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**SAMPLING AND ANALYSIS WORKPLAN FOR
CYANOBACTERIA AND CYANOTOXIN MONITORING
IN SELECTED LAKES IN INDIANA
2017**



PREPARED BY

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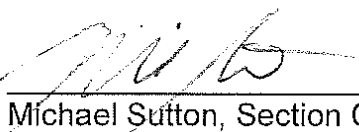
**SAMPLING AND ANALYSIS WORKPLAN FOR
CYANOBACTERIA AND CYANOTOXIN MONITORING IN SELECTED
LAKES IN INDIANA
Rev 2017**

**Indiana Department of Environmental Management
Office of Water Quality
Watershed Assessment & Planning Branch
Indianapolis, Indiana**

TECHNICAL REVIEW

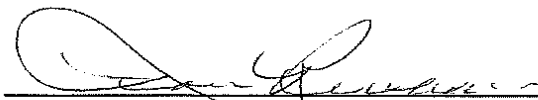
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The IDEM Quality Improvement (QI) Section staff reviewed, and does approve, this
Quality Assurance Project Plan (QAPP).

 Date 3/27/17
Quality Improvement Program Staff, Office of Compliance Support

FOREWORD

Work Plan versus QAPP:

This Sampling and Analysis Work Plan is an extension of the existing Watershed Assessment and Planning Branch, October 2004 “*Quality Assurance Project Plan (QAPP) for Indiana Surface Water Quality Monitoring and Total Maximum Daily (TMDL) Program*” and serves as a link to the existing QAPP as well as an independent QAPP of the project. As per the U.S. EPA QAPP guidance, this Work Plan establishes criteria and specifications pertaining to a specific water quality monitoring project that are usually described in the following four groups (phases) or sections as QAPP elements:

Phase A. Project Management/Planning

The plan documents project history and objectives, and establishes Data Quality Objectives (DQOs).

Phase B. Measurement/Data Acquisition

The plan describes sampling procedures, analytical methods, sample and data acquisition requirements, and the quality control measures specific to the project.

Phase C. Assessment/Oversight

The plan identifies the key elements of external and internal checks, audits, peer reviews, Data Quality Assessments (DQAs), and the preparation of QA/QC Review Reports to management.

Phase D. Data Validation and Usability

The plan describes data handling and associated QA/QC activities including QA/QC Review Reports.

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LIST OF ACRONYMS

| | |
|---------|--|
| ADDA: | 4 <i>E</i> ,6 <i>E</i> -3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid |
| AIMS: | Assessment Information Management System |
| AB: | Assessment Branch |
| CEES: | Center for Earth and Environmental Science |
| CFR: | Code of Federal Regulations |
| CLP: | Contract Laboratory Program |
| CRQL: | Contract Required Quantification Limit |
| CWA: | Clean Water Act |
| DO: | Dissolved Oxygen |
| DQA: | Data Quality Assessment |
| ELISA: | Enzyme Linked Immunosorbant Assay |
| EPA: | Environmental Protection Agency |
| GPS: | Global Positioning System |
| HUC: | Hydrologic Unit Code |
| IAC: | Indiana Administrative Code |
| IC: | Indiana Code |
| IDEM: | Indiana Department of Environmental Management |
| IUPUI: | Indiana University Purdue University at Indianapolis |
| MDL: | Method Detection Limit |
| mg/L | Milligram gram per liter |
| µmho/cm | Micromho per centimeter |
| ml | Milliliter |
| NHD: | National Hydrography Dataset |
| NTU: | Nephelometric Turbidity Unit(s) |
| OWQ: | Office of Water Quality |
| QA: | Quality Assurance |
| QAC: | Quality Assurance Coordinator |
| QAM: | Quality Assurance Manager |
| QAO: | Quality Assurance Officer |
| QA/QC: | Quality Assurance/Quality Control |
| QC: | Quality Control |
| QAPP: | Quality Assurance Project Plan |
| RL: | Reporting Limit |
| RPD: | Relative Percent Difference |
| SM: | Standard Methods |
| SOP: | Standard Operating Procedures |
| SS: | Stainless Steel |
| SU: | Standard Units |
| µg/l: | Micrograms per liter |
| U.S.: | United States |
| USEPA: | United States Environmental Protection Agency |
| WAPB: | Watershed Assessment and Planning Branch |
| WHO: | World Health Organization |

Cyanobacteria and Cyanotoxin Program Objective

The Indiana Department of Environmental Management's (IDEM) Office of Water Quality (OWQ) is responsible for sampling and assessing Indiana's surface water pursuant to the Clean Water Act (CWA) Section 305(b). IDEM has developed a surveillance program for lakes to warn the public when cyanobacteria and cyanotoxins are present in large enough quantities to render recreation unsafe.

I. Project Management/Planning

(QAPP Elements A4, A5, A6, A7, A8)

Project/Task Organization and Schedule: (QAPP Element A4)

The IDEM Watershed Assessment and Planning Branch (WAPB) staff will sample for cyanobacteria and assess for high cell counts and concentrations of the cyanotoxins microcystins, cylindrospermopsin and anatoxin-a during the summer recreational season (May through August) at designated swimming beaches managed by the Indiana Department of Natural Resources (DNR). Samples may be collected outside of this timeframe at the request of DNR. Water samples will be collected for the following nutrient parameters: ammonia nitrogen, nitrate/nitrite, total Kjeldahl nitrogen, and total phosphorous. The following reservoirs/lakes will be sampled: Worster Lake at Potato Creek State Park, Sand Lake at Chain O'Lakes State Park, Lake James at Pokagon State Park, Salamonie Reservoir (Lost Bridge West State Recreation Area), Mississinewa Reservoir (Miami State Recreation Area), Cecil M. Harden Lake (Raccoon State Recreation Area), Monroe Lake (Paynetown and Fairfax State Recreation Areas), Hardy Lake at Hardy Lake State Recreation Area, Whitewater Lake at Whitewater Memorial State Park, Deam Lake at Deam Lake State Recreation Area, Starve-Hollow Lake at Starve-Hollow State Recreation Area, Brookville Reservoir (Mounds and Quakertown State Recreation Areas) and Kunkel Lake at Ouabache State Park. These sites will be sampled monthly unless cyanobacteria cell counts exceed 100,000 cells/ml, at which point sampling will occur biweekly. Additionally, the Dog Park Lake at Fort Harrison State Park will be sampled only for cyanobacteria identification and enumeration and cyanotoxin analysis. Additional sites may be added depending on time and staffing constraints.

Background and Project/Task Description: (QAPP Elements A5, A6)

The presence of sufficient numbers of cyanobacteria in surface waters serves as an indicator that cyanotoxins may be produced. Exposure to high levels of cyanobacterial cells and cyanotoxins may lead to various illnesses in humans and animals when exposed through contact with or ingestion of surface water. Indiana has not established cyanobacteria or cyanotoxin water quality criteria or standards for full body contact recreational uses to protect human health, however, the World Health Organization (WHO) guidelines are commonly used for this purpose and the United States Environmental Protection Agency (USEPA) has recently come out with recreation guidelines. Indiana will use a combination of WHO and EPA recommended values as described in Section 5.

The ELISA microcystins, cylindrospermopsin and anatoxin-a analysis will be performed using an Abraxis™ ADDA ELISA Immunoassay Test Kit. The ELISA analysis will be performed in the IDEM WAPB laboratory. In addition to cyanobacteria sampling, field parameters of pH, DO, DO saturation, temperature, turbidity and specific conductivity will be recorded. Water samples will be collected for ammonia nitrogen, nitrate/nitrite, total

Kjeldahl nitrogen and total phosphorous. Samples will be analyzed for dissolved reactive phosphorous at such time as the laboratory has the capability to do so. Atmospheric observations and lake/beach conditions will be recorded during each sampling event. Only cyanobacteria and cyanotoxin samples will be collected at the Dog Lake. Results will be entered into the WAPB AIMS database and posted on www.algae.IN.gov.

Data Quality Objectives (DQOs): (QAPP Element A7)

The Data Quality Objective (DQO) process (USEPA 2000) is a planning tool for data collection activities. It provides a basis for balancing decision uncertainty with available resources. The DQO process is required for all significant data collection efforts for a project. It is a seven step systematic planning process used to clarify study objectives, define the appropriate types of data, and establish decision criteria on which to base the final use of the data. The DQO for this project is identified in the following seven steps.

1. State the Problem

The waters in the previously identified recreational lakes have either a documented history of, or the potential to support cyanobacteria blooms which may present a human and animal health hazard.

2. Identify the Goals and Decisions of the Study

This project will provide information regarding the nature and extent of cyanobacteria populations in the selected swimming areas, and will measure microcystins, cylindrospermopsin and anatoxin-a concentrations. By routine sampling for cyanobacteria in these lakes, IDEM can determine if the conditions in the water pose an unreasonable risk to swimmers and dogs, and can take steps to adequately warn the public of those risks.

3. Identify Information Inputs

Composite water samples will be collected at each swimming beach water sampling location for cyanobacteria identification and enumeration, and cyanotoxin levels. Field parameters (pH, Dissolved Oxygen (DO), DO saturation, temperature, turbidity, and specific conductance) will be measured at each site during each sampling event. Water samples will be collected for ammonia nitrogen, nitrate/nitrite, total Kjeldahl nitrogen and total phosphorous. Only cyanobacteria and cyanotoxin samples will be collected at the Dog Park Lake. Visual field observations will include weather conditions and lake/beach conditions. All samples collected will be identified and enumerated. Samples will be analyzed for the cyanotoxins microcystins, cylindrospermopsin and anatoxin-a using the Abraxis™ ADDA ELISA ,ELISA, and RBA Immunoassay Test Kits respectively.

4. Define the Boundaries of the Study Area

The study areas consist of the designated swimming beaches at Worster Lake at Potato Creek State Park, Sand Lake at Chain O'Lakes State Park, Lake James at Pokagon State Park, Salamonie Reservoir (Lost Bridge West State Recreation Area), Mississinewa Reservoir (Miami State Recreation Area), Cecil M. Harden Lake (Raccoon State Recreation Area), Monroe Lake (Paynetown and Fairfax State Recreation Areas), Hardy Lake at Hardy Lake State Recreation Area, Whitewater Lake at Whitewater Memorial State Park, Deam Lake at Deam Lake State Recreation Area, Starve-Hollow Lake at Starve-Hollow State Recreation Area, Brookville Reservoir (Mounds and Quakertown State Recreation Areas), Kunkel Lake at Ouabache State Park and the Dog Park Lake at Fort Harrison State Park.

5. Develop a Decision Rule

Cyanobacteria will be compared to the WHO and EPA guidelines. WHO guidelines recommend using an action level of 100,000 cells/ml of cyanobacteria to post recreational advisory signs. New EPA guidelines for microcystins recommend posting recreational advisories at 4 µg/l and DNR will issue an additional advisory at 4µg/l. Swimming will be prohibited at 20 µg/l, consistent with WHO guidelines. DNR will use the EPA recommended cylindrospermopsin level of 8 µg/l. As EPA and WHO have not set levels for anatoxin-a, an anatoxin-a level of 80 µg/l will be used as an additional advisory threshold, based on the State of Ohio's recommended thresholds. For cyanotoxin exposure for dogs, the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment has developed action levels for microcystin, anatoxin-a and cylindrospermopsin. A warning to dog owners will occur whenever any cyanotoxins are detected and dogs will be prohibited from swimming at the values of 0.8 µg/l microcystin, any anatoxin-a and 1.0 µg/l of cylindrospermopsin. (Email correspondence with Regina Linville, PhD, Toxicologist Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.)

Table 1. Decision Levels

| Action Thresholds for Cyanobacteria and Associated Toxins | | | | |
|--|---|---------------------------------|----------------------|---------------------------------|
| | WHO Level | | IN DNR Limits | |
| Constituent of Concern | Advisory levels | Swimming /exposure prohibitions | Advisory levels | Swimming /exposure prohibitions |
| Cyanobacteria | 100,000 cells/ml = Recreational advisory warning | | | |
| Humans | | | | |
| Cylindrosermopsin | | 20 ug/l | | 8 ug/l * |
| Microcystin | | | 4 ug/l | 4 ug/l |
| Anatoxin-a | | | | 80 ug/l ** |
| Dogs *** | | | | |
| Cylindrosermopsin | | | | 1.0 ug/l |
| Microcystin | | | | 0.8 ug/l |
| Anatoxin-a | | | | 0.8 ug/l |

ug/l = Microgram (1/1,000,000 gram) per liter

* US EPA Recommended level

** Based on Ohio EPA recommendations

*** Based on the California EPA Standard

6. Specify Tolerable Limits on Decision Error and Performance or Acceptance Criteria

Good quality data are essential for minimizing decision error. By minimizing both sampling design error and laboratory error more confidence can be placed in the cell counts and cyanotoxin levels reported to the public through this program. Program-specific controls will be used to minimize the introduction of errors. Controls for field sampling include the collection of field duplicates and blanks. Laboratory controls include thorough

verification of species identifications and cyanotoxin analyses. Photographs of cyanobacterial identification slides will be taken and stored on file.

7. Optimize the Design for Obtaining Data

Each beach swimming area will have composite samples taken in order to better represent the cyanobacteria populations in the swimming area. Staff will wade in to about waist high, will stand for a minute to let the water/sediment settle and take the sample using the integrated water column sampler. Samples will be collected in the morning and early afternoon when the cyanobacteria are believed to be the most prevalent. Sampling at the Ft. Harrison Dog Park Lake will be modified to reflect the characteristics of that lake.

