Quality Exchange submissions, and perform field work on all aspects of project
John Abatemarco	NJDEP BFBM	Field personnel	Perform field work on all aspects of project
Kelly Krolik	NJDEP BFBM	Field personnel	Perform field work on all aspects of project
Frank Klapinski	NJDEP BEARS	Data user	Assessment of water quality data for Integrated Reports

* *The project QAM has the authority to access and discuss quality-related issues with senior management outside of their direct supervisory chain as necessary.*

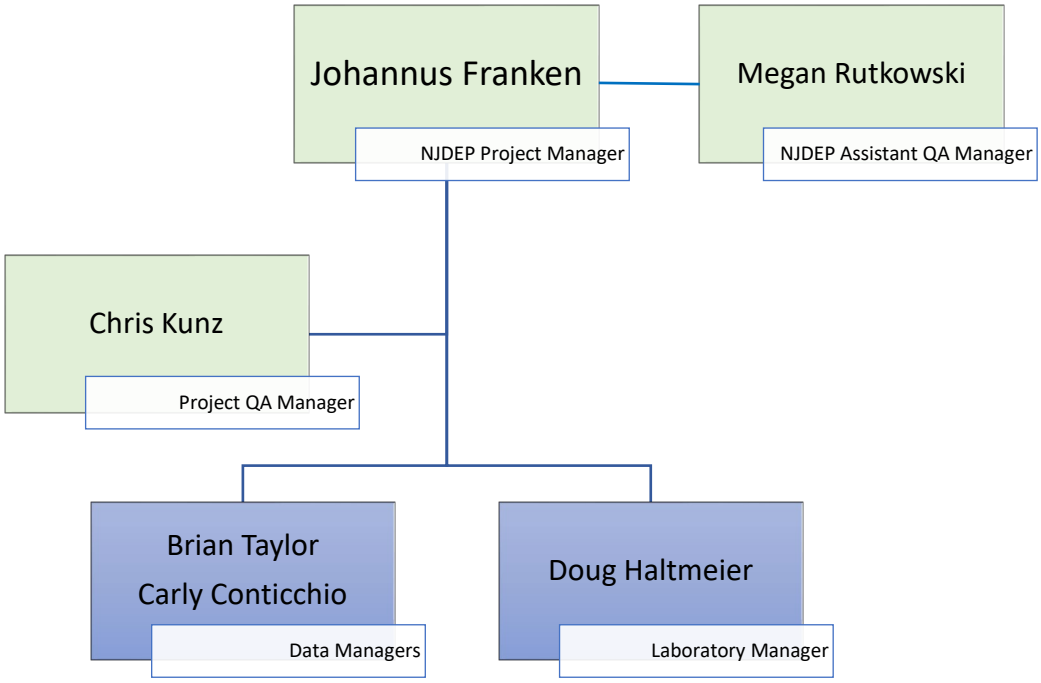
** *The assistant QAM from the NJDEP OQA has been delegated QAPP signature authority from the EPA as described in the Department's Quality Management Plan (QMP).*

A9 Project QAM Independence

The Project QAM is a separate individual from the Project Manager and their role in this project relates to planning, documenting, coordinating and assessing the effectiveness of this QAPP. The Project QAM will not perform data collection activities during this project.

A10 Project Organizational Chart and Communications

Figure 1: Organizational Chart



A11 Personnel Training / Certification

Training of new or additional staff, and/or retraining of experienced staff will be documented in the following ways depending on the type of training necessary. Training of new staff requires that they complete formal and/or informal training before they can conduct sampling or tasks without supervision. Any formal training of staff such as reading training manuals, SOPs, video or online training or training by outside professionals will be documented via email which includes the staff member being trained, the staff member's supervisor, the project manager and the project QA

manager. Informal training, such as on the job training or in the field training, will be documented by the trainer in their field notebook noting the date, time and type of training conducted. The trainer will provide training progress/completion to the staff member's supervisor, project manager and project QA manager via email.

To ensure that training is completed, an initial observation/audit will be conducted by the staff member's supervisor, project manager and/or the project QA manager. The auditor will provide an email to the staff member, the staff member's supervisor, the project manager and the project QA manager stating that training has been completed and that the staff member can conduct sampling without supervision. Routine audits (at least every two years for each staff person) will be conducted by the staff member's supervisor, the project manager or the project QA manager. Any deficiencies, recommended actions or additional training requests will be conveyed via email to the staff member, the staff member's supervisor, the project manager and the project QA manager.

Assistants to the project will be trained by the Project Manager or designee in the operation and use of all sampling equipment including proper boating and trailering safety. A NJ Boating Safety Certificate is preferred although not required. The training will have three components: trailering/boating, lab and field. Training will be on the job and include, but not be limited to, calibration, data collection, sampling techniques, sample processing, data retrieval, documentation, data entry and data processing.

All crew members are required to adhere to the BFBM Field Work Health and Safety Plan developed in cooperation with NJDEP Office of Occupational Health and Safety (OOHS). Crew members must also adhere to any guidance provided by OOHS during the QAPP period. The project manager will be present/available to provide guidance or corrective action during sampling events.

The Project Manager or designee will be responsible for any necessary training and will confirm trainees are qualified prior to their performing environmental information operations without oversight. The Project Manager will document the training in the notes section of the bound field and calibration notebooks.

BFBM is certified by the Office of Quality Assurance (certified lab ID # 11896) for all parameters to be measured in the field. New Jersey Department of Health is certified by the Office of Quality Assurance (certified lab ID # 11036) for all parameters to be measured in the laboratory.

A12 Documents and Records

All physical data collected will be recorded on the NJDEP Lake Monitoring Network field data sheet (Lake Field Sheet, Appendix G), bound field logs and water chemistry will also be electronically recorded on the YSI ProDSS handheld display. Field data required for analysis and result tracking will be recorded on a NJDOH CHEM 44 Organic and Inorganic Chemistry Sample Submittal form (NJDOH Sample Form, Appendix H). An electronic file with results will be sent to our office via shared access to a Microsoft OneDrive folder.

Program QA will be responsible for Document Control.

- When sampling is complete and the Laboratory has received all samples, results will be provided within their contracted turnaround time period. Once received, Program QA will verify all data.

- NJDEP/BFBM will retain records of data for no less than 5 years. They are disposed of in accordance with the Department's record disposition policy.

B1 Identification of Project Environmental Information Operations

This project will employ the environmental information operations identified with an "x" in the table below. These operations will satisfy the project purpose through the implementation of the tasks described in A5. This task implementation will ensure satisfaction of the data quality objectives and performance, and acceptance criteria described in sections A4 and A6.

Environmental Information	x	direct measurements of environmental parameters or processes.
	x	analytical testing results of environmental conditions (e.g., geophysical or hydrological conditions).
	x	information on physical parameters or processes collected using environmental technologies.
	x	calculations or analyses of environmental information.
		information provided by models.
		information compiled or obtained from databases, software applications, decision support tools, websites, existing literature, and other sources.
		development of environmental software, tools, models, methods, applications;
Environmental Technology		systems, devices and their components applicable to both hardware and methods or techniques that measure and/or remove pollutants or contaminants and/or prevent them from entering the environment
		pollution prevention: measurement, monitoring, reduction, control, and/or treatment processes, such as wet scrubbers (air), granulated activated carbon unit (water), filtration (air, water).
		Contamination: containment to prevent further movement of the contaminants, such as capping, and solidification or vitrification, and biological treatment.
		Storage containers, methods, or facilities, such as drums, tanks, and ponds or lagoons.
		Design, construction, and operation or application of environmental technology.
		Remediation processes and their components, and/or technologies, such as soil washing (soil), pump and treatment, soil vapor extraction (soil), land farming and other bioremediation processes.
Other		

B2 Methods for Environmental Information Acquisition

Field Activities

Sampling Station Selection

Water quality monitoring will take place, via boat, at the number of monitoring stations that best represent the limnological aspects of the lake. If the lake is relatively small and uniform in shape, then one or two stations will be centrally positioned and equidistant from the shoreline (if depth allows). If the lake is expected to exhibit relatively uniform water quality characteristics, then one sample station will be located

approximately in the center of the lake. Additional monitoring stations, as needed, will be positioned in sections of the lake which may be expected to exhibit differing water quality. Factors include, but are not limited to, morphology, incoming streams, dams/outlets and any site-dependent factors deemed valid by best professional judgement upon first site visit.

Monitoring station geolocations will be collected using ArcGIS Field Maps while paired to a Juniper Geode Global Navigation Satellite System (GNSS) receiver. Field Maps allows for real-time data capture, editing, display and navigation. Station numbers will be assigned as sampling is performed and begin at 1 and increase to the number needed to sufficiently monitor the lake.

Lakes that have been monitored previously have established stations and will be displayed in Field Maps. Existing stations will be re-used for the current year. Any added stations will follow the existing alphanumeric naming convention and use the proceeding number. (e.g. NJLM-0000-1 or NJW04459-000-1).

In the event a lake must be eliminated due to insufficient depth, water quality monitoring stations at the replacement lake will be selected in the same manner. Replacement lakes are chosen from the Oversample Lakes as described in A6 Information/Data Quality Objectives and Performance/Acceptance Criteria/Representativeness.

Field Analysis

Analyze-immediately measurements will be taken by BFBM staff, concurrent with faunal sampling, in accordance with N.J.A.C. 7:18 *Regulations Governing the Certification of Laboratories and Environmental Measures (NJDEP, 2018).* These parameters will be measured in situ. BFBM (certified lab ID # 11896) is certified by the OQA for the parameters tabulated below. All calibrations, quality control checks, and record keeping will be performed in accordance with the BFBM laboratories standard operating procedures which are available upon request.

BFBM Certified Parameter	Method	SOP #/Revision Date
Dissolved oxygen	Hach 10360 – 10/2011 Rev 1.2	AM008.0713a -- Rev. 2 – November 2022
pH	SM 4500-H B-11	AM005.0315 -- Rev. 2 - August, 2017
Specific conductance	SM 2510 B-11	AM008.1293 – Rev. 2 – August 2017
Water temperature	SM 2550 B-10	AM007.1293 -- Rev. 2 - August 2017
Turbidity	SM 2130 B-11	AM009.0614 -- Rev. 2 – August 2017

Prior to field sampling, all sample collection equipment will be cleaned using the protocol outlined in Appendix I, Submerged Water Sampler Cleaning Method. If two lakes are sampled in one day, a separate, clean submerged water sampler will be used on the second lake. Sampling crews will carry multiple clean submerged water samplers in their vehicles to avoid cross contamination from previous sample collection.

A total depth reading will be obtained to determine how the top-to-bottom profile will be collected at each in-lake station for optical dissolved oxygen (ODO), pH, chlorophyll a, phycocyanin, specific conductance, and water temperature and depth. Readings for these parameters will be collected as stated below.

All stations require a reading at 0.1 meters below surface and determined sample depth.
Stations with total depths ≤ 1.0 meter require readings at half of the total depth.
Stations with total depths < 3.0 meters require readings at 0.5-meter intervals.
Stations with total depths ≥ 3.0 meters require readings at 1.0-meter intervals to 20 meters.
After 20 meters, readings are required at 2.0-meter intervals.

Stations with total depths > 1.0 meters require a reading at 0.5 meters above the bottom.

Readings and sample depths will not necessarily be recorded at whole numbers due to the high accuracy (±0.004m for 10-meter cables and ±0.04m for 30-meter cables) of the depth sensor used.

Total depth and reading interval/sample depth will be determined using a YSI Pro DSS. The YSI Pro DSS is a multi-parameter water quality system that combines depth, temperature, pH, conductance, ODO, and Total Algae probes into one meter that is submersible to the desired depth in the lake. The YSI Pro DSS Total Algae Sensor measures phycocyanin and chlorophyll a.

Sample collection

Samples will be collected as per NJDEP FSPM 2024. Samples will be collected using a horizontal submerged sampler, which will be cleaned at the BFBM laboratory prior to use at each lake (see previous section) . The submerged sampler will be field rinsed with "water of interest" once prior to collecting a sample at each station and/or depth for the lake. Each individual lake will require one clean submerged sampler.

Total depth, measured in the top-to-bottom profile described in the previous section, will determine sample depth at each station.

Sample will be collected from one (1) meter depth at stations ≥1.5 meters total depth.
Sample will be collected from mid-depth at stations <1.5 meters total depth.

The sample will be analyzed for the parameters listed in Appendix E, Lake Sampling Parameters according to the lake type. Turbidity will be measured from an aliquot of the grab sampled collected at required depth using a Hach 2100Q Turbidity meter.

Secchi Depth: Universal 20cm secchi disc to measure water transparency. All lake monitoring stations require a secchi depth measurement and shall be recorded following guidance below.

Secchi depth measurement <1.0 meter, record to the nearest 0.05 meter.
Secchi depth measurement >1.0 meter, record to the nearest 0.1 meter.

Forel-Ule Color: Forel-Ule Color Comparator. Forel-Ule Color is measured at half secchi depth. The measurement is for general information purposes and not used for the project data objectives.

Ambient Air Temperature: Fisher Brand Traceable Flip-Stick Thermometer is calibrated with a NIST-certified thermometer before the sampling run. Air temperature is measured for general information purposes only and not used for project's data objectives.

Phycocyanin

BFBM will screen for cyanobacteria in the field using a Turner Designs Fluorosense handheld fluorometer and the YSI Pro DSS Total Algae Sensor. This testing is not certified, and as such, any results obtained cannot be used for regulatory purposes. The Fluorosense and Total Algae Sensor will assist with the in-situ detection of phycocyanin, a pigment unique to cyanobacteria, a naturally occurring component of a lake phytoplankton community. Under certain conditions, a cyanobacteria population can rapidly increase in density, forming a bloom which can potentially produce toxins, called cyanotoxins. These cyanobacteria blooms are commonly known as Harmful Algal blooms (HABs). The Fluorosense will be used at the water surface (0.1m) and sample

depth (determined by total depth). A top-to-bottom profile (as stated above) of chlorophyll a and phycocyanin will also be collected using the Total Algae Sensor to determine concentrations of these compounds throughout the water column. If phycocyanin levels are approaching HAB conditions, Fluorosense reading of 12 micrograms per liter (µg/L) or higher, a sample will be collected and analyzed for cell identification, enumeration and cyanotoxins. HAB sampling and analysis will follow guidance in the latest version of the NJ Cyanobacterial Harmful Algal Bloom (HABs) Freshwater Recreational Response Strategy (https://dep.nj.gov/wms/bfbm/cyanohabs/#response-strategy), Standard Operating Procedures Partner Phycocyanin Meter Loan Program, 2024 (Appendix L) and HAB Sample Lab Processing Procedure (Appendix M).

Laboratory Analyses

The following parameters will be analyzed by New Jersey certified laboratories – NJDOH and NJDEP BFBM. Single-use plastic bottles, of the volume indicated, will be used for each parameter listed. Laboratories used shall be certified by DEP's OQA for the requested parameters. The reporting levels, listed in the table below, are **required** for this project.