Training and Staffing Requirements: (QAPP Element A8)

The Watershed Assessment and Planning Branch (WAPB) staff has written Standard Operating Procedures (SOPs) and staff is trained by experienced peers on how to collect surface water samples, operate field and lab equipment and perform required QA/QC procedures. Staff training and SOP techniques are based on proven EPA sampling methods and recommendations for containers, preservation techniques, and holding times as cited in the CFR (Code of Federal Regulations, 1999). IDEM staff and summer interns are provided review and initial field instruction sessions by senior staff before the actual sampling begins.

Selected staff from the WAPB has received training in algal identification and enumeration from the staff at CEES and PhycoTech in St. Joseph, Michigan. A representative from Abraxis™ has trained selected WAPB staff on the use of the Abraxis™ ELISA System. Staff trained in cyanobacteria identification and enumeration and the use of the Abraxis™ ELISA System will be performing those tasks at the IDEM WAPB lab.

Two staff will be assigned to each field sampling run, however one staff can sample if necessary. There also will be a review for all participants on how to collect surface water samples, operate field and lab equipment, and perform QA/QC Procedures.

Data handling and report development will be shared by staff of the Targeted Monitoring, Technical and Logistical Services and Watershed Planning and Restoration Sections. Input of field observations and field data into the Assessment Information Management System (AIMS) database will be conducted by staff of the Targeted Monitoring Section. Likewise, QA/QC of all field data entry will be handled by the Targeted Monitoring Section staff. Chemists from the Technical and Logistical Services Section will validate the data through QA/QC procedures as cited in Section IV of this workplan.

II. MEASUREMENT/DATA ACQUISITION

Sampling Process Design/ Methods, Sample Handling and Custody

(QAPP Elements B1, B2, B3, B4, B5, B6, B7)

Sampling Sites/Sampling Design: (QAPP Element B1)

The initial sample site locations were selected by the Targeted Monitoring Section Chief based on the availability of a public swimming beach at a state or federal managed recreation area. Table 1 lists the selected sampling sites, plus lake names, AIMS Number, County Name, 8-Digit HUC, Quad Map, the latitudes and longitudes of each sampling site. Figures 1 through 14 depict all sampling sites for each lake. The site numbers in Table 1 and the site maps in the Figures are used as quick reference points corresponding to the actual sampling site locations of L-Site numbers in AIMS and will be referred to by the Site ID number rather than the AIMS number. Final coordinates for each site were determined using a Trimble Juno™ Series GPS (Global Positioning System) with an accuracy of 1-3 meters during the pre-survey phase of this project.

Table 2. Sampling Sites

| Site # | Lake Name | AIMS Numbers | County | HUC | Latitude | Longitude |
|--------|-----------------------------|--------------|------------|-------------|---------------|----------------|
| 1 | Whitewater | GMW-07-0001 | Union | 50800030712 | 39° 36' 54.9" | -84° 57' 58.1" |
| 2 | Hardy | WEM-06-0001 | Scott | 51202070604 | 38° 46' 55.2" | -85° 41' 53.8" |
| 3 | Cecil M Harden Raccoon | WLV-12-0002 | Parke | 51201081208 | 39° 43' 12.1" | -87° 04' 22.5" |
| 4 | Monroe Paynetown | WEL-07-0001 | Monroe | 51202080702 | 39° 04' 49.1" | -86° 25' 51.1" |
| 5 | Monroe Fairfax | WEL-07-0003 | Monroe | 51202080703 | 39° 01' 02.0" | -86° 28' 53.9" |
| 6 | Mississinewa Miami | WMI-06-0001 | Wabash | 51201030605 | 40° 42' 09.2" | -85° 56' 08.0" |
| 7 | Worster | UMK-01-0005 | St Joseph | 71200010104 | 41° 33' 03.2" | -86° 01' 41.8" |
| 8 | Salamonie Lost Bridge W | WSA-04-0001 | Huntington | 51201020405 | 40° 46' 46.9" | -85° 38' 14.8" |
| 9 | Sand | LMJ-16-0024 | Noble | 40500011601 | 41° 19' 50.0" | -85° 23' 00.9" |
| 10 | Lake James | LMJ-08-0033 | Steuben | 40500010803 | 41° 42' 35.5" | -85° 02' 10.7" |
| 11 | Lake James Inn | LMJ-08-0033 | Steuben | 40500010803 | 41° 42' 09.3" | -85° 02' 10.6" |
| 12 | Brookville Quakertown | GMW-07-0005 | Union | 50800030715 | 39° 35' 10.9" | -84° 59' 41.5" |
| 13 | Brookville Mounds | GMW-07-0004 | Franklin | 50800030717 | 39° 29' 53.2" | -84° 59' 12.6" |
| 14 | Deam Lake | OSK-07-0001 | Clark | 51401010702 | 38° 27' 58.5" | -85° 51' 40.8" |
| 15 | Starve-Hollow Lake | WEM-09-0007 | Jackson | 51202070905 | 38° 48' 50.4" | -86° 04' 50.3" |
| 16 | Kunkel Lake | WUW-06-0005 | Wells | 51201010604 | 39° 52' 41.2" | -86° 01' 03.9" |
| 17 | Fort Harrison Dog Park Lake | WWU-09-0006 | Marion | 51202010904 | 40° 43' 12.4" | -85° 06' 21.0" |

Figure 1. Site Map of Worster Lake

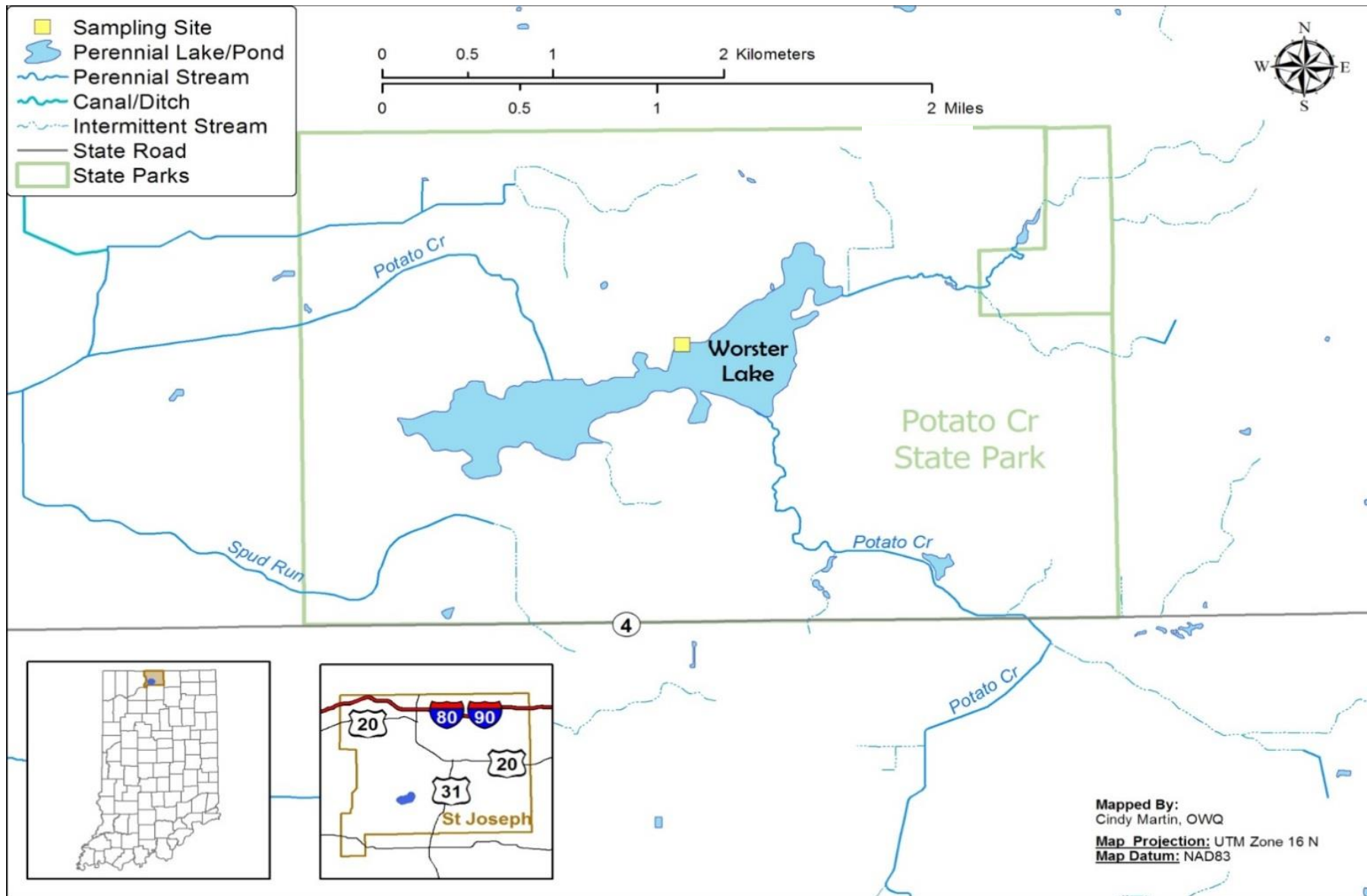


Figure 2. Salamonie Lake Site Map

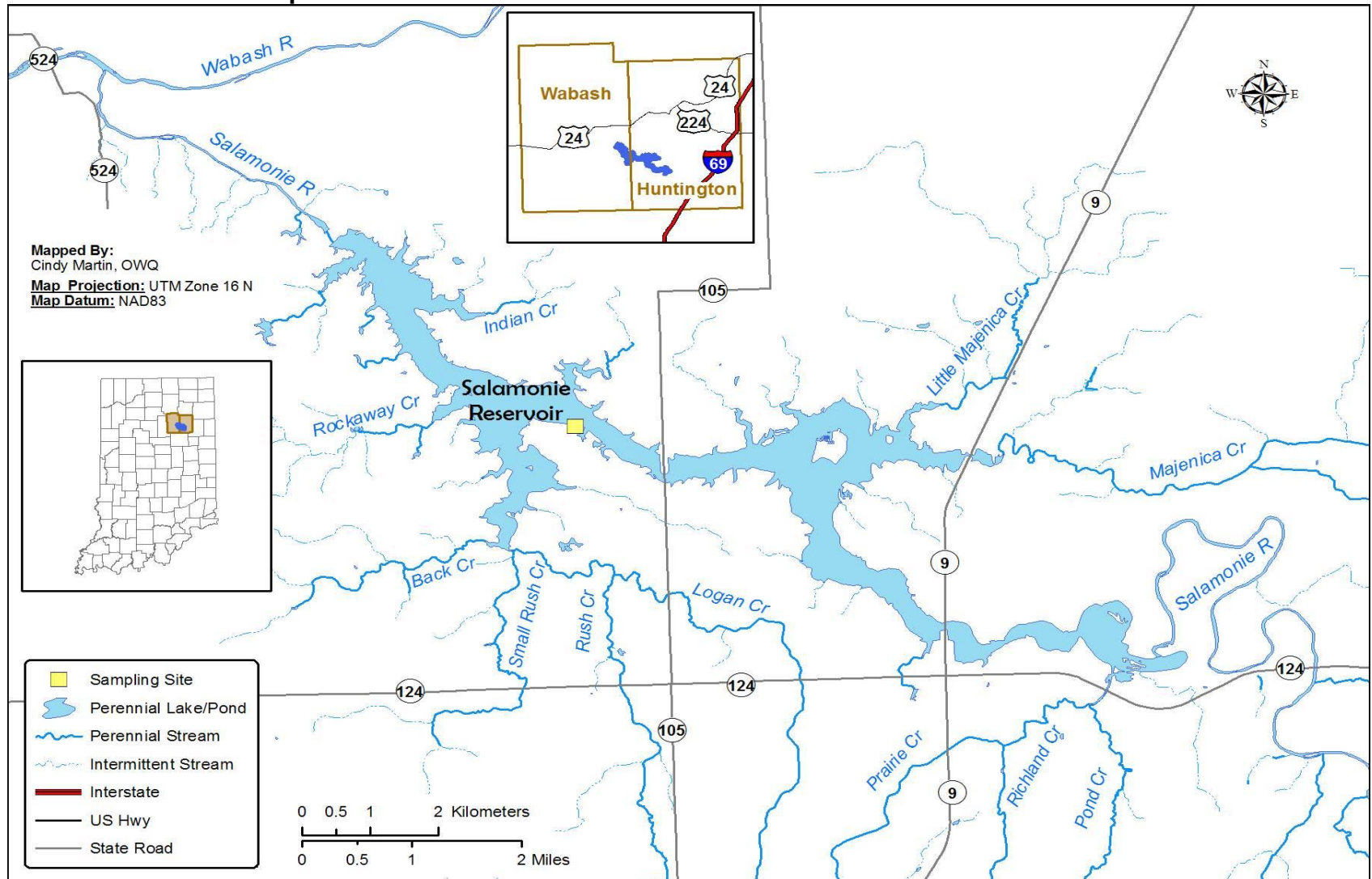


Figure 3. Mississinewa Lake Site Map

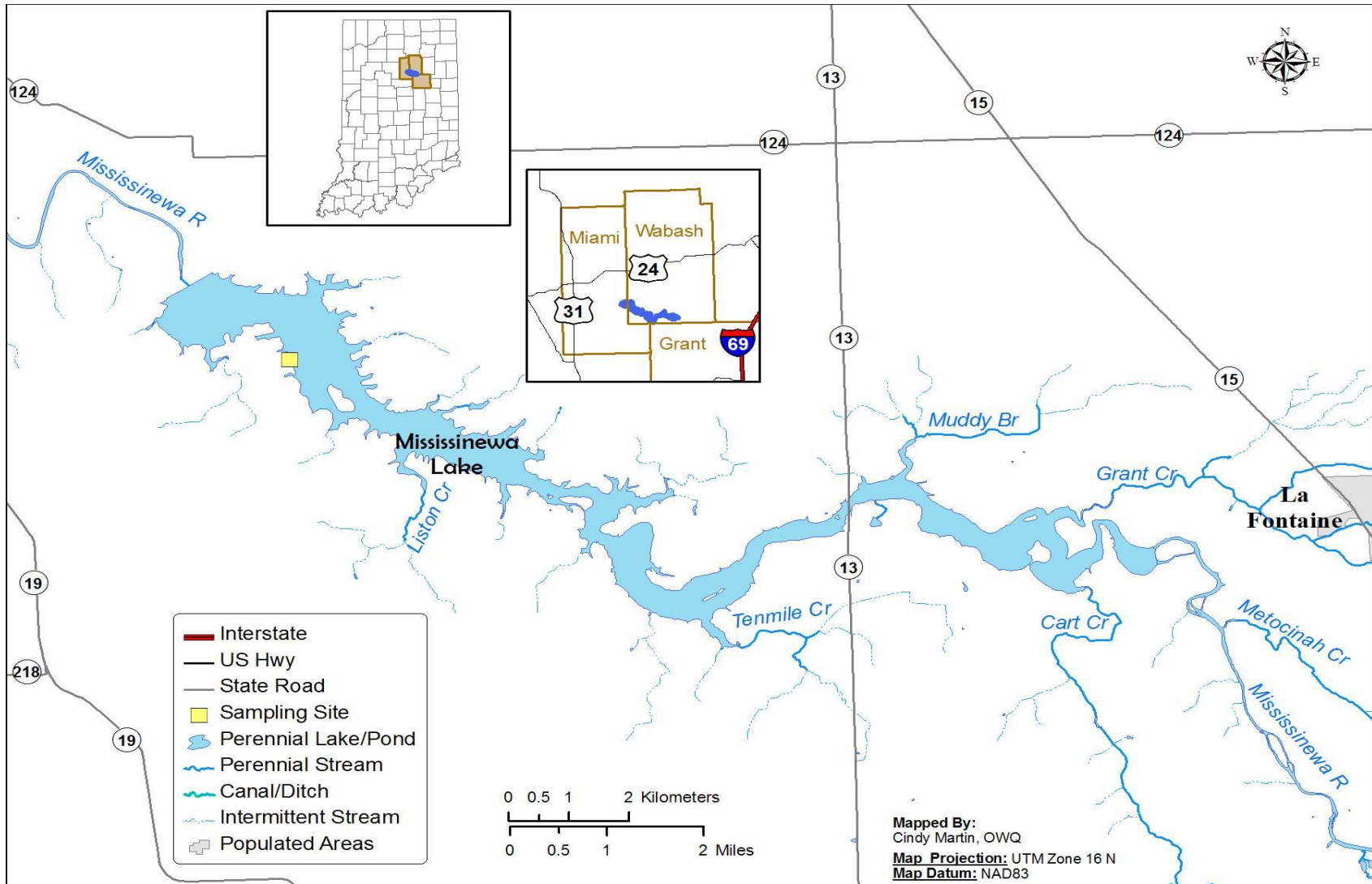


Figure 4. Brookville Reservoir Site Map

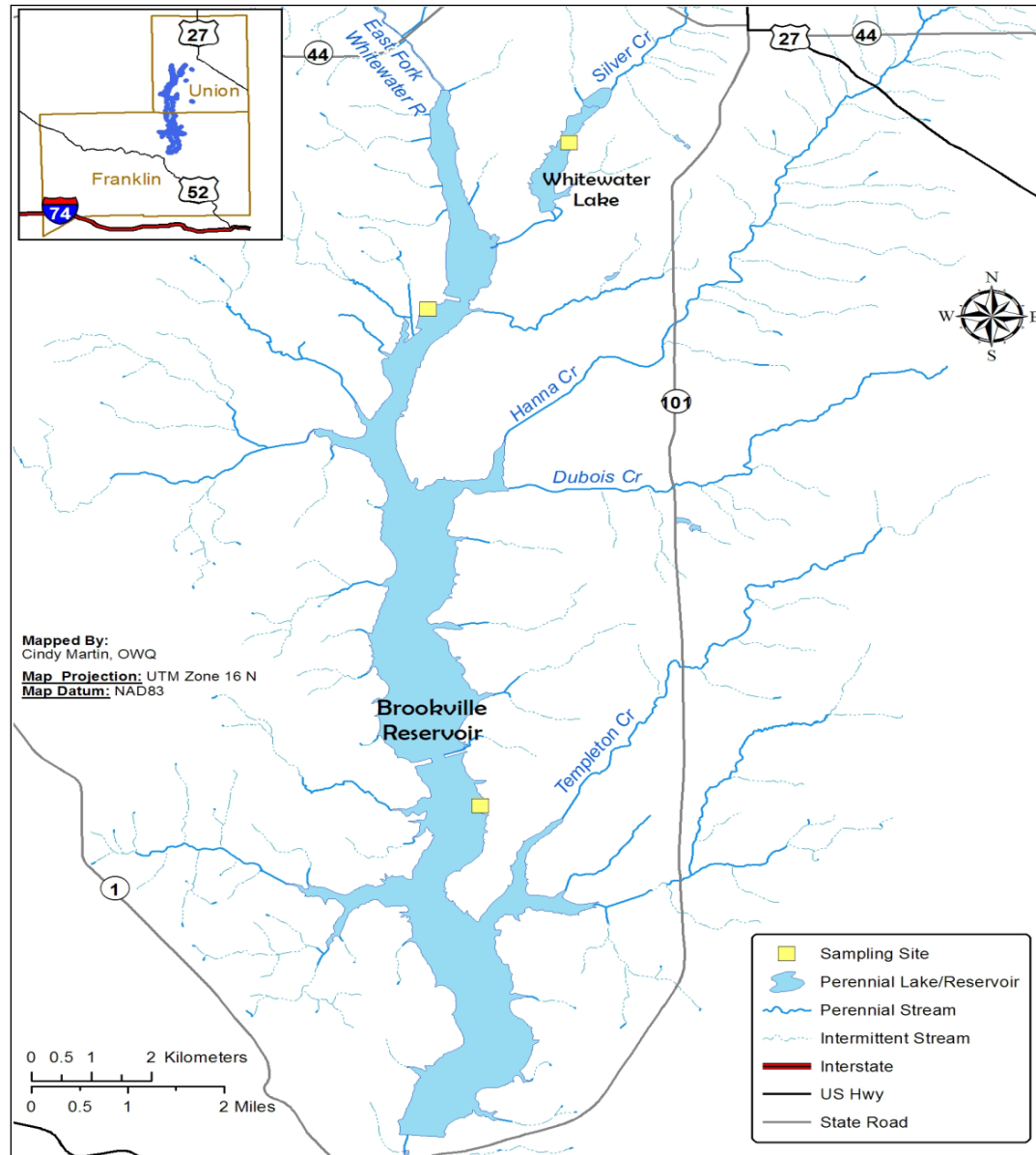


Figure 5. Whitewater Lake Site Map

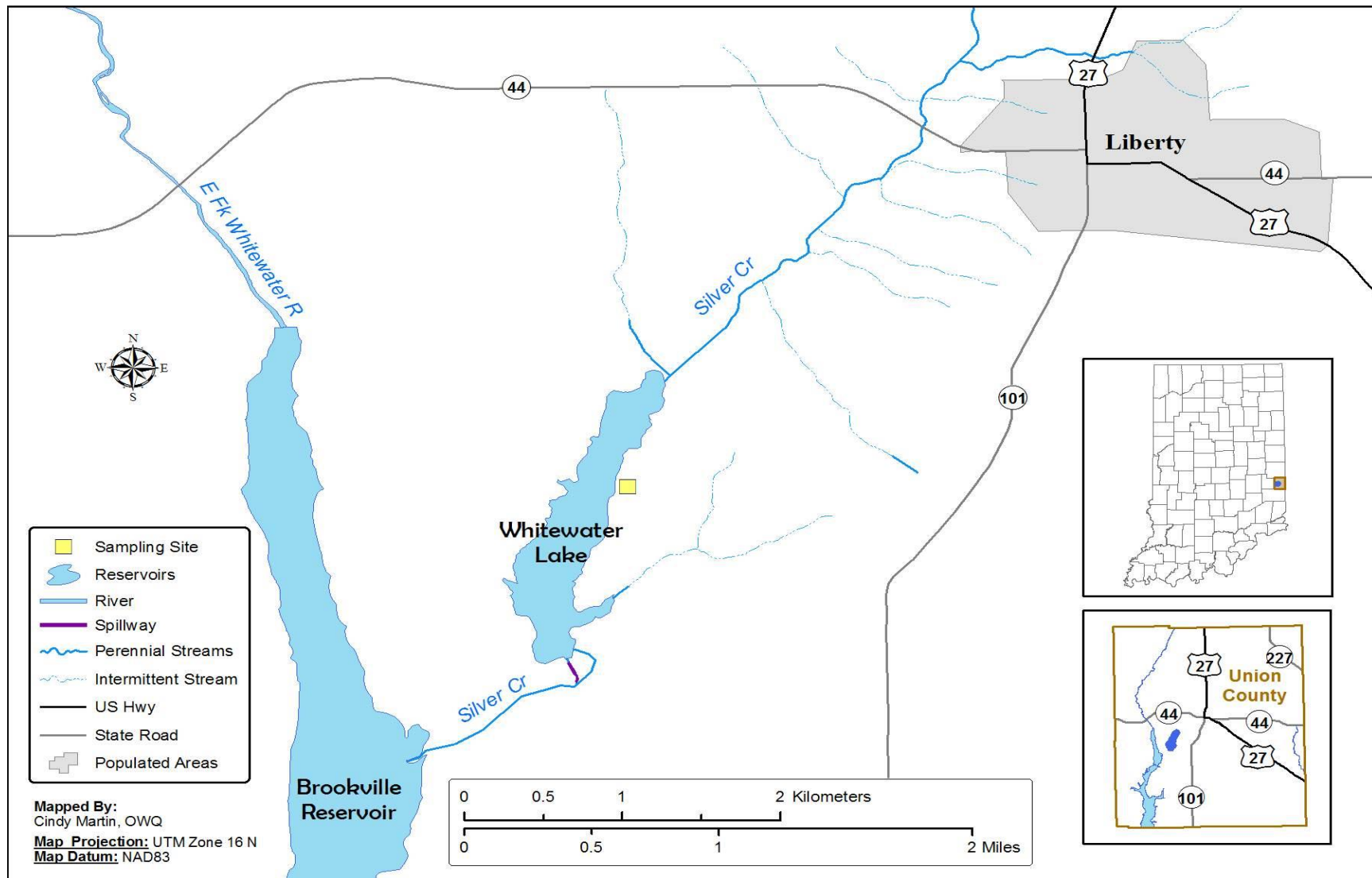


Figure 6. Sand Lake Site Map

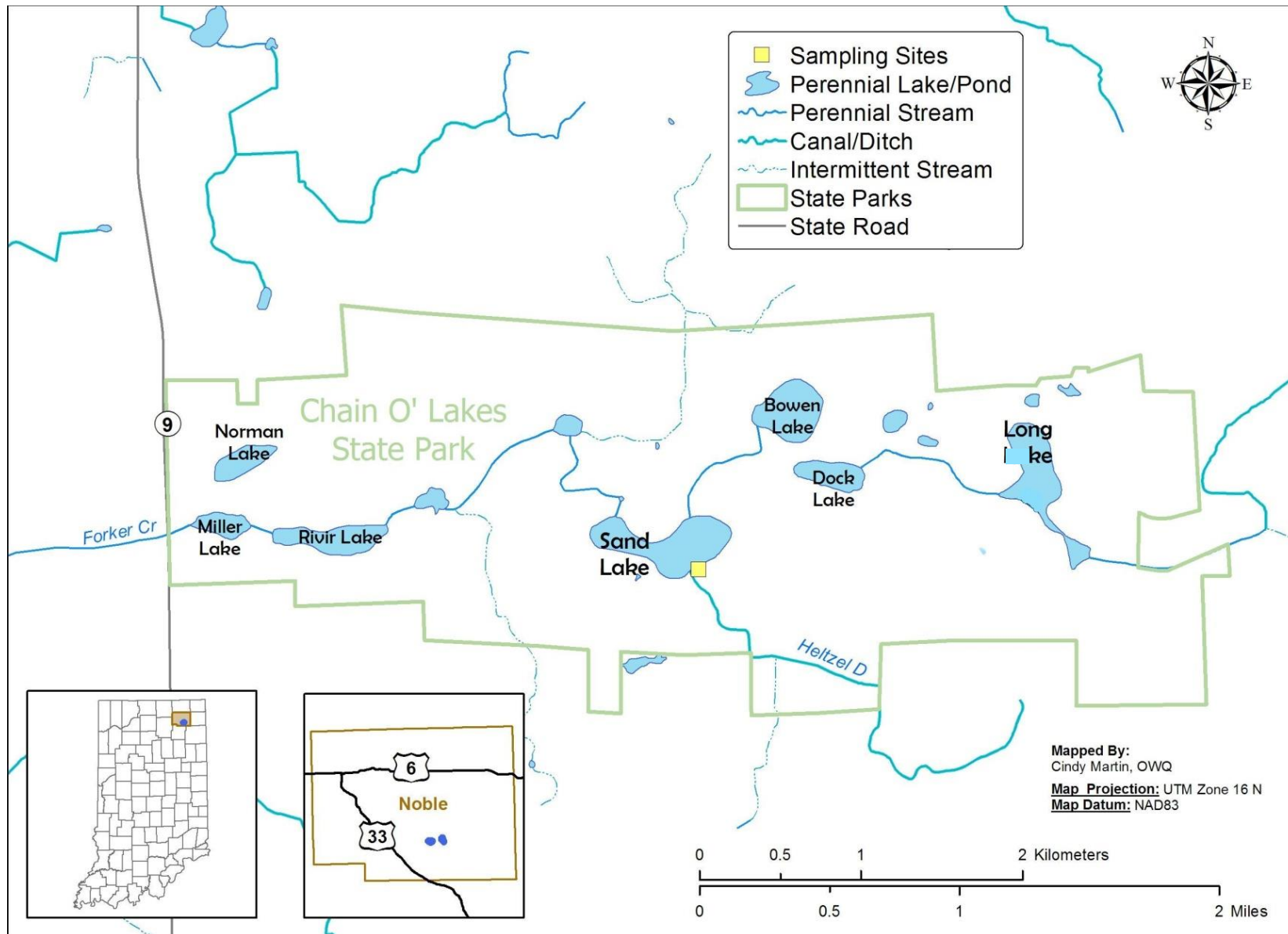


Figure 7: Lake James Site Map

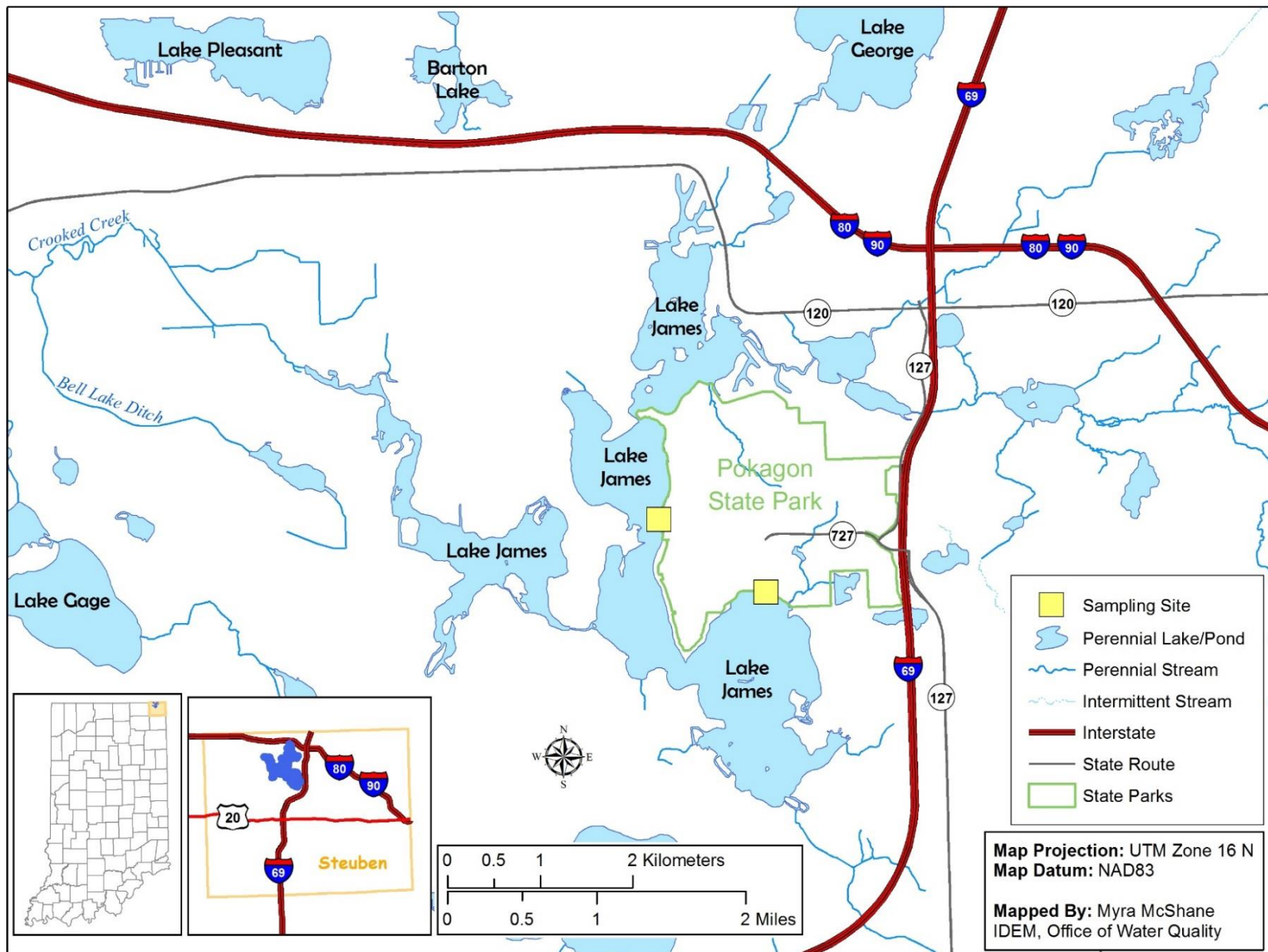


Figure 8: Raccoon Lake (Cecil M. Hardin) Site Map

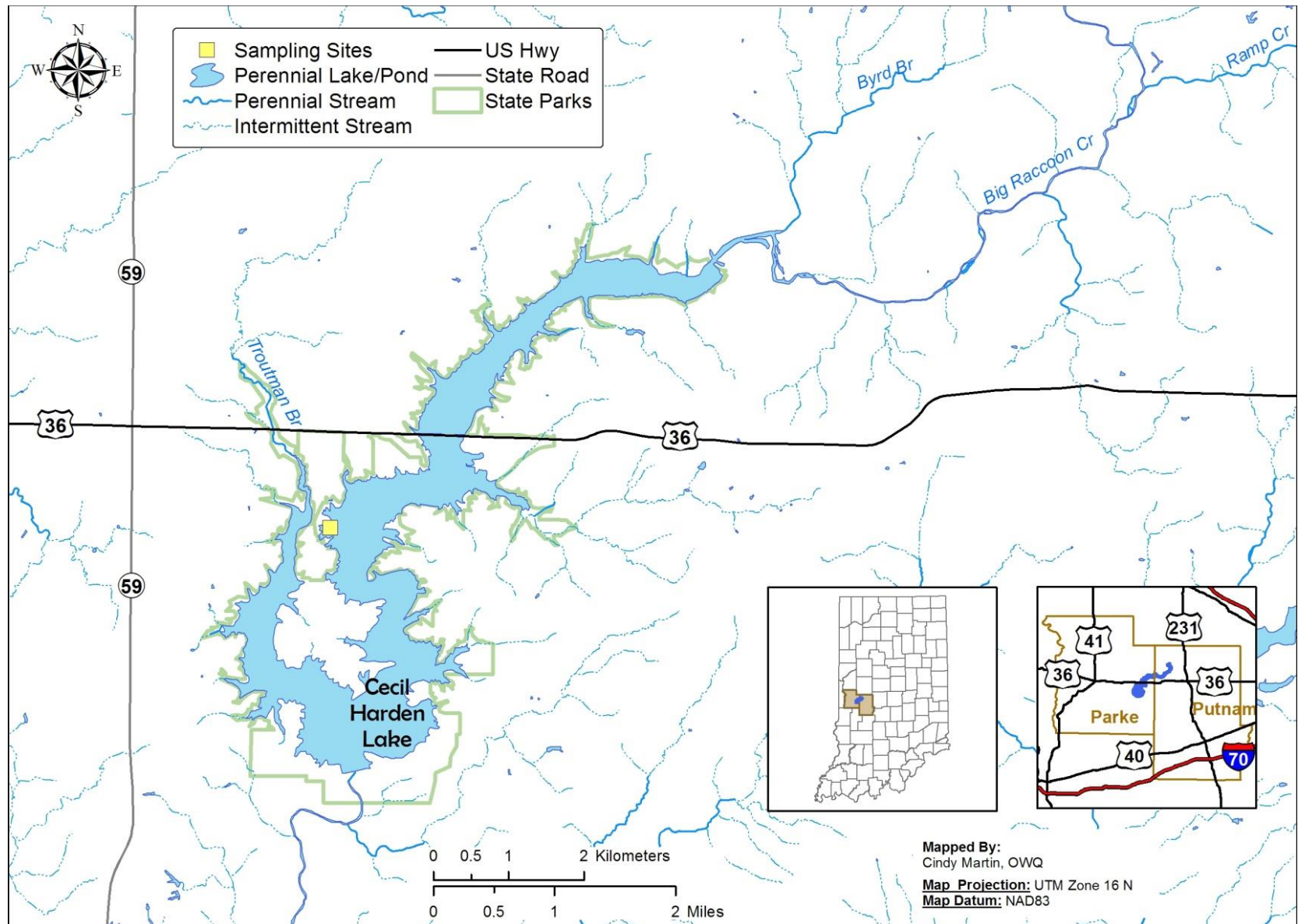


Figure 9: Monroe Reservoir Site Map

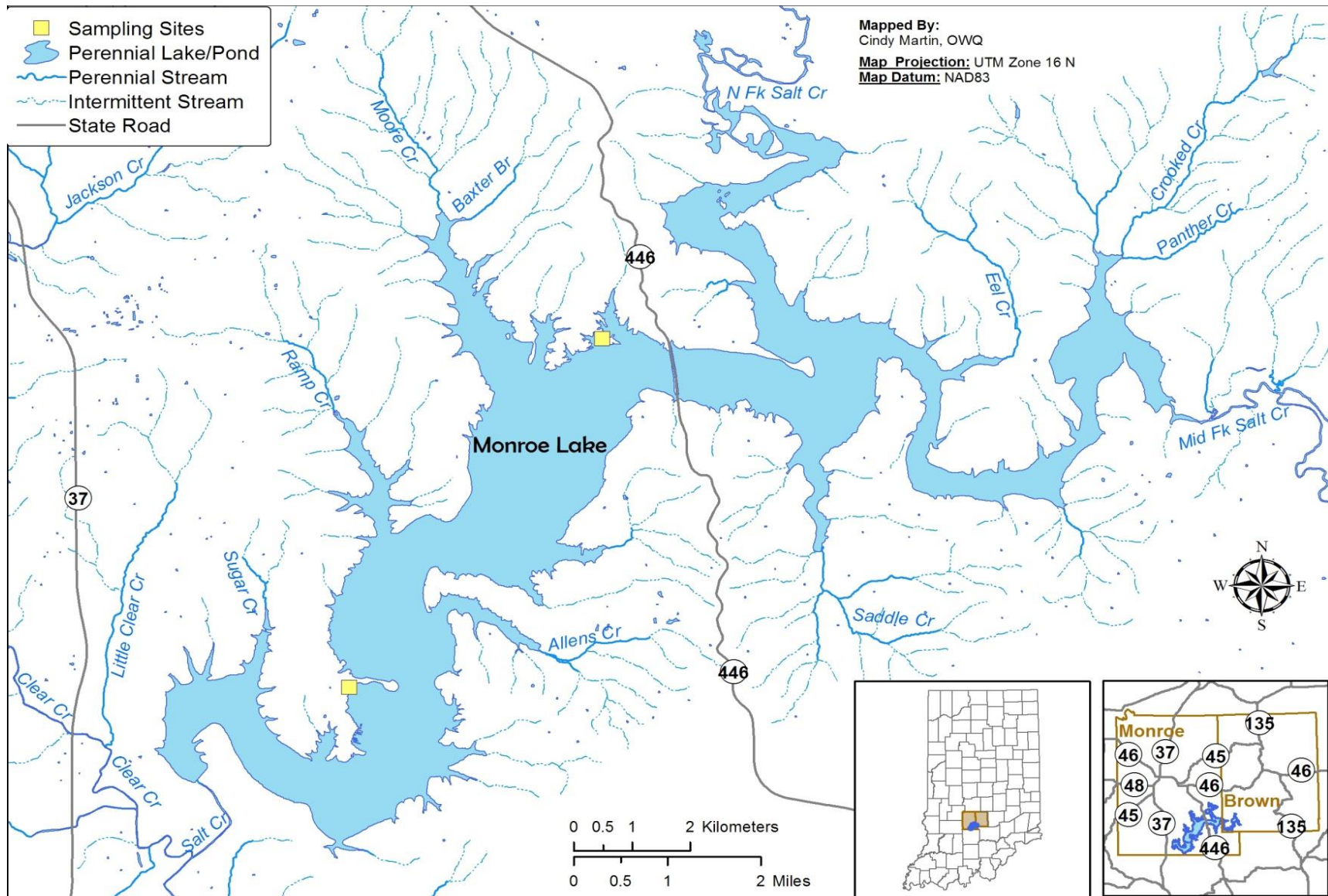


Figure 10: Hardy Lake Site Map

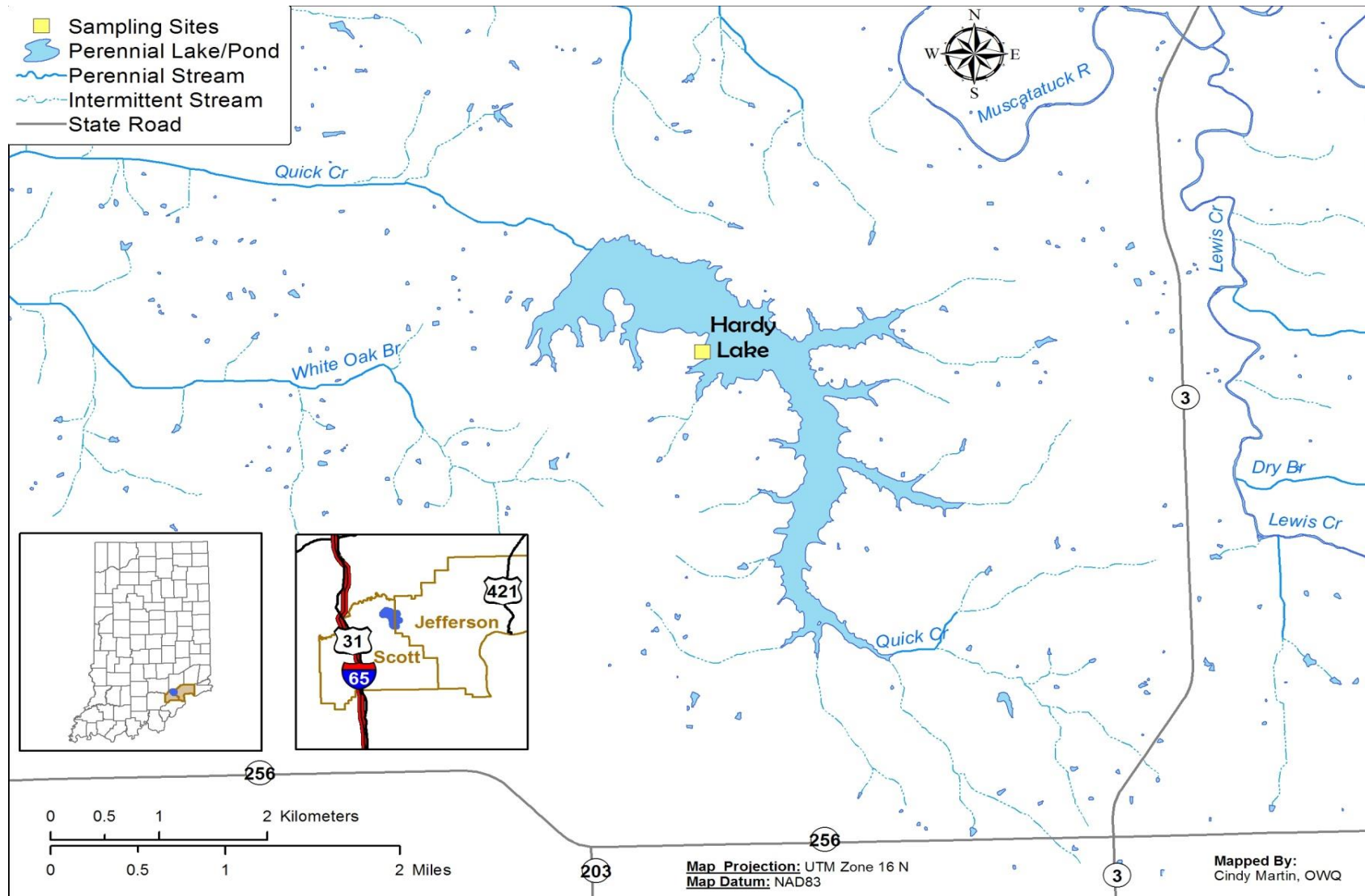


Figure 11: Deam Lake Site Map

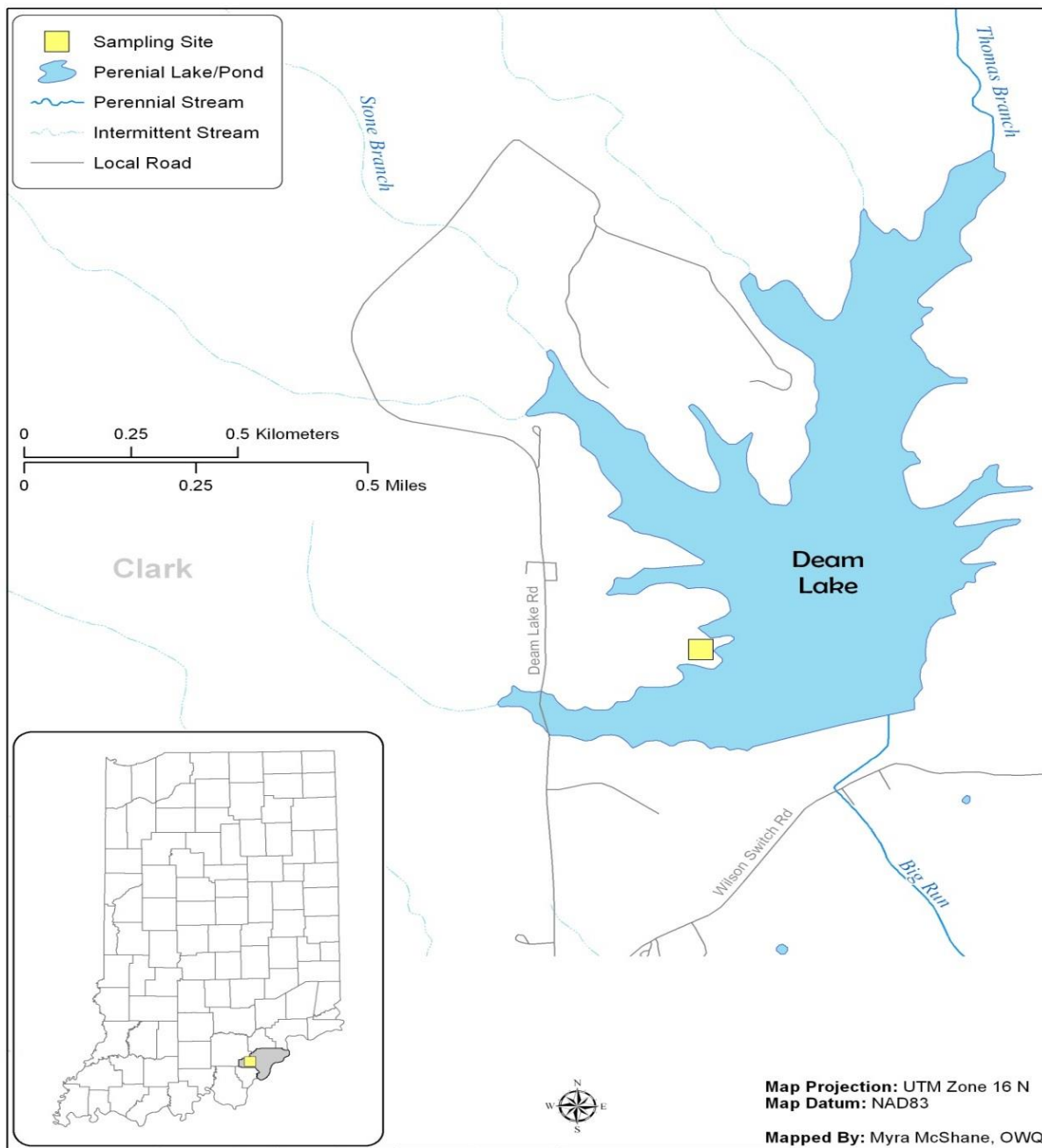


Figure 12: Starve-Hollow Lake Site Map

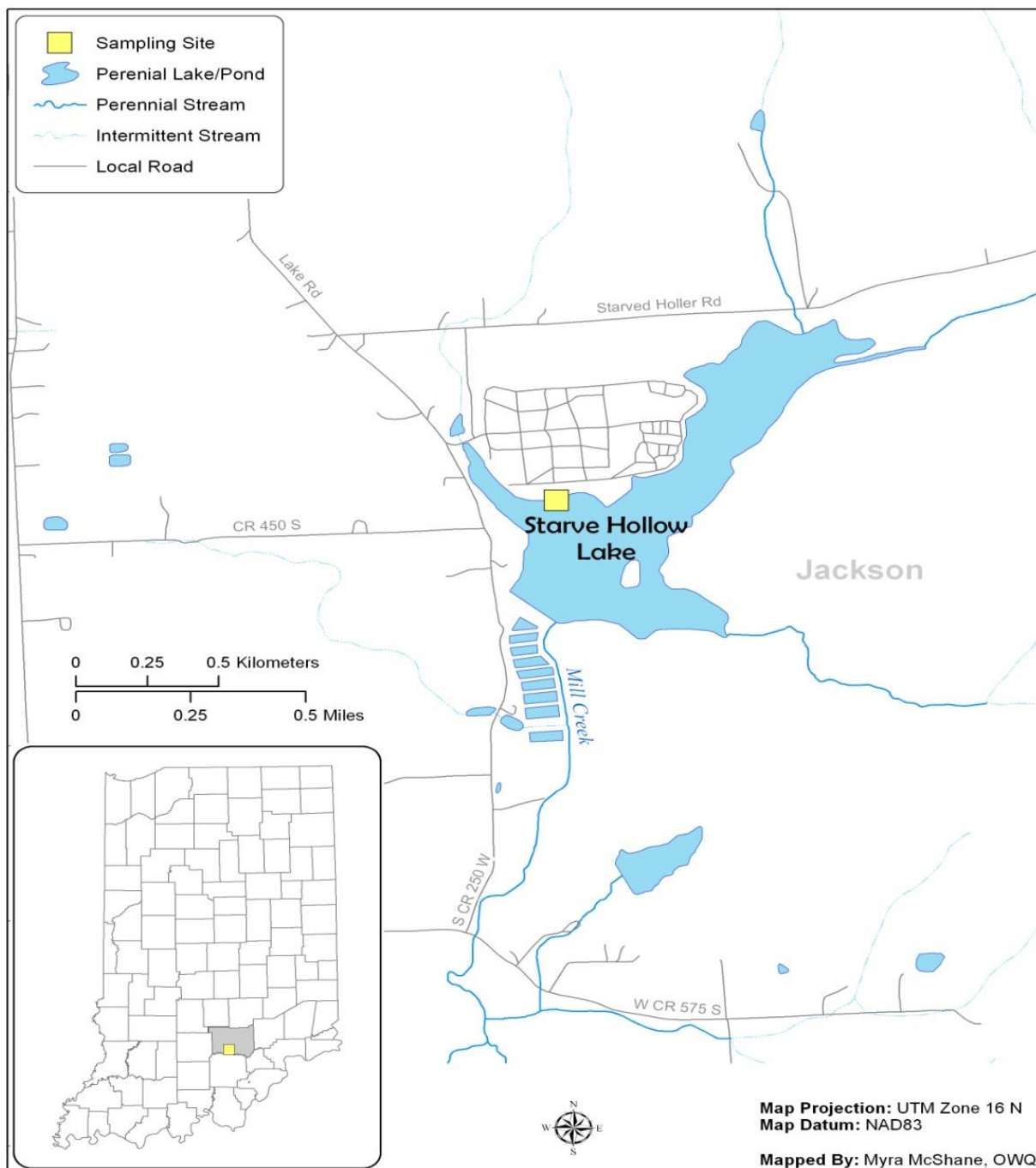


Figure 13: Kunkel Lake Site Map

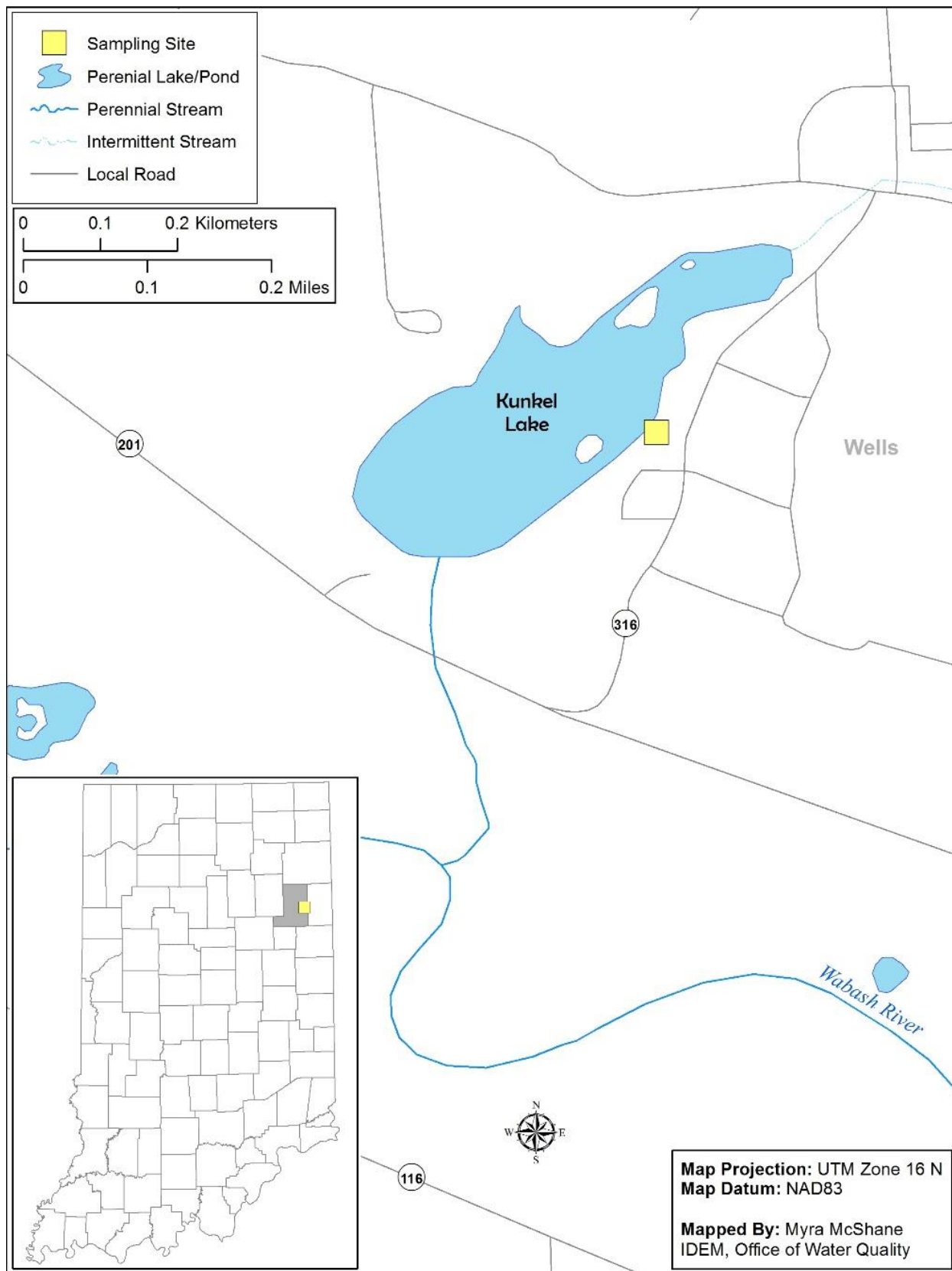
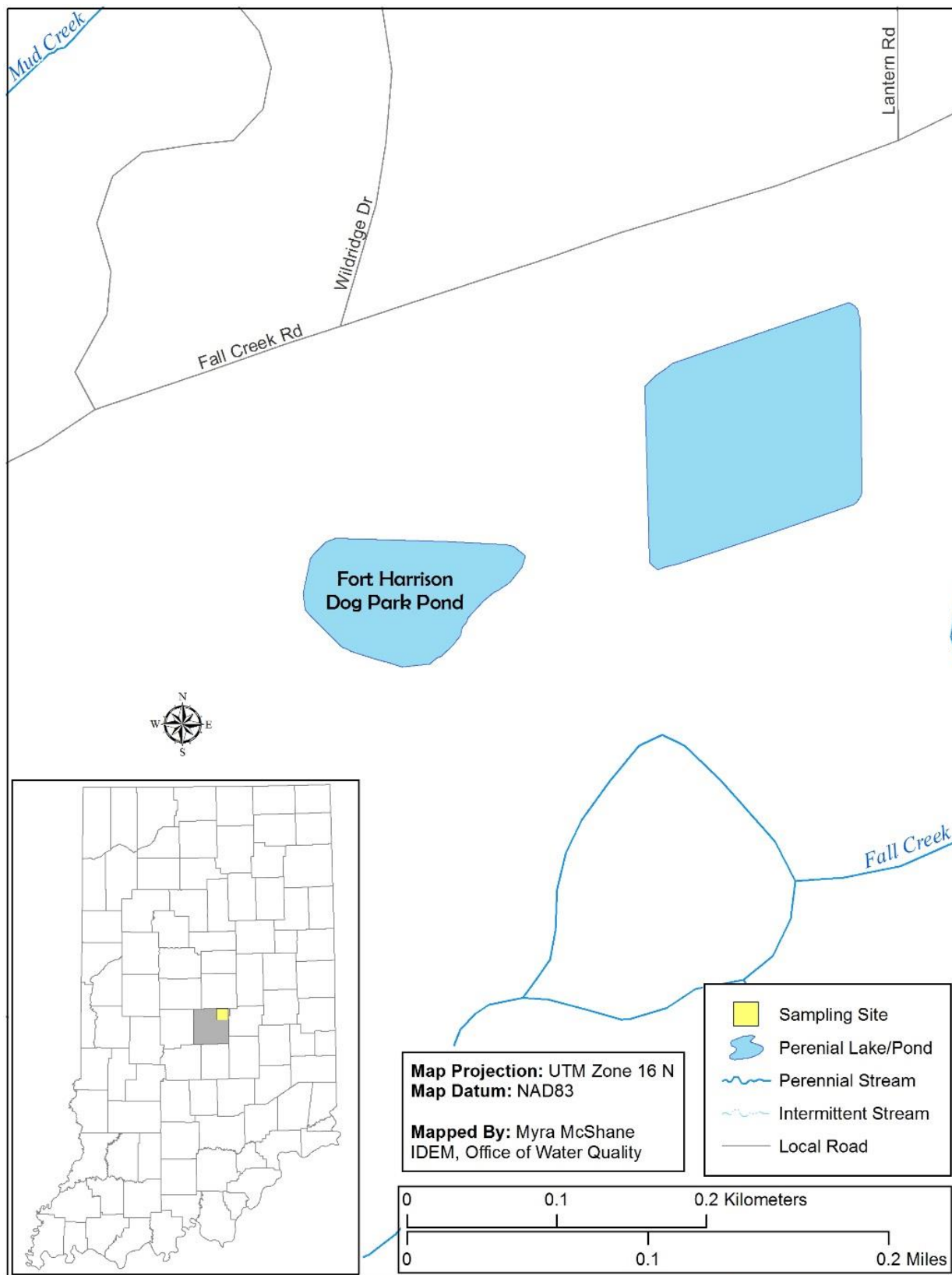


Figure 14: Dog Park Lake Site Map



Sampling Methods and Sample Handling: (QAPP Elements B2, B3)

Collecting Surface Water Samples for Cyanobacteria and Cyanotoxin Analysis

See Appendix A.

QA/QC Samples

For quality control, field blanks, equipment blanks and duplicates samples will be collected as generated by AIMS. One field blank will be collected per sampling trip from a carboy with deionized water. Field duplicates will be generated by filling two sample bottles from the churn. For each sampling event a Chain-of-Custody Form is filled for quality assurance/quality control (QA/QC) and accompanies the sample bottles to the IDEM lab and the CEES lab. More details about sampling procedures are described in the "Water Quality Surveys Section Field Procedure Manual", IDEM 2002.

The field parameters will be measured directly from the lake water using either a Hydrolab Quanta or YSI data sonde. An optional method would be to use lake water collected in a separate stainless steel bucket from the last sampling area at each beach. Notations on the field sheet will be made if the latter method is used.

Analytical Methods: (QAPP Element B4)

Laboratory Procedure for Cyanobacteria Identification and Enumeration

See Appendix B.

Laboratory Procedure for Microcystins, Cylindrospermopsin and Anatoxin-a Analysis

See Appendix C

Field Parameter Measurements:

The field measurements will be measured for DO, temperature, pH, conductivity, and turbidity each time a water sample is collected. The field parameters to be measured at each sampling site and their respective test methods and quantification limits are identified below in Table 2.

In addition to sampling, during each sampling run, field observations from each site are documented on Field Sheets.