Parameter	Laboratory	Analytical Method	SOP Revision # / Date	Detection Limit (mg/L)	Reporting Level (mg/L)	Holding Time	Preservative	Volume
Nitrite + Nitrate	DOH cert # 11036	SM 4500-NO3 F-16	10 02/25/25	0.008	0.025	28 days	See Note 1	500ml
Ammonia (Non-distillation)	DOH cert # 11036	SM 4500-NH3 B + H-11	6 01/03/25	0.005	0.010	28 days	See Notes 1 & 2	
Ammonia (Distillation)	DOH cert # 11036	SM 4500-NH3 B + H-11	6 01/03/25	0.023	0.05	28 days	See Notes 1 & 2	
Total Kjeldahl Nitrogen	DOH cert # 11036	EPA 351.2	2 12/12/24	0.085	0.100	28 days	See Note 1	
Total Phosphorus	DOH cert # 11036	EPA 365.1	2 12/18/22	0.007	0.010	28 days	See Note 1	
Dissolved Organic Carbon (DOC)	DOH cert # 11036	SM 5310 C-14	6 03/07/25	0.45	1.00	28 days	See Note 3 and 1	500ml
Color Dissolved Organic Matter (True Color, see Note 5)	DOH cert # 11036	SM 2120 B-11	4 03/10/25	2.5 PCU	2.5 PCU	48 hours	See Note 3 Ice to 4ºC	500ml
Dissolved Silica (see Note 5)	DOH cert # 11036	EPA 200.7	10 03/06/25	0.26	0.5	180 days	See Note 3 and 4	500ml
Alkalinity	DOH cert # 11036	SM 2320 B-11	2 03/06/22	1	1	14 days	Ice to 4ºC	1000ml

Parameter	Laboratory	Analytical Method	SOP Revision # / Date	Detection Limit (mg/L)	Reporting Level (mg/L)	Holding Time	Preservative	Volume
Apparent Color (Qualified True Color method, see Note 5)	DOH cert # 11036	SM 2120 B-11	4 03/10/25	2.5 PCU	2.5 PCU	48 hours	Ice to 4ºC	
Chloride (flow injection)	DOH cert # 11036	SM 4500-Cl E-11	8 02/25/25	1.3	2.5	28 days	Ice to 4ºC	
Total Suspended Solids (see Note 5)	DOH cert # 11036	SM 2540 D-15	3 06/27/22	1	1	7 days	Ice to 4ºC	
Sulfate (flow injection)	DOH cert # 11036	EPA 375.2	8 02/25/25	2.8	10	28 days	Ice to 4ºC	
Ortho Phosphorus (see Note 5)	DOH cert # 11036	EPA 365.1	3 03/07/25	0.004	0.005	48 hours	Ice to 4ºC	
Hardness – total as CaC03	DOH cert # 11036	SM 2340 B-11	10 03/06/25	0.069	3.31	180 days	See Note 4	500ml
Calcium (see Note 5)	DOH cert # 11036	EPA 200.7	10 03/06/25	0.038	0.5	180 days	See Note 4	
Magnesium (see Note 5)	DOH cert # 11036	EPA 200.7	10 03/06/25	0.035	0.5	180 days	See Note 4	
Sodium (see Note 5)	DOH cert # 11036	EPA 200.7	10 03/06/25	0.05	0.5	180 days	See Note 4	
Potassium (see Note 5)	DOH cert # 11036	EPA 200.7	10 03/06/25	0.127	0.5	180 days	See Note 4	
Chlorophyll a	BFBM cert # 11896	EPA 445.0	2 02/23	0.1 (µg/L)	N/A	21 days	Ice to 4ºC and filter within 24 hours.	500ml
Microcystins	BFBM cert # 11896	EPA 546	1.1 04/24	0.10 ppb	0.15 ppb	30 days	Ice to 4ºC and freeze within 24 hours.	50ml
Cylindrospermopsin (see Note 6)	BFBM cert # NA	N/A	N/A	0.040 ppb	0.05 ppb	30 days	Ice to 4ºC and freeze within 24 hours.	50ml
Anatoxin-a (see Note 6)	BFBM cert # NA	N/A	N/A	0.10 ppb	0.15 ppb	30 days	Ice to 4ºC and freeze within 24 hours.	50ml

Parameter	Laboratory	Analytical Method	SOP Revision # / Date	Detection Limit (mg/L)	Reporting Level (mg/L)	Holding Time	Preservative	Volume
Saxitoxin (see Note 6)	BFBM cert # NA	N/A	N/A	0.015 ppb	0.02 ppb	30 days	Ice to 4ºC and freeze within 24 hours.	50ml

1. Concentrated H_2SO_4 to pH 2; ice to 4°C
2. Samples with turbidity levels >10NTU's must be distilled. The turbidity level determined by the BFBM will be noted on the chain of custody forms and the sample bottle labels relinquished to the NJ certified laboratory for ammonia testing.
3. Field filter volume listed for sample using peristaltic pump, dedicated tubing and 0.45µm capsule filter after following procedure in 16.3 Filtration Blanks
4. Conc. HNO_3 to pH of <2; ice to 4°C
5. Reference sites only.
6. Not a NJ-certified parameter. Samples can be analyzed by BFBM using a microtiter plate Enzyme-Linked Immuno-Sorbent Assay (ELISA) using the Abraxis kits for Microcystins, Anatoxin, Cylindrospermopsin and Saxitoxin. Instructions for these kits are included in Appendix J, HAB Toxin Analysis Kit Instructions.

Equipment Blanks

Equipment blanks will be collected annually for each horizontal sampler used for the lake monitoring network. This sample is collected by completely filling a clean submerged sampler with ULTRA-PURE water. An equipment blank, of a volume necessary for analysis of all network parameters, is then taken from the submerged sampler by filling the sample containers. The equipment blank is preserved in the same manner as routine samples. Additional equipment blanks will be collected, as needed, if contamination is detected or suspected.

Filter Blanks

Each 0.45µm capsule filter will be flushed with 3 liters of ULTRA-PURE water prior to sample collection in the field. The filtration blank, of a volume necessary for DOC analysis, will then be collected after passing through the filter. The filtration blank is then preserved, as detailed in the above table, for each parameter. Analysis will include dissolved parameters based on lake type. A filter blank will be collected for each lot of filters received from the supplier. The filter blank date, filter manufacturer, model number and lot will be recorded in a spreadsheet for future reference.

Existing Information

NJDEP GIS data is central to desktop reconnaissance of network lakes. GIS data will help determine many characteristics of a lake including location, Geographic Naming Information System (GNIS) name, surface area, public or private ownership, presence of swimming beach/dam, history of pesticide use and some degree of accessibility. In addition, Google searches are used for finding parcel ownership and contact information.

The NJDEP Lake Survey Design is central to the project because it assigned each lake in the lake monitoring universe, a unique identification number (e.g. NJW04459-000 or NJLM-0000). This is used to identify the lake and track all monitoring activities.

ArcGIS Field Maps will warehouse all lake geolocation information. The data is central to field work when monitoring a lake. The Sample Station Selection section explains the process to identify existing sample stations for repeat monitoring.

Existing analytical data from these monitoring stations is ancillary to field work but central to the project. Ancillary uses include referencing water chemistry data and depths while central uses include appending water quality data to the water region of interest for Targeted or Panel for Statistical.

B3 Integrity of Environmental Information

Field Labeling

Careful handling of all samples and accurate recording of field data are required to ensure integrity of results. After filling each sample bottle, caps will be marked with lake name and station number in pencil then stored on ice. Pencil is used because the markings are compatible with the plastic caps. Bottle caps are often wet, which renders permanent markers unusable. A waterproof pen is used to record pertinent data on the Lakes Field Sheet (Appendix G) which is printed on waterproof paper.

Once all samples are collected, staff will return to the vehicle and immediately append each station number to the lake unique identification number. This combination creates the lake station identification number which is used to log the location and track analysis results. The following two paragraphs will refer to the images provided in Appendix K, Laboratory Analysis Labels.

The lake station identification number is then transcribed to a waterproof label for chlorophyll a and HAB analysis at the BFBM lab. In addition, lake name, date, time, Fluorosense reading and collectors initials are recorded.

NJDOH uses a proprietary system for laboratory tracking called Field ID Number. Field ID Numbers are pre-printed waterproof labels provided by the lab and assigned by BFBM staff to each lake station. The assigned Field ID Number is written on the Lakes Field Sheet for the corresponding lake station and applied to the waterproof label indicating the parameter and preservative for each sample bottle. The Lake Field Sheet and the NJDOH Sample Form are described in A12 Documents and Records and provided as appendices.

Chain of Custody

The NJDOH Sample Form has a chain of custody at the bottom. Field required include sampler name (printed), sampler signature and affiliation. If the sampler does not deliver the samples, the sampler will sign over to the delivery person. The delivery person will then sign them over to the receiving person at the laboratory.

BFBM does not require a chain of custody form. The waterproof label affixed to the chlorophyll a/HAB sample provides all pertinent information required by analysts for proper tracking of results.

Sample Storage/Holding Times/Laboratory Turnaround

Samples will be stored in a cooler on ice in a BFBM vehicle after collection and until delivery to each laboratory. Delivery to laboratory is typically same day as collection via BFBM vehicle. If delivery cannot be made on the same day as collection, samples will be delivered prior to other duties the next morning. This is critical for parameters with short holding time. Sample storage/preservation and holding times for each parameter required by this project can be found in the previous section in the table under Laboratory.

Laboratory turnaround time is typically 30 days from analysis date.

B4 Quality Control

NJDOH (certification number 11036) and NJDEP BFBM (certification number 11896)

Any existing data to be used was systematically reviewed by BFBM staff prior to finalizing it in the Lake Monitoring Database.

B5 Instrument / Equipment Calibration, Testing, Inspection and Maintenance

BFBM is certified by the DEP-OQA (certified lab ID # 11896) for all parameters listed below:

Temperature, pH, Conductance and DO will be measured using a YSI Pro DSS. The YSI Pro DSS is a multi-parameter water quality system that combines temperature, pH, conductance, and ODO probes into one meter that is submersible to the desired /depth of the lake. Maintenance of the unit will follow manufacturer's instructions and recommendations unless otherwise detailed by BFBM SOP.

Turbidity is measured using a HACH Model 2100Q turbidimeter which is calibrated once every three months per OQA recommendations. The meter is then checked with certified standards for accuracy within the calibration range during each day of use. Records of all calibrations and calibration checks shall be maintained in the field logbook.

B6 Inspection/Acceptance of Supplies and Services

Sampling consumables (YSI ProDSS parts, pH and conductance standards, single-use sample bottles, gloves, tubing, etc.) will be inspected after delivery from vendor for integrity by BFBM staff receiving items. Any materials with defects will be replaced. Sampling equipment will be washed as described in Appendix I, Submerged Sampler Cleaning Method, between sampling events using ULTRA-PURE water and phosphate-free detergents. Consumables and equipment will be stored in the BFBM laboratory and field vehicles. Consumables and equipment will be sealed to prevent accumulation of dust or accidental cross contamination between sampling events.

B7 Environmental Information Management

Field data and analysis results will be stored locally in a Microsoft Access database housing all lake monitoring data. Data will be entered into EPA's Water Quality Data Exchange (WQX) by staff within 12 months of receiving all the results and will be accessible through the USEPA, USGS and National Water Monitoring Council's Water Quality Portal. Data will be entered by the Data Manager(s) and transferred to WQX by BFBM staff familiar with the data to check for accuracy. All raw data records shall be maintained for a period of no less than five years.

C1 Assessments and Response Actions

The Project Manager will be responsible for the oversight of all activities covered under this QAPP, including sample collection, lab coordination, stakeholder communication and data review with Data Manager(s). The Project Manager will assess field collection functions and make corrections when necessary to maintain the data accuracy as defined in this plan.

If any changes or modifications are made to this plan regarding data collection, as it relates to the objectives(s) and data accuracy required in this project, all original signatories of the QAPP will

be notified by the project manager. If a laboratory cannot be secured for analysis, or the contract award is delayed and frozen samples will expire per method requirements, all signees of the QAPP will be notified. All NJ certified laboratories used are subject to audits and to the requirements of the OQA Laboratory Certification Program as well as internal performance evaluations. At any point during the project, the NJDEP Office of Quality Assurance may audit compliance with the required elements of this QAPP. The project manager will be responsible for corrective actions

During the project, it is important to assess the project's activities to ensure that the QAPP is being implemented as planned. This helps to ensure that everything is occurring appropriately and serves to minimize learning about critical deviations toward the end of the project when it may be too late to remedy the situation. Ongoing assessments that will occur during this project include: field oversight, laboratory oversight, and annual review. If any of the described reviews reveal necessary changes to this QAPP, an addendum will be prepared and circulated for signature.

In addition, at any point during the project, the NJDEP Office of Quality Assurance may audit compliance with the required elements of this QAPP.

Field Oversight

- *Readiness review of the field team prior to starting field efforts*
  Sampling personnel will be properly trained by qualified personnel before any sampling begins. Equipment maintenance records will be checked to ensure all field instruments are in proper working order and any required field calibrations will be performed. Adequate supplies of all preservatives and bottles will be obtained and stored appropriately before heading to the field. Sampling devices will be checked to ensure that they were properly cleaned. Required logbooks, log sheets, chain-of-custody forms, etc. will be assembled by the sampling personnel. The field team lead will review all field equipment, instruments, containers, and paperwork to ensure that all is ready prior to each sampling event. Any problems observed will be corrected before the sampling team departs for the sampling event.

- *Field activity audits*
  During at least one annual sampling event, the project QAM or project manager will assess the sample collection methodologies, field measurement procedures, and record keeping of the field team to ensure activities are being conducted as described in this QAPP. Any deviations noted will be corrected immediately to ensure all subsequent samples and field measurements and observations are valid. If any deviations are associated with technical changes and/or improvements made to the procedures, the project manager will verify that the changes have been documented and addressed in an amendment to this QAPP. The project manager may stop any field activity that could potentially compromise data quality.

  The project QAM or project manager will discuss any noted issues or concerns informally and openly with the field team while on-site. Any findings will be recorded while on-site in the field notebook. Once back in the office, they will formalize the audit findings and corrections

thereof in an e-mail to the project QAM, project manager and project field staff. In this e-mail, the person responsible for correcting the issue or concern will be identified, as applicable. A corrective action response will then be prepared as described under "Response Actions," below.

- *Post-field activity reviews*
  Following each field event, the field team lead will review field datasheets and records to ensure that all information is complete and any deviations from planned methodologies are documented. The results of this review, as well as comments associated with potential impacts on field samples and field measurement integrity will be documented and used as a guide to identify areas requiring improvement prior to the next sampling event. If any identified findings have potential to impact subsequent field activities, a corrective action response will be prepared as described under "Response Actions," below.

Laboratory Oversight

Following receipt of the laboratory data package for the year of sampling events, the project manager and project data manager will review the data package for completeness, as well as to ensure that all planned methodologies were followed and that project data quality objectives were met. The results of the review will be documented. The project manager or project data manager has the authority to request re-testing or other corrective measures if the laboratory has not met the project's data quality objectives and/or has not provided a complete data package.

The NJDOH (certification number 11036) and NJDEP BFBM (certification number 11896) laboratory's QA Manual describes the policies and procedures for QA assessment and response in the laboratory.

Annual Review

The Lake Monitoring Network QAPP needs to be amended each year due to the rotation of the various networks. An annual review will be performed by the project manager to confirm the suitability of and evaluate the effectiveness of the QAPP. During this annual review, project manager will verify that NJDOH (certification number 11036) and NJDEP BFBM (certification number 11896) laboratories continue to be certified for the methods specified herein. The results of the annual review will be incorporated into the addendum, if needed.