Table 3: Field Parameters Test Methods

| Parameter | Method * | Limits of Quantitation | Units |
|-------------------------|-----------------|-------------------------------|--------------|
| Dissolved Oxygen (D.O.) | ASTM D888-09(C) | 0.03 | mg/L |
| D.O. Saturation | ASTM D888-09(C) | 0.01 | % |
| Turbidity | SM 2130B Mod | 0.30 | NTU |
| Specific | SM 2510B | 3.00 | µmho/cm |

| | | | |
|-------------|-------------|----------|---------|
| Conductance | | | |
| Temperature | SM 2550B(2) | 0.1 | Celsius |
| pH | EPA 150.2 | +/- 0.01 | SU |

* Clesceri et al., 2012.

Nutrient Parameters Measurements:

Nutrient measurement analysis is performed at the TestAmerica-Chicago Lab, 2417 Bond Street, University Park, IL 60484 in accordance with pre-approved test methods and allotted time frames. A chain-of-custody form accompanies each sample set through the analytical process. The nutrient parameters and their respective test methods and quantification limits are identified below in Table 3.

Table 4 Nutrient Parameters Test Methods

| Parameter | Method | Limits of Quantification | Units | Preservative | Holding Times |
|------------------|-------------------|--------------------------|-------|---------------------------------------|---------------|
| Ammonia Nitrogen | SM4500NH3-G | 0.1 | mg/L | H ₂ SO ₄ < pH 2 | 28 days |
| Total Phosphorus | SM4500P-E | 0.03 | mg/L | H ₂ SO ₄ < pH 2 | 28 days |
| Nitrate/Nitrite | SM4500NO3-F | 0.5 | mg/L | H ₂ SO ₄ < pH 2 | 28 days |
| TKN | SM4500N(Org) - | 0.3 | mg/L | H ₂ SO ₄ < pH 2 | 28 days |

Quality Control and Custody Requirements: (QAPP Element B5)

Analytical results from the Abraxis™ ELISA include quality control checks from which precision, accuracy and completeness can be determined for each batch of samples. Raw data are archived by analytical batch for easy retrieval and review. Chain-of-Custody procedures will be followed including day and time of sample collection, time of sample set-up for microcystins analysis and the identification process, time of reading the results, and time and method of disposal. Any method deviations will be thoroughly documented in the raw data bench sheets and records. All QA/QC samples will be tested according to the following guidelines:

Field Duplicate: Field Duplicates will be collected at a frequency of 1 per batch or at least 1 for every 20 samples collected (≥ 5%).

Field Blank: Field Blanks will be collected at a frequency of 1 per batch or at least 1 for every 20 samples collected (≥ 5%).

Laboratory Blank: Laboratory Blanks (sterile laboratory water blanks) will be tested at a frequency of 1 per day or every time the laboratory is in use.

Nutrient Data