Response Actions

For any findings (deviations from the QAPP) identified during the assessments described above, a root cause analysis will be performed by applicable project personnel (e.g., the project manager, field staff, etc.), depending on the nature of the problem. A root cause analysis is an investigation to determine and address the cause that led to the finding. Once the root cause is identified, the applicable project personnel will develop a plan to correct the identified finding, with a copy of this plan sent to the project QAM in the form of an email. The plan will document steps to be taken to correct the problem and to prevent future occurrences of the problem.

The project manager will periodically review all corrective actions to ensure continued effectiveness at preventing future instances of any identified finding. Any new instances of the initial finding will undergo the response action process described above.

C2 Oversight and Reports to Management

The Project Manager is responsible for the oversight of all activities relating to sample collection, laboratory, contracting and data review with Data Manager(s). The Project Manager will assess field collection functions and make corrections when necessary to maintain the data accuracy as defined in this plan. Final deliverables will include geolocations collected during sampling and data submittal to the Water Quality Exchange. The geolocations and data submittal will be combined to provide updates to the NJDEP Lake Mapper. The NJDEP Lake Mapper is a GIS experience that enables users to visualize and interact with all lakes sampled for the NJDEP Lake Monitoring Network.

https://experience.arcgis.com/experience/a73177c2e69740ada4a8218cecf06f39

Any data obtained through analysis of parameters that are not certified through the NJDEP OQA's environmental laboratory certification program cannot be used for regulatory purposes as defined in N.J.A.C. 7:18-1.7.

D1 Environmental Information Review

The project manager will be responsible for verifying data by comparing requested analyses to the data received from the laboratory and that these data are aligned with the field-collected samples.

Data will be verified by comparing any data qualifiers to expected results as well as blank samples. The proposed analytical methods are well established, and data can be validated by comparing results to spiked matrix samples as well as laboratory blanks.

Data quality is dependent on the credibility of the laboratory running the analysis and ensuring that all methods and procedures are followed correctly. The project manager will be responsible for assessing the quality of submitted data by reviewing the documentation and accreditation of the laboratory.

Establishing procedures for environmental information review helps to ensure that project data are evaluated in an objective and consistent manner. The review will consist of verification, validation and data quality assessment.

Verification is the process of evaluating the completeness, correctness, and compliance of a specific data set to method, procedural, or contractual requirements (e.g., ensuring that method requirements were met during analytical procedures). While on site, field staff will check all field logs for missing data before departing for the next site. Upon return to the office, the post-field activity reviews described above will be carried out by the personnel specified in C1, above, as an additional verification to ensure data are consistent, correct, and complete, with no errors or omissions. The verification will include confirming whether all sites were sampled as planned. In addition, for the analyses performed in contracted laboratories, verification will be performed by

laboratory staff prior to issuing the analytical laboratory report. Decisions on whether to accept, reject, or qualify data during verification will be made based on the nature and significance of any identified deviation. The verification process will be performed by laboratory staff. The results of the process, including any observed deviations, will be recorded where required by the laboratory certification.

Validation involves determining whether the requirements for a specific intended use or application have been fulfilled (e.g., were the project-specific data quality objectives presented in this QAPP met?). For analyses performed in contracted laboratories, data validation will be performed upon receipt of results from the laboratory by project personnel as described in the laboratory oversight activities subsection of section C1, above. For analyses performed in the field, the data quality objective requirements contained herein align with those in the methods being employed by the project field staff, so additional validation beyond the verification described above is not required for this project. Decisions on whether to accept, reject, or qualify data during validation will be made based on the nature of any identified deviation.

Data Quality Assessment is the scientific and statistical evaluation of data to determine if the data obtained from environmental information operations are of the right type, quality, and quantity to support their intended use. Project data quality will be evaluated as detailed in this document, with respect to the sampling design, sampling methods, field and laboratory analyses, quality control, and maintenance.

By adhering to the requirements contained in this document and any documents referenced (e.g., equipment manuals, standard operating procedures, etc.), the data quality will be ensured. If samples or procedures used in this study fail to meet the guidelines listed in this document, the data will be flagged appropriately. Any flagged data will be carefully scrutinized to determine whether the data can be used in decision making. The data quality assessment will be made and documented by the personnel tasked with performing the useability assessment described in section D2.

D2 Useability Determination

The project manager will determine data useability based on the culmination of results from the validation, verification, and data quality assessment process. The project manager may ask the questions referenced in Table 8 below as they determine the usability of data for this project. The QAQC measures put in place will ensure the data quality is consistent with the goals of the Lake Monitoring Network.

Table 8. Data Usability Questionnaire

Item	Assessment Activity
Data Deliverables	Was all necessary information (e.g., results and reports) performed/provided, including validation and verification of results?

Item	Assessment Activity
Deviations from QAPP (e.g., sampling sites, sample handling (preservatives, holding times, etc.), analytical methods, QC sample failures)	Were there any deviations from the QAPP? If so, what impact do these deviations have on usability?
SOP deviations	Were there any deviations from the SOPs that were used? If so, what impact did these have on adhering to the QAPP?
Metrological effects/site conditions	Were there any weather or site conditions that may have affected results?
DQI: Precision	Were precision criteria met for all samples? If not, what percent of samples had precision issues? Is there enough data that met criteria for use in decision making?
DQI: Accuracy (Bias)	Were accuracy criteria met for all QC samples? Is there enough data that met criteria for use in decision making?
DQI: Representativeness	Was the data collected in a manner that ensured representativeness (e.g., if it was planned that samples would be taken every month to ensure seasonal differences were captured, but three samples were missed, can you confirm that the 3 samples that were missed were not all from the winter months?)
DQI: Completeness	Was any planned data collection omitted? Is there enough data that met criteria for use in decision making?
DQI: Comparability	Did results from different environmental information operations agree in an expected manner?
DQI: Sensitivity	Were the quantitation/ reporting limits specified in the QAPP met?
Usability decision	Based on an evaluation of all criteria tabulated above, is there enough usable data to make a specific decision?

E1 References

NJDEP, 2024. Field Sampling Procedures Manual. Contaminated Site Remediation & Redevelopment, NJDEP. https://dep.nj.gov/srp/guidance/fspm/

NJDEP, 2023. Surface Water Quality Standards, N.J.A.C. 7:9B. Bureau of Environmental Analysis, Restoration and Standards, NJDEP. https://www.nj.gov/dep/wms/bears/swqs-overview.htm

NJDEP, 2014. 2014 Integrated Water Quality Monitoring and Assessment Methods Document. Bureau of Environmental Analysis, Restoration and Standards, NJDEP. https://dep.nj.gov/wms/bears/technical-support-docs/#wqa

NJDEP, 2024. NJDEP Lake Monitoring Network QAPPs (2005-2024). Bureau of Freshwater & Biological Monitoring, NJDEP.

NJDEP, 2024. Aquatic Macrophyte Survey Program, FY24-33. Bureau of Freshwater & Biological Monitoring, NJDEP.

NJDEP, 2015. BFBM Lake Survey Design. Bureau of Freshwater & Biological Monitoring, NJDEP.

E2 Appendices

Appendix A, List of Statewide Statistical Survey Lakes

Site ID	GNIS Name	County	Municipality
NJLM-0705	"Strawberry Lake"	ATLANTIC	HAMILTON TWP
NJW04459-254	"Overpeck Creek"	BERGEN	LEONIA BORO
NJLM-0085	Country Lake	BURLINGTON	PEMBERTON TWP
NJW04459-151	Lower Aetna Lake	BURLINGTON	MEDFORD LAKES BORO
NJW04459-331	Camp Inawendiwin Lake	BURLINGTON	TABERNACLE TWP
NJLM-0198	"Batona Trail"	BURLINGTON	WASHINGTON TWP
NJW04459-430	Grenloch Lake	GLOUCESTER	WASHINGTON TWP
NJW04459-231	Conines Millpond (Allentown Lake)	MONMOUTH	UPPER FREEHOLD TWP
NJW04459-109	Green Pond	MORRIS	ROCKAWAY TWP
NJLM-0416	"Mirror Lake"	MORRIS	HANOVER TWP
NJW04459-116	Hainsville Pond	SUSSEX	MONTAGUE TWP
NJW04459-064	Lake Saginaw	SUSSEX	SPARTA TWP
NJLM-1359	Marshall Pond "Laurel Lake"	SUSSEX	VERNON TWP

Appendix B, List of Potential Oversample Lakes

Site ID	GNIS Name	County	Municipality
NJLM-0198	"Batona Trail"	Burlington	Washington Twp
NJW04459-116	Hainsville Pond	Sussex	Montague Twp
NJLM-0416		Morris	Hanover Twp
NJW04459-425	Lawrence Lake	Sussex	Andover Twp
NJLM-1171		Morris	Rockaway Twp
NJLM-1176	Lake My-An-Ho	Camden	Winslow Twp
NJLM-0706	Cloverleaf Lake	Atlantic	Hamilton Twp
NJLM-0957		Sussex	Sparta Twp
NJW04459-097	Great Gorge Lake	Sussex	Vernon Twp
NJLM-1271	Dun-rite Pond	Camden	Gloucester Twp

Appendix C, List of Reference Lakes

Site ID	GNIS Name	County	Municipality
NJW04459-058	Mount Misery Lake	Burlington	Pemberton Twp
NJW04459-352	Hands Mill Pond	Cumberland	Maurice River Twp
NJLM-0028	Green Turtle Lake	Passaic	West Milford Twp
NJW04459-134	Silver Lake	Sussex	Hardyston Twp
NJW04459-097	Great Gorge	Sussex	Vernon Twp
NJW00459-233	Watchu Pond	Sussex	Byram Twp
NJW04459-339	Mashipacong Pond	Sussex	Montague Twp
~~NJW04459-009~~	~~Deer Park Pond~~	~~Warren~~	~~Allamuchy Twp~~

Deer Park Pond eliminated due to multiple access issues and possible dam removal.

Appendix D, List of Regional Targeted Lakes

Site ID	GNIS Name	County	Municipality
NJLM-0626	Big Pine Lake	Burlington	Pemberton Twp
NJLM-0086	Country Lake	Burlington	Pemberton Twp
NJLM-1224	Newton Lake	Camden	Collingswood Boro
NJLM-1279	Shaws Mill Pond	Cumberland	Lawrence Twp
NJW04459-120	Sunset Lake	Cumberland	Upper Deerfield Twp
NJW04459-378	Almonesson Lake	Gloucester	Deptford Twp
NJLM-0985	Malaga Lake	Gloucester	Franklin Twp
NJW04459-430	Grenloch Lake	Gloucester	Washington Twp
NJLM-0456	Gropp Lake	Mercer	Hamilton Twp
NJLM-0285	Elmer Lake	Salem	Pittsgrove Twp

APPENDIX E, Lake Sampling Parameters

Indicator Type	Indicator	Statistical Lakes	Statewide Reference (by Ecoregion)	Targeted Regional (Water Region)
Trophic Indicators	Vertical profile measurements (DO, Temperature, pH)	x	x	x
	Secchi Disk transparency	x	x	x
	Water chemistry	Chloride Conductivity Turbidity Alkalinity Hardness Sulfate	Chloride, Conductivity, Turbidity, Alkalinity Hardness, Calcium, Magnesium, Sodium, Potassium, Sulfate, True Color, Apparent Color, Total Suspended Solids	Chloride Conductivity Turbidity Alkalinity Hardness Sulfate
	Nutrients	TP, NH3, TKN, NO2-NO3, DOC	TP, Ortho P, NH3, TKN, NO2-NO3, DOC	TP, NH3, TKN, NO2-NO3, DOC
	Chlorophyll-a	x	x	x
Ecological Integrity	Aquatic macrophyte survey (separate QAPP)	N/A	As needed	5 of 10
	Zooplankton Monitoring (separate pilot study)	N/A	As needed	5 of 10
	Physical habitat characterization	As needed		
	Visual Assessment	As needed		
Human Use	Phytoplankton (cyanobacteria)	Checked during each visit with handheld fluorometer		
	Algal toxins (cyanotoxins) per section 11.6	x	x	x

Number of Lakes	Total Number of lakes sampled/ category	250; 5 panels of 50 lakes sampled over 40 years	8	10
Duration	Time period to sample all lakes	8 years/ 50 lake statewide panel	Annually/statewide	2 years/ region
Frequency	samples /year	12-13 lakes per year, 4X Mar-Nov	1X July 24-Aug 7 (± one week)	4X March-Nov
Sites/lake	in-lake stations	As needed	As needed	As needed

Appendix F, Laboratory Analysis

The following parameters will be analyzed by New Jersey certified laboratories – NJDOH (certification number 11036) and NJDEP BFBM (certification number 11896). Single use plastic bottles, of the volume indicated, will be used for the analytes listed. Laboratories shall be certified by DEP's OQA for the requested parameters. The reporting levels, listed in the table below, are **required** for this project. Project action levels are determined by the NJDEP SWQS Surface Water Quality Criteria.

Analyte	Method	Method Detection Limit (mg/L)	Reporting Level (mg/L)	Project Action Level	Holding Time	Preservative	Volume
Nitrite + Nitrate	4500-NO3(F)	0.008	0.025	N/A	28 days	See Note 1	500ml
Ammonia (Non-distillation)	4500-NH3(H)	0.005	0.010	N/A	28 days	See Notes 1 & 2	
Ammonia (Distillation)	4500-NH3(H)	0.023	0.05	N/A	28 days	See Notes 1 & 2	
Total Kjeldahl Nitrogen	351.2	0.04	0.100	N/A	28 days	See Note 1	
Total Phosphorus	365.1	0.005	0.010		28 days	See Note 1	
Dissolved Organic Carbon (DOC)	5310-C	0.45	1.00	N/A	28 days	See Note 3 & 1	500ml
Color Dissolved Organic Matter (True Color, see Note 5)	SM 2120B	2.5 PCU	2.5 PCU	N/A	48 hours	See Note 3 Ice to 4ºC	500ml
Dissolved Silica (see Note 5)	200.7W	0.26	0.5	N/A	180 days	See Note 3 & 4	500ml
Alkalinity	2320-B	1	1	N/A	14 days	Ice to 4ºC	1000ml
Apparent Color (Qualified True Color method, see Note 5)	SM 2120B	2.5 PCU	2.5 PCU	N/A	48 hours	Ice to 4ºC	
Chloride (flow injection)	4500-CL(E)	1.3	2.5	N/A	28 days	Ice to 4ºC	
Total Suspended Solids (see Note 5)	2540-D	1	1	N/A	7 days	Ice to 4ºC	
Sulfate (flow injection)	375.2	2.8	10	N/A	28 days	Ice to 4ºC	
Ortho Phosphorus (see Note 5)	365.1	0.004	0.005	N/A	48 hours	Ice to 4ºC	

Analyte	Method	Method Detection Limit (mg/L)	Reporting Level (mg/L)	Project Action Level	Holding Time	Preservative	Volume
Hardness	200.7(W)	0.069	3.31	N/A	180 days	See Note 4	500ml
Calcium (see Note 5)	200.7(W)	0.05	0.5	N/A	180 days	See Note 4	
Magnesium (see Note 5)	200.7(W)	0.035	0.5	N/A	180 days	See Note 4	
Sodium (see Note 5)	200.7(W)	0.045	0.5	N/A	180 days	See Note 4	
Potassium (see Note 5)	200.7(W)	0.132	0.5	N/A	180 days	See Note 4	

7. Concentrated H_2SO_4 to pH 2; ice to 4°C
8. Samples with turbidity levels >10NTU's must be distilled. The turbidity level determined by the BFBM will be noted on the chain of custody forms and the sample bottle labels relinquished to the NJ certified laboratory for ammonia testing.
9. Filter volume listed for sample using peristaltic pump, dedicated tubing and 0.45µm capsule filter after following procedure in 16.3 Filtration Blanks
10. Conc. HNO_3 to pH of <2; ice to 4°C
11. Reference sites only.