Sample bottles and preservatives certified for purity will be used. Sample collection containers for each parameter, preservative and holding times will adhere to U.S. EPA requirements. Field duplicates and matrix spike/matrix spike duplicates (MS/MSD) shall be collected at the rate of one per sample analysis set or one per every 20 samples, whichever is greater. Additionally, field blank samples using ASTM D1193-91 Type I water will be taken at a rate of one set per sample analysis set or one per every 20 samples, whichever is greater.

Quality assurance documentation for each batch of samples consists of a Chain-of-Custody Form, bench sheets, spreadsheets of results, and the Quality Control Report generated by staff from the Technical and Logistical Services Section.

Field Instrument Testing and Calibrations: (QAPP Elements B6, B7)

This project will benefit from the planned upkeep of the measurement instruments, which enhances the instrument performance and ensures accurate and precise readings. Equipment logs are maintained that record equipment calibration and status.

Measurement equipment requires periodic calibration or standardization to reliably produce accurate results. The multi-parameter water analysis unit will be calibrated immediately prior to each week's sampling (IDEM 2002). Calibration results and drift values will be recorded, maintained, stored and archived in the calibration laboratories at the WAPB laboratories. The drift value is the difference between two successive calibrations. Field parameter calibrations will conform to the procedures as described in the instrument users' manuals (Hydrolab Corporation 2002; YSI 2002). The DO component of the calibration procedure will be conducted using the air calibration method. When used in conjunction with bacteriological and water chemistry sampling, the unit will be field checked for accuracy once during the week by comparison with a Winkler DO test, as well as Hach™ turbidity, pH and temperature meters. Weekly calibration records will be kept in the database field sheets. A Winkler DO test will also be conducted at sites where the DO concentration is 4.0 mg/L or less. Procedures for field instrument calibration are documented in the Surveys Section Field Procedure Manual. Revised June 2002, (Appendix G), Section 2 and will be followed by all staff participating in data collection for this study.

III. ASSESSMENT/OVERSIGHT

(QAPP Elements C1, C2)

Field and laboratory performance and system audits will be conducted to ensure good quality data. The field and laboratory performance includes precision measurements by relative percent difference (RPD) of field and laboratory duplicate, accuracy measurements by percent of recovery of MS/MSD samples analyzed in the laboratory, and completeness measurements by the percent of planned samples that are actually collected, analyzed, reported, and usable for the project.