Analyte	Method	Method Detection Limit (mg/L)	Reporting Level (mg/L)	Holding Time	Preservative	Volume
Chlorophyll a	445	0.1 (µg/L)	N/A	21 days	Ice to 4ºC & filter within 24 hours.	500ml
Microcystins	EPA 546	0.10 ppb	0.15 ppb	30 days	Ice to 4ºC & freeze within 24 hours.	
Cylindrospermopsin (see Note 1)	N/A	0.040 ppb	0.05 ppb	30 days	Ice to 4ºC & freeze within 24 hours.	
Anatoxin-a (see Note 1)	N/A	0.10 ppb	0.15 ppb	30 days	Ice to 4ºC & freeze within 24 hours.	
Saxitoxin (see Note 1)	N/A	0.015 ppb	0.02 ppb	30 days	Ice to 4ºC & freeze within 24 hours.	

1. Not an NJ-certified parameter. Samples can be analyzed by BFBM using a microtiter plate Enzyme-Linked Immuno-Sorbent Assay (ELISA) using the Abraxis kits for Microcystins, Anatoxin and Cylindrospermopsin. This method was utilized by the USEPA as part of the NLA. QA/QC procedures are outlined in: *USEPA. 2009 (Final). Survey of the Nation's Lakes: IntegratedQuality Assurance Project Plan. EPA/841-B-07-003. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.*

Appendix G, Lake Field Data Sheet

NJDEP Lakes Monitoring Network [Prob Ref Targ]

Site ID:	NJ	**DOC Station:**	________	**Sampler /Meter:**	___/___	**DOC Sample ID:**	________
Lake Name:	______________	**Barom, mmHg:**	________	**Sampler Number:**	________	**Panel:**	________
Date:	/ /24	**Air Temp, °C:**	________	**Meter SN:**	________	**Prob ID:**	________

Outlet flowing? Y / N / NA **Sediment cored?** Y / N **Station #:** **Coring Equipment:**

STATION **Time:** : **Total Depth, m:** **Secchi Depth, m:** **Forel Ule Color:** **Sample ID:**

Depth, m	0.1											
Temp, °C												
% Sat												
DO, mg/L												
SpC, µS/cm												
pH												
YSI, Chla RFU												
YSI, PC RFU												

Depth, m	**Turb, NTU**	**Fl PC, µg/L**
0.1		

STATION **Time:** : **Total Depth, m:** **Secchi Depth, m:** **Forel Ule Color:** **Sample ID:**

Depth, m	0.1											
Temp, °C												
% Sat												
DO, mg/L												
SpC, µS/cm												
pH												
YSI, Chla RFU												
YSI, PC RFU												

Depth, m	**Turb, NTU**	**Fl PC, µg/L**
0.1		

STATION **Time:** : **Total Depth, m:** **Secchi Depth, m:** **Forel Ule Color:** **Sample ID:**

Depth, m	0.1											
Temp, °C												
% Sat												
DO, mg/L												
SpC, µS/cm												
pH												
YSI, Chla RFU												
YSI, PC RFU												

Depth, m	**Turb, NTU**	**Fl PC, µg/L**
0.1		

Comments/Observations:

Duplicates at Station:
SpC, µS/cm:
Turb, NTU:

Field ID Number		Lab Sample Number (For Lab Use Only)

New Jersey Department of Health
Environmental and Chemical Laboratory Services
PO Box 361, Trenton, NJ 08625-0361
Phone: 609-530-2820

ORGANIC AND INORGANIC CHEMISTRY SAMPLE SUBMITTAL

(See Instructions)

AGENCY INFORMATION

Submitting Agency	Send Results To	Agency No.	Project Name
NJDEP-BFBM	Johannus Franken	207	Lakes
Street Address	**Final Report Option** ☐ Tier 1 ☐ Tier 2	**Would you like copies of the internal chain of custody forms sent with your report?** ☐ Yes ☒ No	**Project Code** V4PC
35 Arctic Parkway	**Electronic Report Option** ☐ EDD ☐ E-2		**Memo Number**
City, State, Zip Code	**Phone**	**Fax**	**Email**
Trenton, NJ 08625	609-292-0427	609-633-1095	johannus.franken@dep.nj.gov

SAMPLE INFORMATION

Sample Point/Station ID Number/Water Facility ID	Collection Date (YY/MM/DD) __ __ / __ __ / __ __	**Sample Type**
Sampling Site/Facility/Supply/Location/Sampling Point ID	Coll. Time (24h) Start __ __ __ __ Coll. Time (24h) End __ __ __ __	**Non-Potable:** ☒ Stream/Surface ☐ Ground Water ☐ Private Well ☐ Septic ☐ Ocean/Saline ☐ Sediment ☐ Tissue ☐ Sewage: ☐ Raw ☐ Effluent ☐ Industrial: ☐ Raw ☐ Effluent
Waterbody Name	Sample Retention Retain? ☒ No ☐ Yes Duration _____	
Municipality/County	Type of Sampling Event ☒ Regular ☐ Compliance ☐ Repeat ☐ Non-Regulatory ☐ Other	**Potable:** ☐ Groundwater Rule ☐ Source ☐ Confirmation ☐ Raw ☐ Finished ☐ Private Well ☐ At Source ☐ Flushed ☐ 1st Draw ☐ Lead Source Line ☐ Surface H_2O Intake ☐ Distribution System
Sampling Point Street Address	If Repeat or GWR, List Original Lab Sample No.	
	Sample Collector	**Fraction:** ☐ Total ☐ Dissolved
PWSID	Trip #	**Other:** ☐ _____ **Priority:** ☐ Routine ☐ Priority ☐ Emergency

FIELD INFORMATION

Air Temp °C	Water Temp °C	Stream Flow-CFS
Weather Conditions	Sample pH (Field)	Gage Height-Ft.
Preserved in: ☒ Field ☐ Lab Date: __ __ / __ __ / __ __ Time: __ __ __ __	DO (mg/l)	Spec.Cond. (µS/CM)
	DO% Sat	Salinity (ppm)
Chlorine Residual	Sample Depth (meters)	Tide Stage
Comments/Field Checks Network Type	Barometric Pressure (mmHg)	Turbidity (NTU)

ANALYSIS REQUESTS

Metals

☐ Ag..... Silver	☐ Mg..... Magnesium
☐ Al.....Aluminum	☐ Mn..... Manganese
☐ As..... Arsenic	☐ Mo... Molybdenum
☐ B.....Boron	☐ Na .....Sodium
☐ Ba.....Barium	☐ Ni .....Nickel
☐ Be.....Beryllium	☐ Pb .....Lead
☐ Ca..... Calcium	☐ Sb .....Antimony
☐ Cd.....Cadmium	☐ Se .....Selenium
☐ Co.....Cobalt	☐ Si..... Silica
☐ CR-T....Chromium	☐ Tl..... Thallium
☐ Cu.....Copper	☐ U ..... Uranium
☐ Fe ..... Iron	☐ V .....Vanadium
☐ K.....Potassium	☐ Zn.....Zinc

Preferred Methodology
☐ EPA 200.7 / 200.9 ☐ EPA 200.8

Residues
☐ Total Suspended Solids (TSS)
☐ Total Solids (TS)
☐ Total Dissolved Solids (TDS)
☐ Settleable Solids (SS)
☐ Total Volatile Solids (TVS)

General
☐ Alkalinity
☐ Bromide by IC
☐ Chloride
☐ Chloride by IC
☐ Chromium, Hexavalent
☐ Chromium, Hexavalent by IC
☐ Color
☐ Conductance
☐ Cyanide
☐ Dissolved Oxygen
☐ Fluoride
☐ Fluoride by IC
☐ Hardness
☐ MBAS
☐ Odor
☐ pH
☐ Phenols (PW)
☐ Phenols (NPW)
☐ Sulfate by IC
☐ Sulfate Lachat
☐ Turbidity

Mercury
☐ Mercury by EPA 245.1
☐ Low Level Mercury EPA 1631E

Nutrients
☐ Nitrite
☐ Total Phosphorus
☐ Ammonia
☐ Nitrate (Calculated)
☐ Nitrogen, Total (Calculated)
☐ Nitrite + Nitrate
☐ Ortho Phosphorus
☐ Total Kjeldahl Nitrogen (TKN)

Organics (Drinking Water)
☐ EPA 504.1 - EDB, DBCP,123TCP
☐ EPA 505 - Chlordane
☐ EPA 505 - Toxaphene
☐ EPA 507 - N and P containing Pesticides
☐ EPA 515.3 - Chlorinated Acid Herbicides
☐ EPA 524.2 - Purgeables
☐ EPA 525.2 - Liquid-Solid Extractables
☐ EPA 531.1 - N-Methylcarbamoyloximes and N-Methylcarbamates

Organics (Non-Potable Water)
☐ EPA 624 – Purgeables
☐ EPA 625 - Base/Neutral and Acid Extractables

Demands
☐ Total Organic Carbon (TOC)
☐ Dissolved Organic Carbon (DOC)
☐ Chemical Oxygen Demand (COD)

Suggested Dilutions
☐ BOD5 ☐ BOD20 ____ ____ ____
☐ CBOD5 ☐ CBOD20 ____ ____ ____

Other
☐ _____ ☐ _____ ☐ _____

Relinquished By:	Affiliation:	Received By:	Affiliation:	Date/Time	Reason for Custody Change
Name (Print): _____		Name (Print): _____			
Signature: _____	NJDEP	Signature: _____	_____	_____	_____
Name (Print): _____		Name (Print): _____			
Signature: _____	_____	Signature: _____	_____	_____	_____

CHEM-44
FEB 16

Appendix I, Submerged Water Sampler Cleaning Method

1. Fill small, graduated cup with 1oz. of concentrated Liqui-Nox soap. Add this soap into a one-gallon plastic container. Fill container with ULTRA-PURE water and mix well. This makes an approximate 1% "Liqui-Nox solution".

2. Don a set of disposable gloves and rinse them thoroughly with ULTRA-PURE water.

3. With one end sealed and spout(s) in the closed position, fill dirty submerged sampler approximately halfway with Liqui-Nox solution.

4. Scrub the submerged sampler thoroughly, inside and out, using the dedicated submerged sampler cleaning brush. This includes scrubbing of both stoppers.

5. Close the open stopper and vigorously shake the submerged sampler with Liqui-Nox solution inside for approximately 10 seconds.

6. Open spout(s) and allow equal amounts of the contained solution to flow through each spout until the bottle is empty.

7. Put on a new set of disposable gloves and rinse them thoroughly with ULTRA-PURE water.

8. Open both stoppers and rinse bottle with ULTRA-PURE water a minimum of three times.

9. Re-seal one end of the bottle and fill sampler to capacity with ULTRA-PURE water. Close the other end and shake vigorously. Open spout(s) and allow rinsate to flow through spout(s) until empty.

10. Repeat step 9 two more times.

11. Place a new polyethylene bag into the submerged sampler carrying case. Place cleaned submerged sampler into bag and seal case with painters tape. Write "CLEAN, Date of cleaning, and INITIALS of cleaning person" on tape.

12. Rinse cleaning brush three times with ULTRA-PURE water, replace in bag and store in lakes cabinet.

If a trace nutrient problem arises upon analysis while using the above method, a final step will be added. This step will add an acid rinse, followed by several rinses with ULTRA-PURE water to ensure that all trace nutrients are eliminated from the submerged sampler.

Importance of Anatoxin-a Determination

Anatoxin-a is an alkaloid neurotoxin produced by some species of cyanobacteria (blue-green algae). It is one of the most toxic of the cyanobacterial toxins. In humans and other animals, the skeletal neuromuscular junction constitutes a primary target for Anatoxin-a (Anatoxin-a can also cross the blood-brain barrier). The neuromuscular junction is specialized for the rapid transmission of neuronal information from the pre-synaptic nerve terminal to the post-synaptic muscle fiber. This transmission is mediated by the synchronous release of the neurotransmitter acetylcholine (ACh), which activates nicotinic acetylcholine receptors (nAChRs) in the muscle endplate, triggering a series of events that lead to muscle contraction. Most ACh molecules are hydrolyzed by acetylcholinesterases, which are highly concentrated at the neuromuscular junction. Anatoxin-a functions as an agonist of nAChRs, like ACh, but is about 20 times more potent. Unlike ACh, it is not degraded by acetylcholinesterases and produces sustained depolarization of the muscle endplate, causing over stimulation of the muscles, leading to muscle fatigue and ultimately paralysis. Symptoms begin within 5 minutes of ingestion of Anatoxin-a and progress rapidly, resulting in cyanosis, convulsions, cardiac arrhythmia, and respiratory paralysis, which ultimately results in death due to suffocation.

Humans and other animals may be exposed to Anatoxin-a through ingestion of contaminated water, through drinking or during recreational activities in which water is swallowed. Due to the potential for serious harm and even death, many countries are expanding monitoring programs to include Anatoxin-a and are establishing regulations regarding the amount of Anatoxin-a in drinking and recreational waters. New Zealand is among those taking regulatory action, establishing a 6.0 µg/L provisional maximum acceptable value (MAV) for Anatoxin-a.

The Abraxis Anatoxin-a ELISA Assay can be performed in less than 90 minutes. Only a few milliliters of sample are required.

Performance Data

Test sensitivity: The detection limit, based on Anatoxin-a, (90% B/B_0) is approximately 0.1 ppb (µg/L). The middle of the test (50% B/B_0) is approximately 1.38 ppb (ng/mL). Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: Intra and inter assay: < 10%

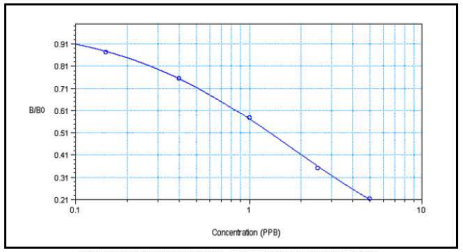
Recoveries:

Level (ppb)	% Recovery
0.25	103.3
0.50	98.0
1.50	104.4
3.00	103.1

Specificity: Cross-reactivity of the Abraxis Anatoxin-a Plate Kit for various congeners:

(+)Anatoxin-a	100.0%
Homoanatoxin-a	124.8%
(-)Anatoxin-a	0.3%

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

General Limited Warranty: Abraxis, Inc. warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

*The monoclonal antibody and enzyme conjugate included in the Abraxis Anatoxin-a ELISA have been licensed (Patent Application P201531661) from the Spanish National Research Council (CSIC) and the University of Valencia (UVEG).

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Email: info@abraxiskits.com
WEB: www.abraxiskits.com

This product is for research use only

R032219

Anatoxin-a ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Anatoxin-a* in Water Samples

Product No. 520060

1. General Description

The Abraxis Anatoxin-a ELISA Plate Kit is an immunoassay for the quantitative and sensitive screening of Anatoxin-a in water samples. This test is suitable for the quantitative and/or qualitative screening of Anatoxin-a in drinking and recreational water samples (please refer to Sample Collection and Handling, section C). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Anatoxin-a. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of these solutions with skin and mucous membranes. If these reagents come in contact with skin, wash thoroughly with water.