Field audits will be conducted to ensure that sampling activities adhere to approved SOPs. Audits are systematically conducted by WAPB Quality Assurance staff to include all WAPB personnel that engage in field sampling activities.

Data Quality Assessment Levels

The samples and various types of data collected by this program are intended to meet the quality assurance criteria and Data Quality Assessment (DQA) Levels as described in the WAPB QAPP (IDEM 2004, pages 128-129). Data collected as specified in this work plan will adhere to DQA Level 3. All field data will comply with DQA Level 2 requirements. All field data are QA/QC'd for completeness, precision and accuracy. QA/QC Review Reports are developed as a final step of QA process/data assessment. The data and their usability identified in the QA/QC Review Reports are used in the decision making for water quality assessments.

IV. DATA VALIDATION AND USABILITY

(QAPP Element D1, D2)

Quality Assurance/Data Qualifiers and Flags:

The various data qualifiers and flags that will be used for quality assurance and validation of the data are found on pages 130-131 of the WAPB QAPP (IDEM 2004).

Data Usability:

The environmental data collected and its usability are finally qualified and classified into one or more of the four Categories: Acceptable Data, Enforcement Capable Results, Estimated Data and Rejected Data.

1. **Enforcement capable** results are DQA Level 3 or 4 data which meet all QC checks.
2. **Acceptable data** are DQA Level 2, 3 or 4 data suitable for decision making. Although a few data may be estimated or even unusable, the sample set as a whole has scientific and statistical integrity. Scientific and statistical decisions may be made with respect to the data quality objectives.
3. **Estimated data** may be suitable for enforcement or decision making on a case by case basis. Estimated data are suitable for determining future sampling needs, locating target parameters, and identifying possible contaminant levels.
4. **Rejected data** are not suitable for enforcement or for decision making.

Laboratory and Estimated Cost:

This project will take four sampling days (day trips) at a minimum of once per month with a maximum of two per month. One vehicle and two staff will be required. Laboratory supplies will cost approximately \$5,375 and have been paid for through the laboratory account.

Laboratory analysis and data reporting for this project will comply with the Quality Assurance Project Plan (QAPP) for Indiana Surface Water Quality Monitoring and TMDL Program (IDEM/100/29/338/073/2004, see IDEM 2004), Request for Proposals (RFP) 16-074 (see IDEM 2016), and the Office of Water Quality Assessment Branch Quality Management Plan (B-001-OWQ-A-00-08-R00, see IDEM 2008c). Analytical tests on the nutrient parameters outlined in Table 3 will be performed by the TestAmerica-Chicago Lab at a cost of \$10,000.

There is no lodging and per diem required. Vehicle fuel cost for one vehicle is approximately \$1000. Excluding staff salaries and lab costs, the total estimated cost of this project is approximately \$1000.

Reference Manuals and Personnel Safety:


All staff who participates in the field component of this study is required to have completed the National Safety Councils basic courses in first aid and CPR. All staff will also have completed the required health and safety training as specified by the WAPB management.

Additionally, field personnel will follow policies and procedures established in the Targeted Monitoring Section:

- Current Operating Procedure
- Chemical Hygiene Plan,
- Material Safety Data Sheets

Hazard Communication Plan
Surveys Section Field Procedure Manual
Quality Assurance Project Plan
Hydrolab Standard Operating Procedure
YSI Standard Operating Procedure

Appendix A- Collecting Surface Water Samples for Cyanobacteria and Cyanotoxin Analysis

| | |
|---|--|
|  | <p>Collecting Surface Water Samples for Cyanobacteria and Cyanotoxin Analysis S-003-OWQ-WAP-TGM-17-T-R0 Technical Standard Operating Procedure (TSOP) Office: Water Quality Branch: Watershed Assessment and Planning Section: Targeted Monitoring</p> <p>Last Revised: N/A Revision Cycle: Every 4 Years Originally Effective: January 23, 2017</p> |
|---|--|

Purpose (of the TSOP)

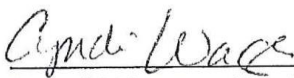
This technical standard operating procedure (TSOP) outlines the process for collecting surface water samples for cyanobacteria and cyanotoxin analysis.

This TSOP should be used by:

Office of Water Quality staff collecting surface water samples for cyanobacteria and cyanotoxin analysis.

Authorizing Signatures

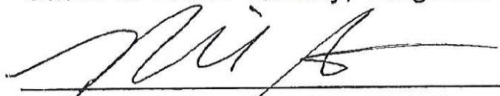
I approve and authorize this technical standard operating procedure:



Cyndi Wagner, Section Chief
Office of Water Quality, Targeted Monitoring Section

1/23/17

Date



Michael Sutton, Section Chief
Office of Water Quality, Technical and Logistical Services Section

1/23/17

Date

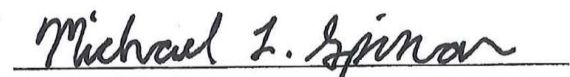


Marylou Renshaw, Chief
Office of Water Quality, Watershed Assessment
And Planning Branch

1-25-17

Date

This technical standard operating procedure is consistent with agency requirements.



Michael Spinar
Quality Assurance Staff
Office of Program Support

1/25/17

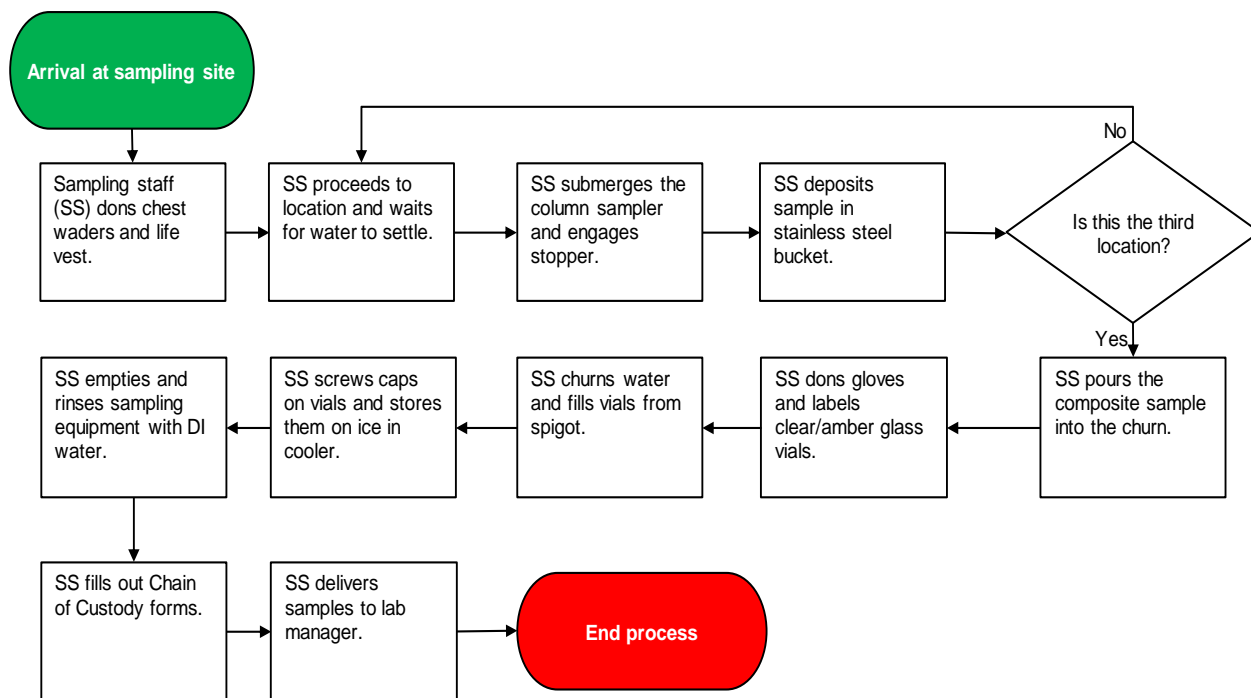
Date

1.0. Overview Flowchart

Not Applicable (N/A)

2.0. Procedure

2.1. Procedural Flowchart:



2.2. Procedural Steps:

Note: One composite sample will be collected from three locations (each side and the middle portion) at each swimming area.

- Step 1. Sampling staff (SS) will don chest waders and a life vest before proceeding into the water.
- Step 2. Holding the integrated water column sampler, SS will walk slowly out into the water to the first location until the water is approximately waist high, and stand to let the water/sediment settle.
- Step 3. SS will hold the depth integrated water column sampler and slowly submerge it vertically until it just touches the wader boot to avoid getting sediment in the sample. SS will move the sampler above and away from the boot and engage the stopper (attached to the sampler via cable) to secure the sample in the tube.
- Step 4. SS will deposit the sample in a stainless steel bucket by holding the bottom of the tube over the center of the bucket and slowly releasing the stopper.
- Step 5. Repeat steps 2 through 4 at the other two locations in the swimming area.

- Step 6. SS will pour the composite sample that has been collected in the stainless steel bucket into the churn.
- Step 7. SS will don latex gloves.
- Step 8. SS will label three 40 milliliter (ml) glass vials (one clear and two amber).
- Step 9. SS will begin to churn the water and fill each vial from the churn spigot. The clear vial will be filled to the base of the collar. The amber vials will be filled to half. Screw the caps on to close the vial.
- Step 10. SS will store all samples on ice in the cooler for transport to the lab.
- Step 11. SS will dump the bucket contents on the ground. Gloves may be removed and disposed of at this point.
- Step 12. SS will rinse all sampling equipment with deionized water.
- Step 13. SS will fill out Chain of Custody forms.
- Step 14. SS will deliver samples to the on-duty WAPB laboratory manager.

2.3. Related Technical Issues:

A. Health and Safety Warnings:

N/A

B. Cautions:

Do not pull up on stopper too firmly or it will break.

Be sure to slowly release the stopper when emptying the composite sampler in order to avoid splashing.

C. Interferences:

Be sure to allow the water sufficient time to settle before sampling in order to avoid getting sediment in the composite sample.

D. Calibration:

N/A

E. Troubleshooting:

N/A

3.0. Roles

3.1. Responsibilities:

A. Sampling Staff (SS)

- 1. Responsible for following this TSOP.

B. Crew Chief

- 1. Responsible for training the SS on this TSOP.

3.2. Training requirements:

- A. Using the integrated water column sampler and churn
 - 1. SS
- B. Water sampling and Chain of Custody
 - 1. SS
- C. Annual branch safety training
 - 1. SS
 - 2. Crew chief

4.0. List forms, equipment, and/or software to be used

4.1. Forms:

- A. Chain of Custody

4.2. Equipment:

- A. Integrated water column sampler
- B. Stainless steel bucket
- C. Churn
- D. 40 ml clear glass vial
- E. 40 ml amber glass vial
- F. Cooler with ice
- G. Deionized water
- H. Latex gloves
- I. Chest waders
- J. Life vest

4.3. Software:

N/A

5.0. Records Management

Records management is covered in the WAPB TSOPs “Analysis for Cyanobacteria Toxins in Water Samples”, S-001-OWQ-WAP-TGM-16-T-R2, and “Cyanobacteria Identification and Enumeration”, B-002-WAP-XX-15-T-R0.

6.0. Definitions

- 6.1. “Composite Sample” – A representative water sample made up of individual smaller samples taken at periodic intervals and composited into one representative sample for analysis.

7.0. Quality Assurance / Quality Control

SS will undergo periodic field audits by a member of the Technical and Logistical Services Section.


8.0. References

N/A

9.0. Appendices

9.1. Chain of Custody

Appendix B- Laboratory Procedure for Cyanobacteria Identification and Enumeration

| | |
|---|--|
|  | <p align="center">Cyanobacteria Identification and Enumeration B-002-WAP-XX-15-T-R0 Technical Standard Operating Procedure Office: Office of Water Quality Branch: Watershed Assessment and Planning Branch Section: All Revised: N/A Revision Cycle: 4 years Effective date: December 21, 2015</p> |
|---|--|

Purpose (of the TSOP)

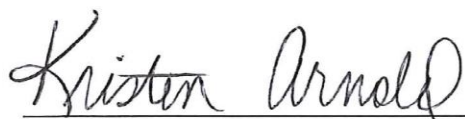
The purpose of this technical standard operating procedure (TSOP) is to describe the method for counting and identifying cyanobacteria, commonly referred to as blue-green algae. Procedures are performed by Targeted Monitoring Section (TMS) and Probabilistic Monitoring Section (PMS) staff of the Watershed Assessment and Planning Branch (WAPB) in the Office of Water Quality (OWQ). Water samples are collected by TMS staff from designated swimming beach waters managed by the Indiana Department of Natural Resources (DNR) which are located in the state of Indiana. Samples are collected monthly unless cyanobacteria cell counts exceed 100,000 cells/milliliter (mL), at which point sampling occurs biweekly. Samples are brought to the WAPB laboratory for identification, quantification, and toxin analyses (refer to the TSOP titled "Analysis of Cyanobacteria Toxins in Water Samples").

This TSOP should be used by:

This TSOP applies to agency staff in OWQ, Targeted and Probabilistic Monitoring Sections responsible for quantifying and identifying cyanobacteria cells. This TSOP covers the processes of loading the nanoplankton counting chamber, counting and identifying cyanobacteria cells using a microscope with Nomarski differential interference contrast (DIC) and epifluorescence capabilities, calculating the cyanobacteria cells present in the sample, and preserving and archiving cyanobacteria samples.

Authorizing Signatures

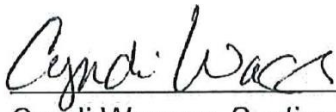
I approve and authorize this technical standard operating procedure:



Kristen Arnold, Senior Environmental Manager
Office of Water Quality, Targeted Monitoring Section



Date



Cyndi Wagner, Section Chief

Office of Water Quality, Targeted Monitoring Section

12-17-15

Date



Mike Sutton, Section Chief

Office of Water Quality, Technical and Logistical Services Section

12/17/2015

Date

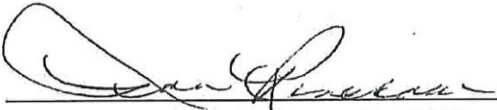


James Stahl, Environmental Technical Specialist

Office of Water Quality, Watershed Assessment and Planning Branch

17 Dec 2015

Date



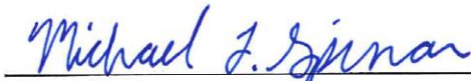
Marylou Renshaw, Branch Chief

Office of Water Quality, Watershed Assessment and Planning Branch

12-16-15

Date

This standard operating procedure is consistent with agency requirements.