3. Storage and Stability

The Anatoxin-a ELISA Kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Anatoxin-a by a monoclonal antibody. Anatoxin-a, when present in a sample, and an Anatoxin-a-enzyme conjugate compete for the binding sites of mouse anti-Anatoxin-a antibodies in solution. The Anatoxin-a antibodies are then bound by a second antibody (anti-mouse) immobilized on the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Anatoxin-a 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.

5. Limitations of the Anatoxin-a ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

Immediately upon collection, fresh water samples must be preserved with the provided Sample Diluent (10X) Concentrate to prevent degradation of Anatoxin-a (please refer to Sample Collection and Handling, section C).

Anatoxin-a will degrade when exposed to natural and artificial light and/or high pH conditions. Samples that have been exposed to natural or artificial light and/or treated with reagents that raise the natural sample pH may produce results that are falsely low. Samples should be adjusted to between pH 5 and pH 7 and protected from light.

Samples containing methanol must be diluted to a concentration < 2.5% methanol to avoid matrix effects.

Seawater samples up to 37 parts per thousand were tested and no matrix effects were detected. Average recovery of spiked seawater samples was 104%.

Anatoxin-a is an intracellular, as well as extracellular, toxin. Therefore, to measure total Anatoxin-a, cell lysing will be required. Once the sample is preserved, three freeze/thaw cycles are recommended for cell lysing.

No matrix effects have been observed with samples that have been treated with ascorbic acid at concentrations ≤ 1 mg/mL. ***Sodium thiosulfate should not be used to treat samples, as sodium thiosulfate will degrade Anatoxin-a, producing inaccurate (falsely low) results.***

Mistakes in handling the test can also cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, exposure to direct or indirect sunlight during the substrate reaction, or extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter plate coated with a secondary antibody (anti-mouse), in a re-sealable aluminum pouch
2. Lyophilized Anatoxin-a-HRP Enzyme Conjugate, 3 vials
3. Conjugate Diluent, 12 mL
4. Lyophilized Anti-Anatoxin-a Antibody, 3 vials
5. Antibody Diluent, 12 mL
6. Empty clear and amber HDPE bottles for combining reconstituted Enzyme Conjugate and Antibody (if necessary)
7. (+)Anatoxin-a Standards (6): 0, 0.15, 0.40, 1.0, 2.5, 5.0 ppb, 1 mL each
8. Control at 0.75 ± 0.185 ppb, 1 mL
9. Sample Diluent (10X) Concentrate, 2 X 25 mL
10. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
11. Substrate (Color) Solution (TMB), 12 mL
12. Stop Solution, 12 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (10-300 µL), stepper pipette (10-300 µL), or electronic repeating pipette with disposable plastic tips (capable of delivering 50-300 µL)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wave length 450 nm)
5. Deionized or distilled water
6. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section D)
7. Paper towels or equivalent absorbent material
8. Timer

C. Sample Collection and Handling

Collect water samples in amber glass sample containers. Drinking water samples should be treated with ascorbic acid (up to 1 mg/mL) immediately after collection to remove residual chlorine. *Do not use sodium thiosulfate. Sodium thiosulfate will degrade Anatoxin-a.*

Immediately upon collection, fresh water samples must be preserved using the Sample Diluent (10X) Concentrate (1 mL of 10X Sample Diluent Concentrate per 9 mL of water sample), to prevent degradation of Anatoxin-a. Samples must be adjusted to between pH 5 and pH 7 and protected from exposure to natural and artificial light, as exposure to light and/or high pH will cause degradation of Anatoxin-a. Store samples refrigerated (up to 28 days). For storage periods greater than 28 days, samples should be stored frozen. Seawater samples do not need to be preserved but the same pH and storage conditions should be applied.

Drinking water samples treated with ascorbic acid (0.1 mg/mL) and sodium bisulfate (1 mg/mL) according to EPA Method 545 do not need to be preserved with Sample Diluent (10X) Concentrate. Samples must be adjusted to between pH 5 and pH 7. The same storage conditions (light, temperature, and duration) apply as described above.

Anatoxin-a is an intracellular, as well as extracellular, toxin. Therefore, to measure total Anatoxin-a, cell lysing will be required. Once the sample is preserved, three freeze/thaw cycles are recommended for cell lysing. This procedure using the three freeze/thaw cycles will not degrade Anatoxin-a.

Preserved fresh water or seawater samples may be filtered following cell lysing and prior to analysis using any of the following syringe filters: Environmental Express 0.2 µm PES (PN SF020E), Pall Acrodisc® 0.2 µm PVDF (PN 4450), Suporⓡ membrane syringe filters (PN 4612), or Environmental Express 1.2 µm Glass Fiber (PN SF012G). *Note: Fresh water samples must be preserved (and lysed) prior to filtration or Anatoxin-a may bind to the filter, removing it from the sample, and producing falsely low sample results.*

D. Test Preparation

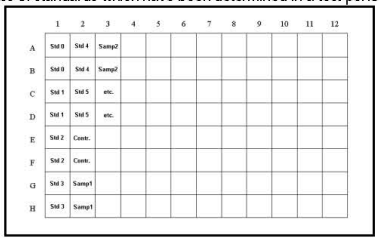
Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. A multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions in order to equalize the incubation periods across the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Allow the microtiter plate, reagents, and samples to reach room temperature before use.
2. The enzyme conjugate and antibody need to be reconstituted prior to use. Add 3 mL of the appropriate diluent to the appropriate vial and vortex well. Let sit for at least 10 minutes and re-vortex prior to use. ***If more than one vial is required for testing, combine the reconstituted enzyme conjugate vials in the amber HDPE bottle and the reconstituted antibody vials in the clear HDPE bottle prior to use.*** The solutions are stable for up to 2 weeks if stored at 4-8°C and up to 1 month if stored frozen.
3. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed.
4. The standard solutions, substrate and stop solutions are ready to use and do not require any further dilutions.
5. Dilute the Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water.
6. Dilute the Sample Diluent (10X) Concentrate at a ratio of 1:10 with deionized or distilled water (i.e. 1 mL of Sample Diluent (10X) Concentrate into 9 mL of deionized water) as needed for sample dilutions.
7. The stop solution must be handled with care as it contains diluted H_2SO_4.
8. After analysis, store the remaining kit components in the refrigerator (4-8°C).

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std5: Standards
Contr.: Control
Samp1, Samp2, etc: Samples



	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp2									
B	Std 0	Std 4	Samp2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Contr.										
F	Std 2	Contr.										
G	Std 3	Samp1										
H	Std 3	Samp1										

F. Assay Procedure

1. Add **50 µL of the standard solutions, control, or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add **50 µL of the reconstituted enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add **50 µL of the reconstituted antibody solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents. Incubate the strips for **60 minutes** at room temperature.
4. Remove the covering and decant the contents of the wells into a sink. Wash the strips **four times** using the diluted wash buffer. Please use at least a **volume of 250 µL of 1X wash buffer** for each well and each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
5. Add **100 µL of substrate (color) solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **20-30 minutes** at room temperature. Protect the strips from sunlight.
6. Add **100 µL of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.
7. Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding Anatoxin-a concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the control and samples will then yield levels in ppb of Anatoxin-a by interpolation using the standard curve. Results can also be determined using a spreadsheet macro available from Abraxis upon request.

Results for fresh water samples which have been preserved with Sample Diluent (10X) Concentrate as described in Sample Collection and Handling (section C) must be multiplied by a factor of 1.1 to account for the initial dilution.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Anatoxin-a than standard 1 (0.15 ppb) should be reported as containing < 0.15 ppb of Anatoxin-a (< 0.165 ppb for preserved water samples). Samples showing a higher concentration than standard 5 (5.0 ppb) should be reported as containing > 5.0 ppb of Anatoxin-a (> 5.5 ppb for preserved water samples) or must be diluted using 1X Sample Diluent to obtain accurate results. The concentration of the positive control provided should be 0.75 ± 0.185 ppb.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of Anatoxin-a greater than that standard. Samples which have higher absorbances than a standard will have concentrations of Anatoxin-a less than that standard.

As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

Importance of Cylindrospermopsin Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Cylindrospermopsin is a toxin produced by several different strains of cyanobacteria (blue-green algae) and has been found in fresh water throughout the world. Certain strains of *Cylindrospermopsis raciborskii* (found in Australia, Hungary, and the United States), *Umezakia natans* (found in Japan), and *Aphanizomenon ovalisporum* (found in Australia and Israel) have been found to produce Cylindrospermopsin. The production of Cylindrospermopsin seems to be strain specific rather than species specific.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms and, in several cases, has lead to death. Human exposure to Cylindrospermopsin can occur through ingestion of contaminated water or food (fish) or during recreational activities in which water is swallowed. Dermal contact with Cylindrospermopsin may occur during showering or bathing, or during recreational activities such as swimming or boating. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of protein synthesis and glutathione, leading to cell death.

To protect against adverse health effects, the U.S. Environmental Protection Agency (EPA) has established guidelines for Cylindrospermosin in drinking water:

-For children pre-school age and younger (less than six years old), 0.7 µg/L (ppb)
-For school-age children and adults, 3.0 µg/L (ppb)

Performance Data

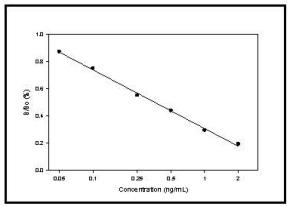
Test sensitivity: The detection limit for this assay is 0.040 ppb (µg/L).

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; for samples: <15%.

Specificity: This ELISA recognizes Cylindrospermopsin and related compounds with varying degrees:

Cylindrospermopsin	100%
Deoxy-Cylindrospermopsin	112%
7-Epi-Cylindrospermopsin	157%

Standard Curve:



Samples: A sample correlation between the ELISA and HPLC methods showed a good correlation.

Recovery

Four (4) groundwater samples were spiked with various levels of Cylindrospermopsin and assayed using the Abraxis Cylindrospermopsin Assay:

Spike Level (ppb)	Recovery Mean (ppb)	Recovery Std.Dev. (ppb)	Recovery (%)
0.1	0.101	0.019	101
0.25	0.269	0.026	108
0.5	0.514	0.038	103
1.0	0.982	0.113	98
Average			103

Precision

Control	1	2	3
Replicates	3	3	3
Days	3	3	3
n	9	9	9
Mean (ppb)	0.198	0.501	1.01
% CV (within assay)	6.2	4.3	5.2
% CV (between assay)	8.3	5.3	4.9

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R032219

Cylindrospermopsin ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Cylindrospermopsin in Water Samples

Product No. 522011

1. General Description

The Abraxis Cylindrospermopsin ELISA is an immunoassay for the quantitative and sensitive detection of Cylindrospermopsin in water samples. No additional sample preparation is required prior to analysis. If necessary, positive samples can be confirmed by HPLC or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Cylindrospermopsin. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

3. Storage and Stability

The Cylindrospermopsin ELISA kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA for the detection of Cylindrospermopsin. It is based on the recognition of Cylindrospermopsin by specific antibodies. Cylindrospermopsin, when present in a sample, and a Cylindrospermopsin-HRP analogue compete for the binding sites of rabbit anti-Cylindrospermopsin antibodies in solution. The anti-Cylindrospermopsin antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Cylindrospermopsin 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.

5. Limitations of the Cylindrospermopsin ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that may be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

The presence of the following substances were found to have no significant effect on the Cylindrospermopsin assay results: aluminum oxide, calcium chloride, calcium sulfate, manganese sulfate, magnesium sulfate, magnesium chloride, sodium chloride, and potassium phosphate up to 10,000 ppm; sodium thiosulfate, sodium nitrate, and zinc sulfate up to 1,000 ppm; humic acid and ferric sulfate up to 100 ppm; copper chloride up to 10 ppm; Lugol's solution up to 0.01%.

Samples containing methanol must be diluted to a concentration ≤ 20% methanol to avoid matrix effects.

Seawater samples must also be diluted to a concentration ≤ 20% to avoid matrix effects. Alternately, if a lower detection limit is required, interfering compounds can be removed from seawater or brackish water samples prior to analysis. Please see the Cylindrospermopsin in Brackish Water or Seawater Sample Preparation Technical Bulletin (available upon request).

No matrix effects have been observed with samples which have been treated with sodium thiosulfate or ascorbic acid at concentrations up to and including 1 mg/mL. Please see Sample Collection and Handling (Section C) for additional information on sample collection, preservation, and storage.

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

A. Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a second antibody (goat anti-rabbit)
2. Standards (7): 0, 0.05, 0.10, 0.25, 0.50, 1.0, 2.0 ppb, 1 mL each
3. Control: 0.75 ± 0.15 ppb, 1 mL, prepared from a secondary source, for use as a Quality Control Standard (QCS)
4. Sample Diluent, 25 mL, for use as a Laboratory Reagent Blank (LRB) and for dilution of samples above the range of the standard curve
5. Cylindrospermopsin-HRP Conjugate Solution *(Vortex before use.)*, 6 mL
6. Antibody Solution (rabbit anti-Cylindrospermopsin), 6 mL
7. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section E)
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 12 mL

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (20-200 µL)
2. Multi-channel pipette (10-300 µL), stepper pipette (10-300 µL), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section E)
5. Graduated cylinder
6. Paper towels or equivalent absorbent material
7. Timer
8. Tape or parafilm
9. Microtiter plate reader (wavelength 450)
10. Microtiter plate washer (optional)

C. Sample Collection and Handling

Water samples should be collected in glass, polyethylene terephthalate glycol (PETG), high density polyethylene (HDPE), polycarbonate (PC), polypropylene (PP), or polystyrene (PS) containers. Samples can be stored refrigerated for up to 5 days. If samples must be held for greater than 5 days, samples should be stored frozen.

Finished (treated) drinking water samples must be preserved (quenched) with sodium thiosulfate or ascorbic acid immediately after collection to remove residual chlorine. ***Samples can be quenched with sodium thiosulfate or ascorbic acid at concentrations up to and including 1 mg/mL (higher concentrations will cause interference with the assay).*** The quenching of residual chlorine is necessary for treated water samples only. Raw (untreated) drinking water samples (samples not treated with chlorine) do not require additional reagents at the time of collection.

The pH tolerance range of the assay for samples is between pH 4 and pH 11. Drinking water samples treated with ascorbic acid (0.1 mg/mL) and sodium bisulfate (1 mg/mL) according to EPA Method 545 will be below this range and must be adjusted (between pH 4 and pH 7 is recommended) to avoid interference with the assay.

D. Notes and Precautions

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary.

The use of a multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for the addition of the antibody, enzyme conjugate, substrate, and stop solutions in order to equalize the incubation periods on the entire microtiter plate.

To avoid drift and obtain accurate results, the addition of the antibody, conjugate, color, and stop solutions should be performed in less than 2 minutes for each reagent. If additions to the entire microtiter plate cannot be completed in less than 2 minutes, run size should be decreased to the number of rows which can be pipetted in less than 2 minutes.

Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

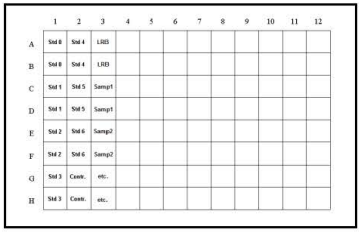
E. Test Preparation

1. Allow the reagents and samples to reach ambient temperature before use.
2. Remove the number of microtiter plate strips required from the resealable pouch. The remaining strips are stored in the pouch with the desiccant (tightly closed).
3. The standards, control, sample diluent (LRB), antibody, enzyme conjugate, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the Wash Buffer (5X) Concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std0-Std6: Standards
Contr.: Control (QCS)
LRB: Laboratory Reagent Blank
Samp1, Samp2, etc.: Samples



G. Assay Procedure

1. Add **50 µL of the standards, control (QCS), LRB, or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. ***Vortex the enzyme conjugate solution.*** Add **50 µL of the enzyme conjugate solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. Add **50 µL of the antibody solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **45 minutes** at room temperature.
4. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips **four times** using the diluted wash buffer. Please use at least a volume of **250 µL of 1X wash buffer** for each well and each washing step. ***Blot the inverted plate after each wash step*** on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
5. Add **100 µL of substrate (color) solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **30-45 minutes** at room temperature. Protect the strips from sunlight.
6. Add **100 µL of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Cylindrospermopsin concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the control (QCS), LRB, and samples will then yield levels in ppb of Cylindrospermopsin by interpolation using the standard curve. Results can also be determined by using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Cylindrospermopsin than standard 1 (0.05 ppb) should be reported as containing < 0.05 ppb of Cylindrospermopsin. Samples showing a higher concentration than standard 6 (2.0 ppb) must be diluted to obtain accurate results. The concentration of the positive control (QCS) provided should be 0.75 ± 0.15 ppb.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of Cylindrospermopsin greater than that standard. Samples which have higher absorbances than a standard will have concentrations of Cylindrospermopsin less than that standard.

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

I. References

(1) Cylindrospermopsin, Review of Toxicological Literature. Prepared by Integrated Laboratory Systems for Scott Masten, National Institute of Health Sciences, RTP, NC. Contract Number N01-ES-65402, December 2000.

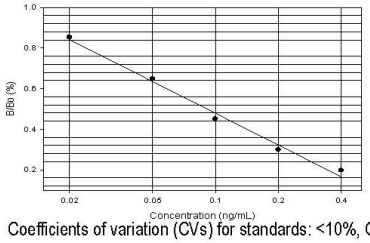
Importance of Saxitoxin Determination
Saxitoxin is one of the "paralytic shellfish poisons" (PSP), produced by several marine dinoflagellates and fresh water algae. Contamination of shellfish with saxitoxin has been associated with harmful algal blooms throughout the world.

In man, PSP causes dose-dependent perioral numbness or tingling sensations and progressive muscular paralysis, which can result in death through respiratory arrest. The maximum tolerance levels established by the EU and FDA are 40-80 µg per 100 g edible portion of fresh, frozen, or tinned shellfish.

The ABRAXIS® Saxitoxin ELISA allows the determination of 42 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in about 1 hour.

Performance Data

Test sensitivity: The detection limit for Saxitoxin is 0.015 ng/mL (mean of 6 blank determinations minus 3 SD). The middle of the test (50% B/B_0) is at approximately 0.09 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.



Test reproducibility: Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.

Selectivity: This ELISA recognizes Saxitoxin and other PSP toxins with varying degrees:

Cross-reactivities:

Saxitoxin (STX)	100% (per definition)
Decarbamoyl STX	29%
GTX 2 & 3	23%
GTX-5B	23%
Lyngbyatoxin	13%
Sulfo GTX 1 & 2	2.0%
Decarbamoyl GTX 2 & 3	1.4%
Neosaxitoxin	1.3%
Decarbamoyl Neo STX	0.6%
GTX 1 & 4	<0.2%

Cross-reactivities with other classes of algal toxins have not been observed.

Samples: Drinking water, ground water, and surface water were tested for matrix effects in the ELISA. No matrix effects were determined.

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Date this User Guide is effective : 20OCT2022
Version: 03

ABRAXIS® Saxitoxin (PSP) ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Saxitoxin (PSP) in Water and Contaminated Samples

Product No. 52255B

1. General Description

The ABRAXIS® Saxitoxin ELISA is an immunoassay for the quantitative and sensitive detection of Saxitoxin. Saxitoxin is one of the toxins associated with paralytic shellfish poisoning (PSP). This test is suitable for the quantitative and/or qualitative detection of Saxitoxin in water samples (please refer to the appropriate technical bulletins for freshwater and seawater samples) as well as other contaminated samples. For shellfish samples, a sample preparation is required. If necessary, positive samples can be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Saxitoxin. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The ABRAXIS® Saxitoxin ELISA Kit should to be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box.

4. Test Principle

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 (anti-rabbit) immobilized on the microtiter 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 plate reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the ABRAXIS® Saxitoxin ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

Samples containing methanol must be diluted to a concentration < 20% methanol to avoid matrix effects.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The ABRAXIS® Saxitoxin ELISA Kit provides screening results. As with any analytical technique (GC/MS, HPLC, etc.), positive samples requiring some action should be confirmed by an alternative method.

6. Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (anti-rabbit)
2. Standards (6) and Control: 0, 0.02, 0.05, 0.1, 0.2, 0.4 ng/mL STX-diHCl, 1.5 mL each
3. Control at 0.075 ± 0.015 ng/mL STX-diHCl, 1.5 mL
4. Antibody Solution (rabbit anti-Saxitoxin), 6 mL
5. Saxitoxin-HRP Conjugate Solution, 6 mL
6. Sample Diluent (10X) Concentrate, 2 X 25 mL, must be diluted prior to use for shellfish or freshwater sample dilutions, see Test Preparation (Section E)
7. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section E)
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 12 mL

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (10-200, and 200-1000 µL)
2. Multi-channel pipette (10-300 µL), stepper pipette with plastic tips (10-300 µL), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section E)
5. Microtiter plate washer (optional)
6. Microtiter plate reader (wave length 450 nm)
7. Shaker for microtiter plates (optional)
8. Materials and reagents for sample preparation
9. Seawater Matrix Saxitoxin Standards (please contact Gold Standard Diagnostics)

C. Sample Preparation (Mussels and other shellfish)

NOTE: If a 100 g sample is needed for regulatory purposes, extraction solution volume should be adjusted accordingly.

1. Remove mussels from shells, wash with deionized water and homogenize.
2. Mix 10 g of homogenized mussels with 10 mL of 0.1M HCl and boil for 5 minutes while stirring.
3. Allow to cool. Centrifuge for 10 minutes at approximately 3500 g.
4. Collect supernatant. Adjust pH to < pH 4.0 with 5 N HCl.
5. Remove 10 µL and dilute in 10 mL of 1X Sample Diluent (this will be a 1:1,000 dilution). Vortex.
6. Analyze as sample (Assay Procedure, step 1).

The STX concentration in the samples is determined by multiplying the ELISA result for the diluted extract by a factor of 2,000. Highly contaminated samples (those outside of the calibration range of the assay), must be diluted further and re-analyzed. We recommend further dilutions of 1:10 with 1X Sample Diluent. The dilution factor will then be 20,000. Samples with low levels of contamination of STX or samples that contain STX congeners with low cross-reactivity (see chart) can be detected in the assay by diluting samples 1:250 before analysis. The assay has low cross-reactivity against GTX 1 & 4, therefore food samples containing these congeners at low concentrations might be underestimated by this ELISA.

D. Alternative Sample Preparation (Mussels and other shellfish)

1. Remove mussels from shells, wash with deionized water and homogenize using a Polytron or equivalent.
2. Mix 1.0 g of homogenized mussels with 6 mL of methanol/DI water (80/20) using a Polytron or equivalent.
3. Centrifuge the mixture for 10 minutes at 3000 g. Collect supernatant.
4. Add 2 mL methanol/deionized water (80/20) to the mussel tissue residue. Re-centrifuge the mixture for 10 minutes. Add supernatant to first portion.
5. Bring the volume of the collected supernatant to 10 mL with methanol/deionized water (80/20). Filter extract through a 0.45 µm filter (Millex HV, Millipore or equivalent).
6. Remove 10 µL and dilute to 1.0 mL with 1X Sample Diluent (1:100 dilution). Vortex. Analyze as sample (Assay Procedure, step 1).

The STX concentration in the samples is determined by multiplying the ELISA result by a factor of 1,000.

E. Test Preparation

Micro-pipetting equipment and disposable pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, conjugate, substrate and stop solutions in order to equalize the incubation periods of the standard solutions and the samples on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

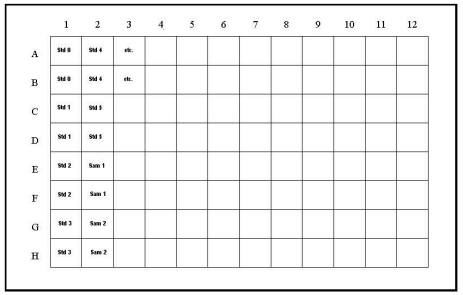
1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, enzyme conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the ABRAXIS® Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water.
5. Dilute the 10X Sample Diluent Concentrate at a ratio of 1:10 with deionized or distilled water (i.e. 1 mL of 10X Sample Diluent Concentrate into 9 mL of deionized water) as needed for sample dilutions.
6. The Stop Solution must be handled with care as it contains diluted H_2SO_4.
7. Freshwater samples must be preserved immediately upon collection to prevent loss of saxitoxin from the samples. Please refer to the Saxitoxin in Freshwater Sample Preparation Bulletin for details.
8. Seawater samples must be analyzed using Seawater Matrix Saxitoxin Standards (available separately, please contact Gold Standard Diagnostics) and an alternate Assay Procedure. Please refer to the Saxitoxin in Seawater Sample Analysis Bulletin for details.

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards
0; 0.02; 0.05; 0.10; 0.20; 0.40 ppb

Sam1, Sam2, etc.: Samples



G. Assay Procedure

1. **Add 50 µL of the standards, control, samples (preserved freshwater), or sample extracts (shellfish)** into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. **Add 50 µL of enzyme conjugate solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. **Add 50 µL of antibody solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 to 60 seconds. Be careful not to spill the contents.
4. **Incubate the strips for 30 minutes at room temperature**.
5. Decant the contents of the wells decant the contents of the wells into a sink. **Wash the strips four times using the diluted wash buffer.** Please use a volume of at least 250 µL of 1X wash buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
6. **Add 100 µL of substrate (color) solution** to the wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 to 60 seconds. Be careful not to spill the contents. **Incubate the strips for 30 minutes at room temperature**, protected from direct sunlight.
7. **Add 100 µL of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

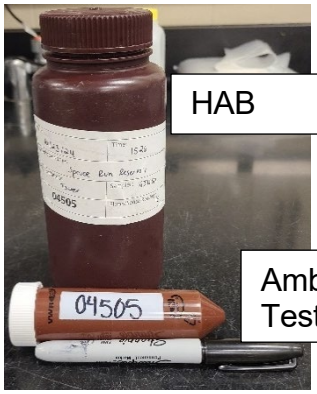
H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding Saxitoxin concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the control and samples will then yield levels in ppb of Saxitoxin by interpolation using the standard curve.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing lower concentrations of Saxitoxin than standard 1 (0.02 ng/mL) are considered as negative. Samples showing a higher concentration than standard 5 (0.4 ng/mL) must be diluted further to obtain accurate results. Results must be multiplied by the appropriate dilution factor for the sample extract (see Sample Preparation, Sections C and D) or preserved freshwater sample (see Saxitoxin in Freshwater Sample Preparation technical bulletin).

As with any analytical technique (GC/MS, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

Quick SOP for collecting Saxitoxin/Anatoxin preserved samples:

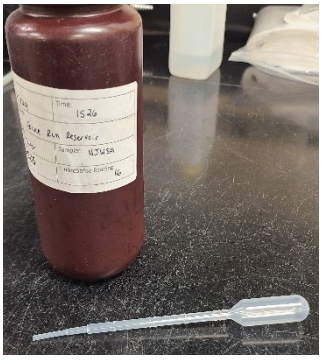


NOTE THE AMBER TEST TUBE HAS PRESERVATIVE IN IT. DO NOT PLACE IT IN THE WATER.

1. Label with a sharpie the amber test tube with the same 5- digit HAB # (example: 04505) used on the 1-L amber HAB bottle.

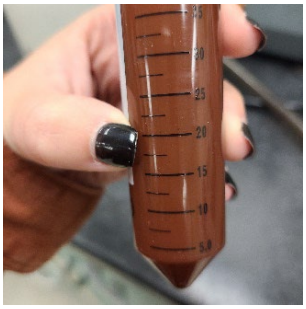
2. Collect water sample into the HAB bottle and log in the sample as usual using app. No need to add info about test tube.

3. **All amber test tube(s) are pre-filled with preservative and must be stored below 86F (store in cooler if needed)**. Mix content of sample in 1-L HAB bottle. Use a disposal syringe/ pipette to extract **18ml and dispense into amber test tube. Do not overfill**. Holding the test tube up to a light/sunlight may help to see where your waterline is. **DO NOT** place the test tube into the waterbody you are testing. The preservative will come out and will ruin your sample.



4. Make sure the lid is on tight. Begin to invert the amber test tube to mix. Put both the HAB bottle and amber test tube in cooler on ice and deliver to BFBM within 24 hours of collection.

5. Make sure all sample lid(s) are on tight**. If the temperature is expected to go above 86F, please put amber test tube sample in a container and place it in a cooler filled with ice. Optionally, for extra security to ensure water sample is not lost, wrap the top (lid attach to tube) of the test tube with electrical tape.**



Fill to 18ml line. Amber test tubes are pre-filled with preservative (2 ml) should total to 20ml.

Preservative in amber test tube – **DO NOT SUBMERGE IN WATER**

NEW JERSEY DEPARTMENT OF HEALTH (&,OR) SENIOR SERVICES
ENVIRONMENTAL and CHEMICAL LABORATORY SERVICRS

PRESERVED NUTRIENTS
POTABLE STREAM NONPOTABLE

FIELD IDENTIFICATION NUMBER

PRESERVATIVE.....**SULFURIC ACID**

NEW JERSEY DEPARTMENT OF HEALTH & SENIOR SERVICES
ENVIRONMENTAL and CHEMICAL LABORATORY SERVICES

METALS
POTABLE STREAM NONPOTABLE

FIELD IDENTIFICATION NUMBER

PRESERVATIVE.....**NITRIC ACID**

NEW JERSEY DEPARTMENT OF HEALTH & SENIOR SERVICES
ENVIRONMENTAL and CHEMICAL LABORATORY SERVICES

GENERAL POLLUTANTS
POTABLE STREAM NONPOTABLE

FIELD IDENTIFICATION NUMBER

PRESERVATIVE.....**NONE**

Date: Time:

Fluorosense(µg/L):

Lake Name:

Station ID:

Lab Number:

Chlorophyll a label (BFBM Lab)

Date:	Time:
Waterbody Name:	
Location/SiteID:	Sampler:
04318	FluoroSense Reading:

HAB sample label (BFBM Lab)

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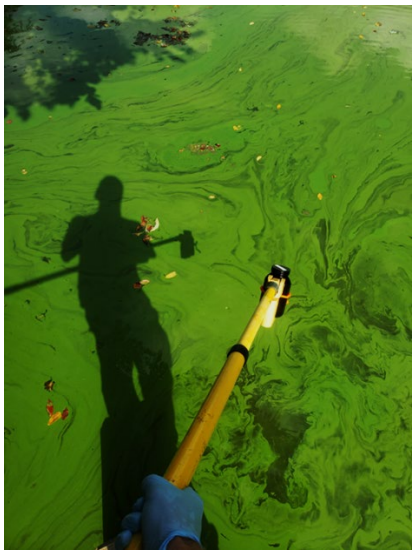
Unique identification numbers (NJDOH Lab)

Standard Operating Procedures
Partner Phycocyanin Meter Loan Program
2024



Prepared By:

Bureau of Freshwater and Biological Monitoring

Table of Contents

1.0 Introduction

1.1 Purpose and Scope

The purpose of this Standard Operating Procedures (SOP) is to outline the specific use of field fluorometers to measure phycocyanin pigment in response to suspected Cyanobacteria Harmful Algal Bloom (HAB) reports. Monitoring will comply with the most recent revision of the New Jersey Cyanobacterial Harmful Algal Bloom Response Strategy (Strategy) and specifically the Agency Responsibilities defined in the Strategy for BFBM. The scope of this SOP, which aligns with the Strategy for lakes, rivers, and streams with potential public access, recreational use, bathing beaches (including licensed beaches), and sources of drinking water. These waterbodies may be owned or operated by state, county, municipal, federal, or private entities. As such, coordination of the investigation and response activities will vary depending on ownership.

Training videos are available and encouraged to view on how to take phycocyanin measurements and reporting a suspected HAB: https://www.state.nj.us/dep/hab/outreach-material.html#vids

1.2 Background

Cyanobacteria are a type of bacteria capable of photosynthesis. Although they are not true algae, they are often referred to as “blue-green algae”. Cyanobacteria frequently impart off-tastes and odors to the water in which they grow. Some species can produce toxins (known as Cyanotoxins) that can be harmful to the health of humans and other animals. Although problems related to cyanobacteria most often occur in freshwaters (lakes and streams), cyanobacteria can also be found in marine waters.

A cyanobacterial Harmful Algal Bloom (HAB) is the name given to the excessive growth, or “bloom”, of cyanobacteria, some of which can produce one or more types of potentially harmful toxins. HABs can occur under suitable environmental conditions of light, temperature, nutrients, and calm water. These “blooms” often result in a thick coating or “mat” on the surface of a waterbody, often in late-summer or early fall.

1.3Cyanobacterial Blooms

Cyanobacterial blooms may vary in species community composition, residence time, toxins they produce, and toxicity and risk to human health, pets, livestock, and wildlife. The distribution and concentration of blooms may be affected by weather and lake conditions such as rain, wind, and currents. Distributions can be lake or waterbody-wide or localized near the shoreline, shallows, or areas affected by flows or the influx of nutrients.

Figure 1. Example of HAB in

Cyanobacteria may maintain a position at a particular depth or may be found throughout the water column where light penetrates (e.g. *Planktothrix, Cylindospermopsis*). Cyanobacteria may migrate vertically to different locations in the photic zone (where light penetrates) throughout the day. Surface accumulations (scum) may develop when cyanobacteria float to the surface during calm, sunny weather and may dissipate within hours as conditions change. Entire cyanobacteria populations may accumulate at 1 or 2 cm below the water surface. Surface accumulations of cyanobacteria may concentrate further when blown by wind to leeward areas like bays, inlets, or near-shore areas (with the direction of the wind). Dense accumulations may extend from the surface to depths more than 1 m.

2.0 Safety

The most common exposures to cyanobacteria and their toxins are believed to occur during recreational activities by mouth, skin, and inhalation routes. Oral exposure may occur from accidental or deliberate ingestion of water. Dermal exposure may occur by direct contact of exposed parts of the body during recreational activity in water containing cyanobacteria. Inhalation may occur through the inhalation of contaminated aerosols while recreating.

Adverse health effects from recreational exposure to cyanobacterial cells and cyanotoxins can cause effects ranging from a mild skin rash to serious illness or death.

Partners must wear gloves provided by BFBM when collecting measurements.

3.0 Response

BFBM receives reports of suspected HABs via the public survey123 reporting application that is part of
the NJDEP HAB Interactive Map Reporting and Communication System.
A suspected HAB may also be received internally from staff during routine monitoring, continuous monitoring stations/ buoys or fight surveillance when scheduled.
DEP continuous monitoring data can be found here: http://njdep.rutgers.edu/continuous/ .

Partners will be notified by BFBM of any reported HABs at their waterbody when phycocyanin measurements and field surveys are needed. Partners may also submit phycocyanin measurements and field surveys at any time through the survy123. Phycocyanin measurements are entered in the comments section.

Upon receipt of suspected HAB report from outside of DEP, the BFBM HAB coordinator or designee will assess the information provided in the suspected HAB report, deploy staff, and/ or coordinate with partners as necessary. The BFBM HAB coordinator will also notify the responsible agency designated for the water body, e.g. a State Park, Watershed Management Area (WMA), local health department. If the responsible agency has trained HAB sampling staff and proper sampling equipment and supplies, they may be requested to perform some of the response activities as in this SOP.

Every effort will be made to respond to reported suspected HABs as soon as possible. In the event resources are limited, the monitoring will be prioritized based on risk to public health.

4.0 Field Survey

A field survey is performed to gather information following reports of suspected HABs. BFBM staff will record site coordinates, observations, take photos, phycocyanin measurements, and determine if sampling is warranted. Upon receipt of the report as HAB survey, BFBM will determine if partner assistance is needed to collect samples for laboratory analysis. If partners are requested to collect samples, BFBM will provide bottles, sampling instructions, and coordinate delivery to the laboratory.

4.1 Site Coordinates and Observations

Sites coordinates and observations will be collected and submitted using the Survey123 reporting application that is part of the NJDEP HAB Interactive Map Reporting and Communication System. Each site on the waterbody will require a unique Survey123 submission. A training video can be found here: HAB Training Video. Part 1: How to report a HAB - YouTube

4.2 Photo Documentation

Photos are used to record conditions at the time of the field screening. Visual observations recorded by photos may be used as part of the screening process to determine the possible presence of a HAB as opposed to other algae or aquatic vegetation that may be confused with a HAB such as duckweed, watermeal, or filamentous algae. Additional example images can be found at: https://www.state.nj.us/dep/hab/photo.html . A series of photographs shall be collected to show the extent of the bloom:

Figure 2. Aquatic Vegetation- Duckweed and Watermeal.

1. Parallel to the shoreline to show a length of approximately 50 feet
2. A picture to indicate how far into the lake evidence of a bloom extends
3. A picture taken while standing in the water or at the edge of the shoreline with the camera pointed straight down to capture the surface.
4. A photo of the beach area (if present)
5. A photo of public access points, e.g. boat launch, fishing pier.
6. Other pertinent photos.

The purpose of the photo series is to demonstrate the appearance of the water which may indicate typical HAB features such as green streaks or pea soup. Color changes from the affected areas of a waterbody compared with the non-affected areas help to indicate the severity and distribution of the bloom.

4.3 Phycocyanin Measurements

Phycocyanin is a unique pigment to cyanobacteria, therefore the presence of phycocyanin is an indicator of cyanobacteria presence. Handheld field fluorometers measure the presence and relative concentration of phycocyanin and may be used to qualitatively demonstrate whether cyanobacteria, if present, are in bloom densities. Phycocyanin measurements cannot predict toxin production and concentration, nor quantify cell density directly. However, these measurements can be used as a preliminary screening tool for suspected HABs and to monitor the status of confirmed HABs.

2023 / 2022 Comparative Summary of FluoroSense (µg/L) and Cell Counts

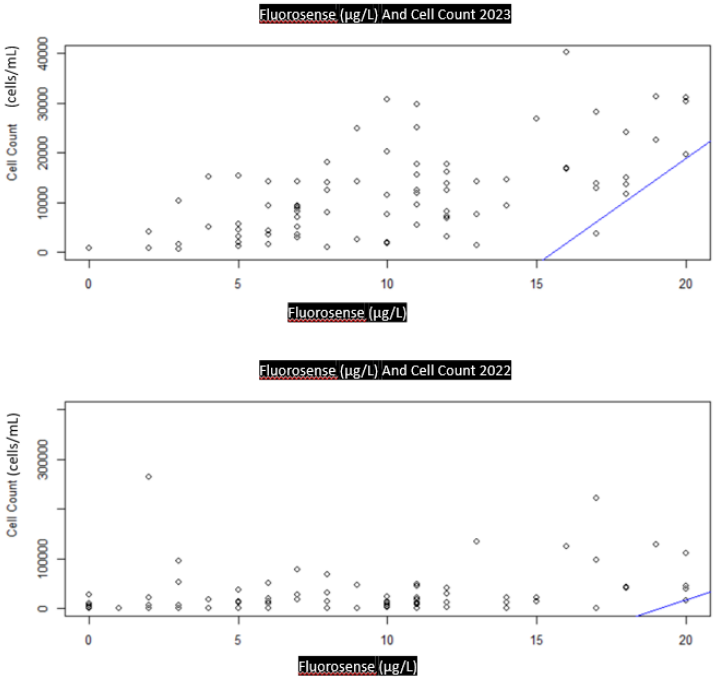


Figure 1

Figure 2

This graph is for the handheld field meter that we will loan to partners and shows correlation developed between cell count and phycocyanin measurements. Correlations are meter specific, different models of meters have different ranges. In recent years, NJDEP has re-evaluated the cell count and FluoroSense meter correlations. The readings to be screening for when monitoring your waterbody is around 13 – 14 µg/L which may be a preliminary indicator to a possible "watch" event.

Cells per ml	**FluoroSense Meter**	**Possible HAB Alerts***
20,000	13	Watch
40,000	23	Advisory
80,000	44	
100,000	55	

***Note**: HAB alerts are determined by cell counts and toxin analysis only. This is to display how the FluoroSense meter is a preliminary screening tool to respond to the potential onset of a HAB.*

Note these measurements are for screening and not intended to replace lab analysis used to determine alerts. In addition, these correlations are based on statewide data, differences may occur at specific waterbodies.

Meter Use

Fluorescence is temperature sensitive. As the temperature of the sample increases, the fluorescence decreases. For greatest accuracy, record the sample temperature and correct the sensor output for changes in temperature if instructed by BFBM. Meter should not be exposed to temperatures beyond the range of 41 to 104°F.

Below are basic procedures. Refer to the Turner Designs manufacturer's manual for any necessary details or troubleshooting.

Calibration (For information purposes. BFBM will provide a fully calibrated meter and recalibrate if necessary). The handheld fluorometer is factory calibrated. However, standard checks and subsequent re-calibration may be necessary.

1. Turn on the power button
2. Place the sensor cap in the standard 200 ppb Rhodamine dye until fully submerged
3. Shake gently so bubbles are purged from the cap
4. Record reading in field notebook and field sheet.
5. 200 ppb Rhodamine dye should read as 100 ppb phycocyanin. If reading is not 100 ppb ± 3% then re-calibration is necessary.
6. To calibrated press and hold "read" while unit is off
7. While continuing to hold "read" press and hold power button until 100 is displayed
8. Place sensor in standard as above
9. The unit will flash "td" four times then automatically turn off; at this point calibration is complete
10. Turn on unit and check with 200 ppb Rhodamine dye as above.
11. If reading is still not 100 ppb ± 3% arrange for service before using

Sample Measurement

A training video on the below procedure is available at: [HAB Training Video. Part 2: Phycocyanin measurements - YouTube]
Measure the temperature of the water using the proved thermometer Record the temperature in the comments section of the Survey123.

1. Turn on the power button.
2. Using a cup filled with tap water, place the sensor cap in the water and submerge it to the designated line.
3. Shake gently so bubbles are purged from the cap.
4. Press the 'Read' button, the unit will flash "td" three times, then the fluorometer reading will be displayed.
5. If the tap water is > zero, contact BFBM as the meter may be out of calibration. If the reading is zero, continue to next step to measure the waterbody.
6. Place the sensor cap in the waterbody until fully submerged. Figure 2.
7. Shake gently so bubbles are purged from the cap.
8. Press the 'Read' button, the unit will flash "td" three times, then the fluorometer reading will be displayed.
9. Repeat three times, record the median (middle) value in the Survey123 comments sections. Make sure that the fluorosense reading is reported first and then add all comments after that.

Figure 3. Submerged

5.0 Loan Expectations

- DEP
  - Coordinate meter maintenance and calibration.
  - Perform confirmation lab analysis as needed.
  - Provide sample bottles and coordinate sample delivery.
- Partners
  - Provide direct contact person
  - Take meter measurements for screening and status as needed.
  - Use the DEP HAB Reporting system to submit measurements and other site information.
  - Inform DEP of any meter issues.

If at any time you have questions regarding the meter use contact BFBM at: 609-292-0427, BFBMHABS@dep.nj.gov and someone will respond within 24 hours.

6.0 Sample Collection for Lab Analysis

A training video on the below procedure is available at: [HAB Training Video. Part 3: Sample collection - YouTube]

Upon receipt of the Survey123, BFBM will deploy samplers based on the information received including photos and other observations.

If collecting samples for BFBM as a partner, a separate Survey123 is used which requires a log in user ID. BFBM will coordinate with partners to assign credentials if necessary and provide bottles, labels, and other specific instructions.

- All PPE safety requirements will comply with Bureau of Freshwater and Biological Monitoring Field Work Health and Safety Plan (HASP) Version #2 August 2019. BFBM will provide this document to partners that collect samples.

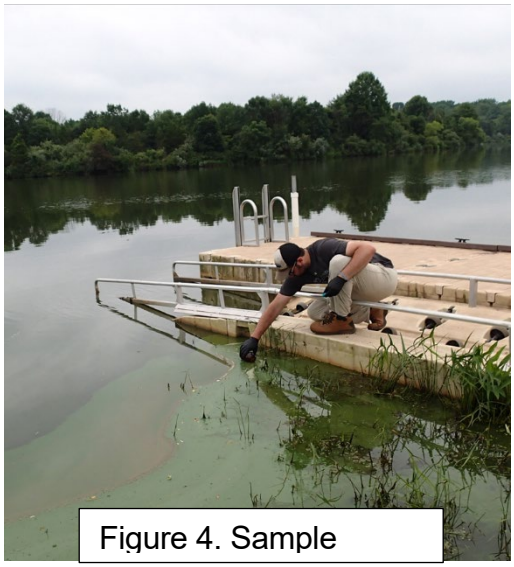


Figure 4. Sample

Samples for HAB analysis include: cyanobacterial IDs, cell counts, toxin analyses (microcystins, anatoxin, saxitoxin, and/ or cylindrospermopsin) and/or chlorophyll a. To collect samples:

1. Complete the Survey123 for sample collection
2. Collect samples at designated locations, filling ¾ of one (1) 500 ml brown plastic bottle made of polyethylene terephthalate glycol (PETG), High Density Polyethylene (HDPE), or amber glass
3. Please write out the entire waterbody name in the waterbody field (e.g. Lake Hopatcong….NOT hopatcong)
4. Site ID should either be a preexisting site ID (from an existing network or previous HAB site) or an accurate short description of the site e.g. beach (include exact name if available such as XYZ Beach Club), boat dock, boat ramp, etc.
5. Samples should be collected just below the surface s immersed approximately 3-6 inches(make sure algae
6. Fill out the HAB sample label and place on bottle
7. Refrigerate samples, or place in cooler with ice
8. Submit to lab ASAP, but not more than 24 hours

Figure 5. Sample

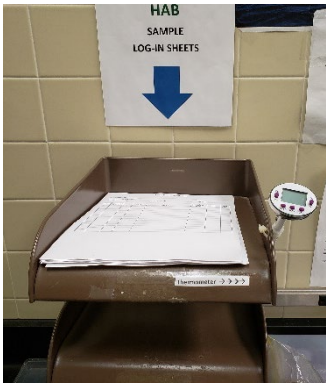
Sample bottle Cleaning
All bottles are cleaned by BFBM in an automated lab washer using phosphate free detergent, several hot tap water rinses, with several final deionized water rinses.

7.0 Public Report of Data and Alerts

Data used for Alert decisions will be uploaded into the DEP HAB Interactive Map Reporting and Communication System by designated BFBM staff. Only validated data and Alert recommendations will be entered and enabled for public access. Data approved for release before 8:00 am will be emailed to designated contacts. The data will be released to the public at 12:00 noon daily. All HAB raw data will be saved in the BFBM HAB database located on the DEP server for security and redundancy. This database may be used for additional summary statistics as requested.

HAB sample Lab Processing Procedure

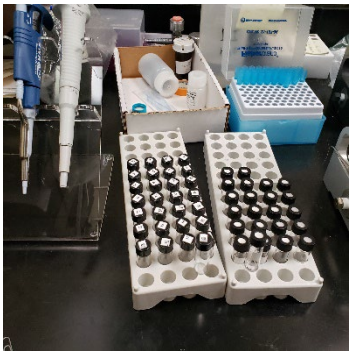
When a HAB sample is delivered/received at the BFBM lab, please follow the steps below for processing:



Filling out HAB sample Form:

- Fill out a blank "Suspected HAB Sample Readings" form found in the labeled inbox at rear left corner of lab 106 benchtop.
    a. Enter waterbody name
    b. Date of sample collection
    c. Sampler initials or affiliate name if not BFBM staff
    d. Enter date/time sample was received at lab
    e. Using the digital thermometer (located next to sample forms), read and record sample temperature on form
    f. Enter your initials as the analyst
    g. Using the HAB label attached to each sample bottle, fill out table on the form with information for each sample/location on separate row, starting with the 5 digit HAB sample #

- Starting with the lowest numbered vial from the vial trays (shown in image below), enter the vial number onto the sheet (under 1st column, "4ml Vial#") consecutively, for each sample location recorded.

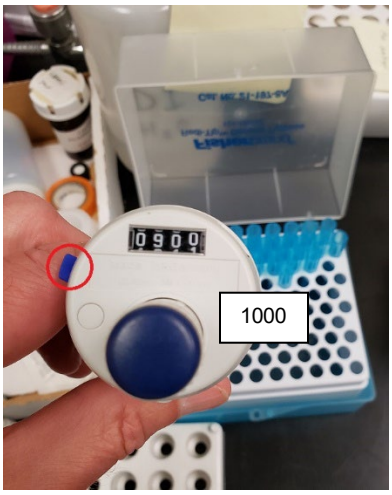


Collecting Toxin sample:

1. For each sample bottle, you will need to collect 2 sample vials (required for diff toxins). The image on the right shows a tray of vials with a number on the lid and the other tray has the corresponding #s on the glass vials with the letter "A" after it. The "A" vial (with the septum lid) contains 0.2 ml of a diluent needed for preservation; the other vial (with the numbered lid) is empty (amber bottles).

2. Select the grey, 1000 ul, Eppendorf repipettor from the pipette rack as seen in photo.

3. Check fill volume, located on the top end of repipettor, it should be set at **1000** as shown in image below. If not set at **1000**, press the blue button circled in red in image below and turn the large blue plunger to the right or left to adjust the value to **1000**. Release blue button and confirm **1000** is displayed.

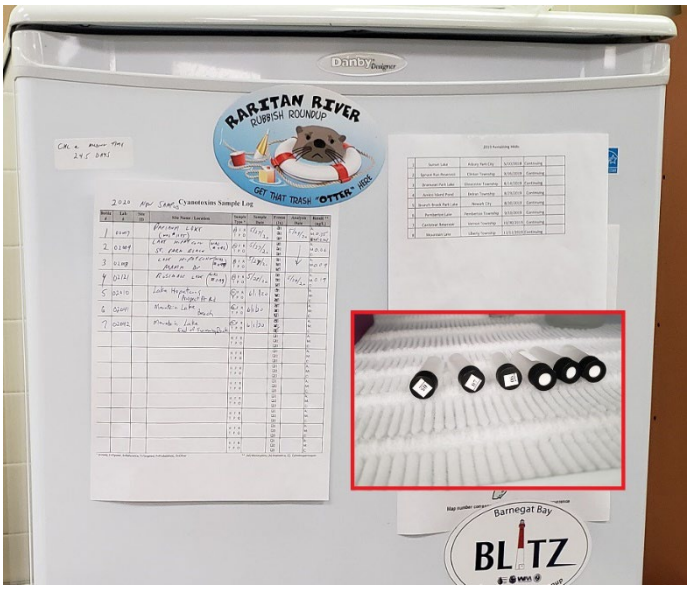


4. Add a clean "blue" tip from tip rack box onto the re-pipettor end by pushing down until firmly attached (see image on far right). Press the blue plunger down to the first stop and slowly release a few times to lubricate plunger.

5. Select the first sample bottle as listed on the Hab sample form, and mix well by inverting several times. Remove the lid from the sample bottle as well as also removing lids from both of the corresponding numbered 4ml vials (from column 1 of your bench sheet). Press the plunger to the first stop, insert the blue tip halfway below the surface of the HAB sample and slowly release plunger. Place sample pipette tip into one of the 4ml vials and slowly press and release the 1000ul sample into the vial. Repeat this step twice for each vial (2000 ul sample total in Each vial). Attach the proper lid to the correct vial (numbered lid on unlabeled glass vial and septum lid onto "A" numbered glass vial) and be sure to securely tighten the lid. Once finished, put lid back on sample bottle and place bottle into water bath to warm to room temp.

6. Hold the repipettor over trash can and press the plunger fully to eject the blue pipette tip for disposal. If you have more than one sample, repeat steps 4 and 5 above for each sample, using a fresh pipette tip.

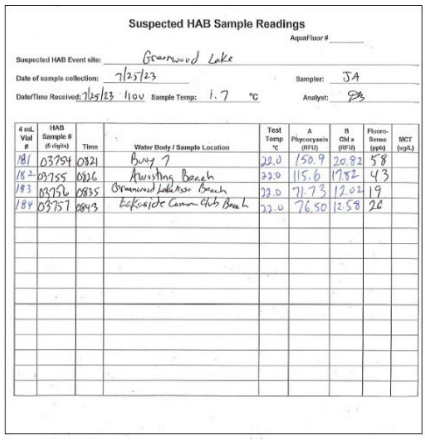
7. Place the 4 ml vials into the small freezer on bench top near **Exit** door (see image). Vials should be placed on a shelf on their sides to safely freeze without cracking (see image inset).



8. Add sample info to the "Cyanotoxin Sample Log" sheet on the outside of freezer door (blank copies in folder to left of freezer). Check off first box for this first freeze.

Taking Aquafluor readings:

1. Place HAB sample bottles into a bath of “warm” water to equilibrate to room temperature.
2. Periodically remove and invert sample bottles several times to speed up temp equilibration.
3. Once samples have reached room temp, turn ON the Aquafluor instrument and select channel **A** (toggle the A/B button to select each channel).
4. With gloved hands, half fill a 10 ml test tube with the sample of interest.
5. Thoroughly wipe the sides of the test tube dry and place the tube into the Aquafluor cell chamber.
6. Press either Read button (there are 2 of them) and the numbers on the Aquafluor screen will begin flashing whiles readings are taken. After about 10 seconds the screen will stop flashing and will display the average result. Record the sample result on the bench sheet under the **A** (Phyco) column.
7. Press the A/B button to select the **B** channel and press the Read button. Once instrument has settled, Record that reading in the column under **B** (Chl).
8. Repeat the above steps for all samples.
9. Once complete, place all HAB sample bottles into sample fridge for storage. Please do not discard sample!



Suspected HAB Sample Readings

AquaFluor #: _____

Suspected HAB Event site: Greenwood Lake

Date of sample collection: 7/25/23 Sampler: JA

Date/Time Received: 7/25/23 1100 Sample Temp: 1.7 °C Analyst: DB

4 mL Vial #	HAB Sample # (6 digits)	Time	Water Body / Sample Location	Test Temp °C	A Phycocyanin (RFU)	B Chl a (RFU)	Fluoro-Probe (µg/L)	MCT (ug/L)
181	037754	0821	Buoy 7	22.0	150.9	20.92	58	
182	037755	0826	Awosting Beach	22.0	115.6	17.82	43	
183	037756	0835	Greenwood Lake Inn Beach	22.0	71.73	12.02	19	
184	037757	0843	Lakeside Canoe Club Beach	22.0	76.50	12.58	26	

For Data Management purposes, Water Chemistry is defined as parameters analyzed by a lab; Field measurements are defined as analyze immediately parameters.

Inventory

Geographic Regions	Statewide
Counties	All
Dates	March 1, 2025 through November 30, 2029
Status	Future/Planned
Sample Frequency	Periodic
Seasons Sampled	Winter, Spring, Summer, Fall
Waterbody Type	Lakes, Reservoirs
Salinity Category	Fresh
Tidal Influence	Non-tidal
Project Description	Statewide Statistical lakes will be monitored to evaluate the trophic state of lakes and assess the ecological health of the State's lentic water resources. Statewide reference lakes will be monitored to document baseline, status, and trend information on minimally or non-impacted water quality, ecological integrity and the trophic state of lakes within each ecoregion. Targeted regional lakes will be monitored to produce a robust assessment of environmental conditions affecting water quality in a selected water region according to the Integrated Water Quality Monitoring and Assessment Report cycle.
Parameters analyzed type	Biological - Algae; Chemical/physical: Nutrients

Data Management Supplement

QAPP network path file location?	V:\LUM\BFBM\Bfbm\Quality Assurance Plans\Calendar Year 2025 QAPPs
Where will data be recorded in field (media)	Field data sheet, YSI Pro DSS, Field Notebook
If data collected electronically, where will it be stored?	V:\LUM\BFBM\Lakes and Fishibi\Lakes Monitoring\Databases
Format to be received from Lab	DOH Text File
Method of receipt from lab/s	Citrix
Personnel receiving outside lab data	Brian Taylor, Carly Conticchio
Will data be uploaded to WQDE/STORET?	Yes

Lake Sample Station locations

Station ID (WQDE compliant & referenced)	Waterbody/ Location	Latitude-dd	Longitude-dd	County	Site exists in WQDE?	Location Type
NJW04459-058-1	Mt. Misery Lake	39.925783	-74.526095	Burlington	Yes	Reference Lake
NJW04459-058-2	Mt. Misery Lake	39.924246	-74.523682	Burlington	Yes	Reference Lake
NJW04459-352-1	Hands Mill Pond	39.243287	-74.901574	Cumberland	Yes	Reference Lake
NJW04459-352-2	Hands Mill Pond	39.245199	-74.904330	Cumberland	Yes	Reference Lake
NJLM-0028-1	Green Turtle Lake	41.143351	-74.329651	Passaic	Yes	Reference Lake
NJLM-0028-2	Green Turtle Lake	41.148516	-74.329033	Passaic	Yes	Reference Lake
NJW04459-233-1	Watchu Pond	40.928053	-74.770434	Sussex	Yes	Reference Lake
NJW04459-233-2	Watchu Pond	40.930143	-74.769398	Sussex	Yes	Reference Lake
NJW04459-233-3	Watchu Pond	40.931352	-74.767907	Sussex	Yes	Reference Lake
NJW04459-134-1	Silver Lake	41.121719	-74.532404	Sussex	Yes	Reference Lake
NJW04459-097-1	Great Gorge Lake	41.161202	-74.523444	Sussex	Yes	Reference Lake
NJW04459-097-2	Great Gorge Lake	41.161322	-74.518986	Sussex	Yes	Reference Lake
NJW04459-339-1	Mashipacong Pond	41.269306	-74.726483	Sussex	Yes	Reference Lake
NJW04459-339-2	Mashipacong Pond	41.265440	-74.729664	Sussex	Yes	Reference Lake
NJW04459-009-1	Deer Park Pond	40.903843	-74.796497	Warren	Yes	Reference Lake
NJW04459-009-2	Deer Park Pond	40.906200	-74.794777	Warren	Yes	Reference Lake
NJLM-1224-1	Newton Lake	39.90257	-75.0931	Camden	Yes	Targeted Lake
NJLM-1224-2	Newton Lake	39.90781	-75.0828	Camden	Yes	Targeted Lake
NJLM-1224-3	Newton Lake	39.91003	-75.0737	Camden	Yes	Targeted Lake
NJLM-1224-4	Newton Lake	39.90471	-75.0694	Camden	Yes	Targeted Lake
NJLM-0626-1	Big Pine Lake	39.98592	-74.5705	Burlington	Yes	Targeted Lake
NJLM-0626-2	Big Pine Lake	39.98283	-74.5724	Burlington	Yes	Targeted Lake
NJLM-0626-3	Big Pine Lake	39.9799	-74.5734	Burlington	Yes	Targeted Lake
NJLM-0985-1	Malaga Lake	39.58352	-75.0591	Gloucester	Yes	Targeted Lake
NJLM-0985-2	Malaga Lake	39.57871	-75.0588	Gloucester	Yes	Targeted Lake
NJLM-0985-3	Malaga Lake	39.57477	-75.0589	Gloucester	Yes	Targeted Lake
NJLM-1279-1	Shaws Mill Pond	39.31663	-75.1538	Cumberland	Yes	Targeted Lake
NJLM-1279-2	Shaws Mill Pond	39.31485	-75.155	Cumberland	Yes	Targeted Lake
NJLM-1279-3	Shaws Mill Pond	39.313	-75.1558	Cumberland	Yes	Targeted Lake
NJLM-0285-1	Elmer Lake	39.58951	-75.1646	Salem	Yes	Targeted Lake
NJLM-0285-2	Elmer Lake	39.59289	-75.165	Salem	Yes	Targeted Lake
NJLM-0285-3	Elmer Lake	39.59584	-75.1656	Salem	Yes	Targeted Lake
NJLM-0086-1	Country Lake	39.95035	-74.5498	Burlington	Yes	Targeted Lake
NJLM-0086-2	Country Lake	39.94755	-74.5535	Burlington	Yes	Targeted Lake
NJLM-0086-3	Country Lake	39.94737	-74.5481	Burlington	Yes	Targeted Lake
NJW04459-430-1	Grenloch Lake	39.7828	-75.0578	Gloucester	Yes	Targeted Lake
NJW04459-430-2	Grenloch Lake	39.78124	-75.0548	Gloucester	Yes	Targeted Lake
NJLM-0456-1	Gropp Lake	40.18828	-74.6875	Mercer	Yes	Targeted Lake
NJLM-0456-2	Gropp Lake	40.19124	-74.6837	Mercer	Yes	Targeted Lake
NJW04459-120-1	Sunset Lake	39.45058	-75.2418	Cumberland	Yes	Targeted Lake
NJW04459-120-2	Sunset Lake	39.44829	-75.2377	Cumberland	Yes	Targeted Lake

Station ID (WQDE compliant & referenced)	Waterbody/ Location	Latitude-dd	Longitude-dd	County	Site exists in WQDE?	Location Type
NJW04459-120-3	Sunset Lake	39.44697	-75.2406	Cumberland	Yes	Targeted Lake
NJW04459-378-1	Almonesson Lake	39.81709	-75.0964	Gloucester	Yes	Targeted Lake
NJW04459-378-2	Almonesson Lake	39.81402	-75.0973	Gloucester	Yes	Targeted Lake
NJW04459-378-3	Almonesson Lake	39.81187	-75.0984	Gloucester	Yes	Targeted Lake

Statistical lakes will be added once Stations are established.