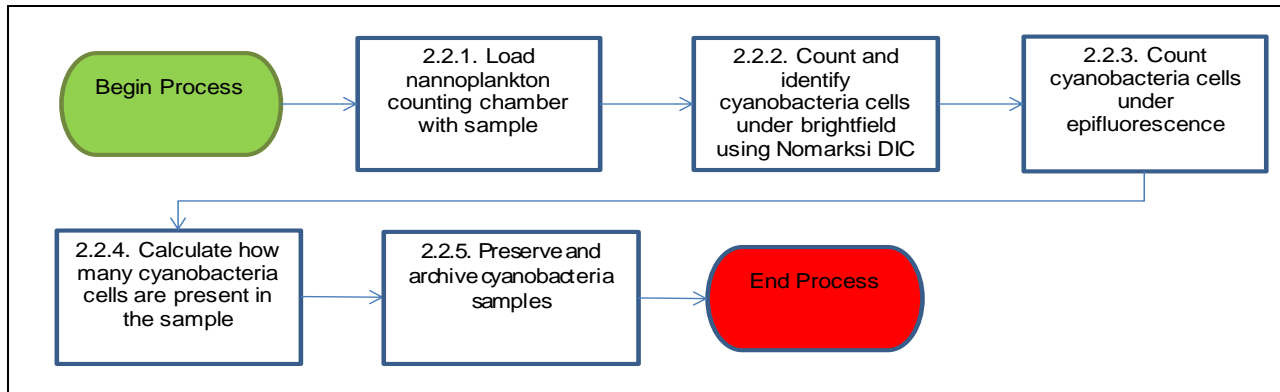
Quality Assurance Staff

Office of Program Support

12/17/15

Date

1.0 Overview Flowchart



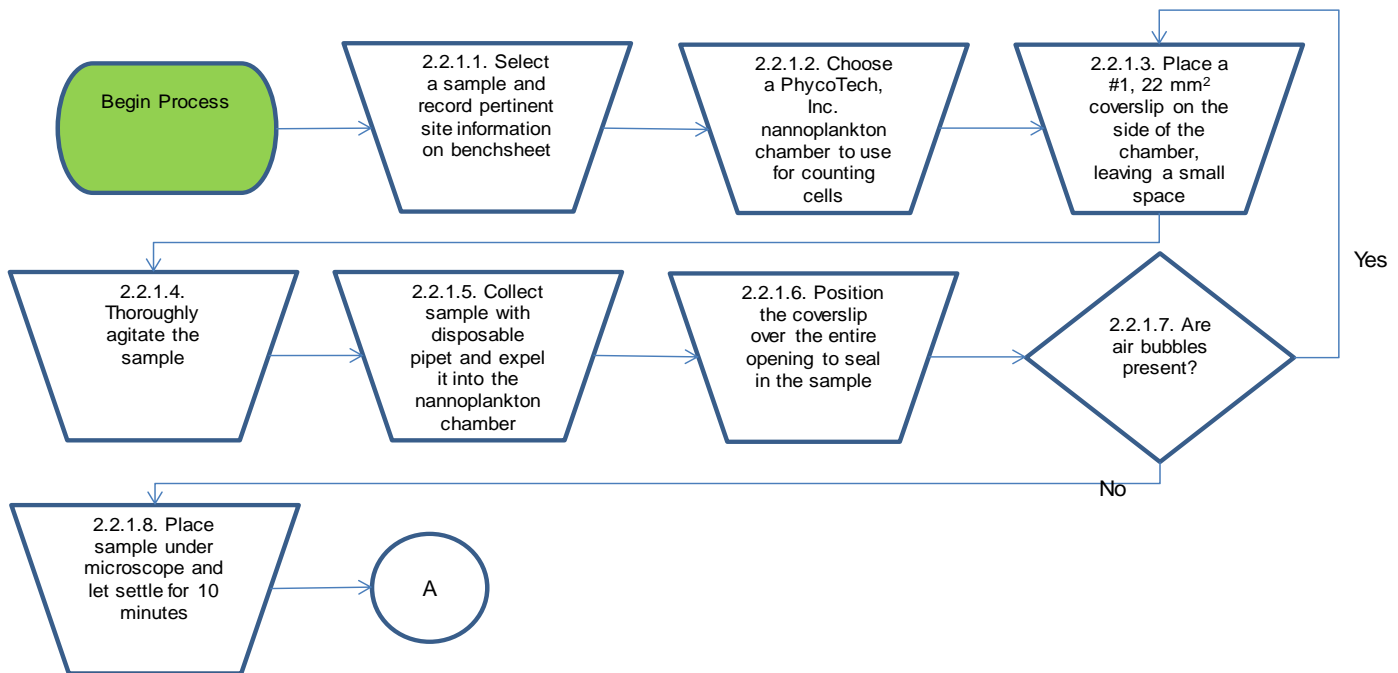
2.0 Procedure

2.1. Procedural Flowchart:

Procedural Flowcharts are shown in Section 2.2., followed by a step-by-step description.

2.2. Procedural Steps:

2.2.1. Loading the PhycoTech, Inc. nanoplankton counting chamber



2.2.1.1. All steps will be done by an IDEM biologist. Select a sample from the refrigerator and record all necessary site information along with date and time on a Cyanobacteria Benchsheet like the one shown in Appendix 1.

2.2.1.2. Choose a PhycoTech, Inc. nanoplankton chamber to use for counting cells and record the chamber's identification number on the Cyanobacteria Benchsheet. It is important to record which chamber is used because each chamber is individually calibrated and holds a slightly different volume than the other chambers. The volume of the chamber is part of the calculation to determine total cyanobacteria cells/mL.

2.2.1.3. Place a 22 x 22 mm² glass coverslip on the side of the counting chamber and leave a small opening to add sample (<http://www.phycotech.com/products.html#Nanno>).

2.2.1.4. Thoroughly agitate the sample.

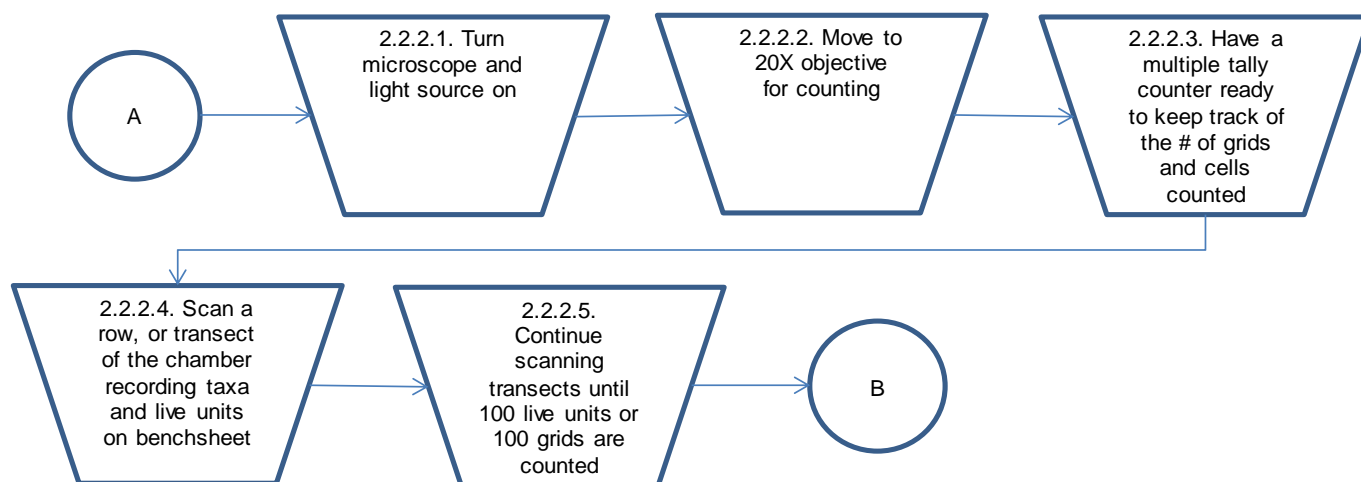
2.2.1.5. Using a disposable plastic pipet, collect an aliquot of the sample and expel it into the open space of the nanoplankton chamber that the coverslip is not covering (<http://www.phycotech.com/products.html#Nanno>).

2.2.1.6. As the sample fills the chamber, it will draw the coverslip over the chamber. Position the coverslip over the entire opening to seal in the sample (<http://www.phycotech.com/products.html#Nanno>).

2.2.1.7. If air bubbles are present, repeat the filling process by starting over at step 2.2.1.3. (Note: The chamber should be thoroughly rinsed and dried before starting over to remove any adherent cyanobacteria from the previous attempt to fill the chamber.)

2.2.1.8. Place the nanoplankton chamber on the microscope stage and let the sample settle for 10 minutes before beginning to count cells.

2.2.2. Cyanobacteria identification and enumeration using Nomarski differential interference contrast (DIC) microscopy



2.2.2.1. Turn on the microscope and the light source. The power switch on the Nikon Eclipse 80i is located on the back left side of the microscope and the light source switch is on the front of the microscope at the bottom. The manual for the Nikon Eclipse 80i microscope is located in Suite #100 of the Shadeland Office in filing cabinet “S” in the Watershed Assessment and Planning Branch area. (Make sure the upper turret is in a position other than #1 or #2).

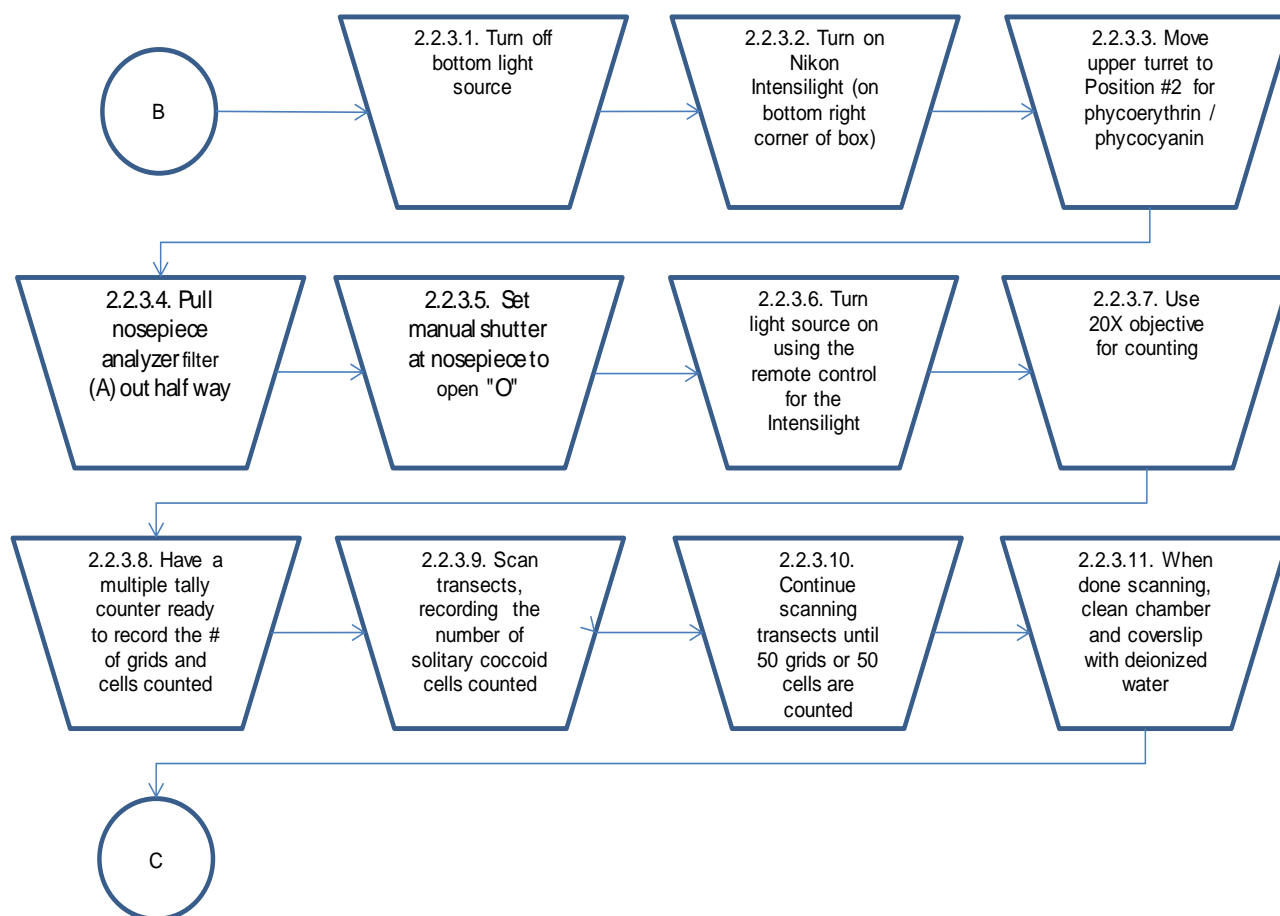
2.2.2.2. Position the 20X objective over the nanoplankton chamber.

2.2.2.3. Make sure the multiple tally counter is set at “0” before counting begins. Be sure to keep track of the number of grids that are counted since this is used in the calculation to determine total cells/mL.

2.2.2.4. Choose a transect, or row, on the nanoplankton chamber to count. Using a gridded reticle in the microscope eyepiece as a boundary, identify and count the number of cyanobacteria cells that occur within that grid. Keep track of the number of cells that are encountered in each of the grids using the multiple tally counter. The total number of cells is needed to determine total cells/mL. Also remember to record the number of grids that are scanned during the enumeration process. Record taxa and the number of natural units of each taxon on a benchsheet. In most cases, multiple transects will need to be scanned. Some colonies and filaments are only partially in the grid. The rule for counting cells that fall partially outside of the boundary is to count them if they touch the upper and left boundaries. They should not be counted if they touch the bottom or right boundaries of the grid.

2.2.2.5. Continue the identification and enumeration process until 100 natural units or 100 grids have been counted. For samples that are dense with cyanobacteria cells, it is common to record 100 natural units before 100 grids have been counted which is fine. If cyanobacteria cells are sparse, it is more likely to count 100 or more grids and potentially not even find 100 natural units. The latter is common at the beginning of the season in May and maybe June when cyanobacteria are not yet blooming. Counting can end once one of these criteria are met.

2.2.3. Cyanobacteria identification and enumeration using epifluorescent microscopy



2.2.3.1. Turn off the bottom light source of the microscope.

2.2.3.2. Turn on the Nikon Intensilight (switch is located on the bottom right corner of the Nikon Intensilight power box).

2.2.3.3. Move the upper turret to Position #2 for Phycoerythrin/Phycocyanin (you will see green light emitting from the objective). Phycoerythrin will fluoresce bright orange-red. Phycocyanin will fluoresce orange-yellow. (Epi filters for phycoerythrin/phycocyanin – Exciter: D546/10x, 25mm, Dichroic: 560 DCLP, Emitter: 580/30m)

2.2.3.4. Pull the nosepiece analyzer filter (A) half-way out.

2.2.3.5. Be sure that the manual shutter at the nosepiece is set to open “O” (moved to the right).

2.2.3.6. Turn light source on using the remote control. The intensity of the light can also be controlled by this. (To return to DIC microscopy do the opposite of the steps listed above.)

2.2.3.7. Position the 20X objective over the nanoplankton chamber.

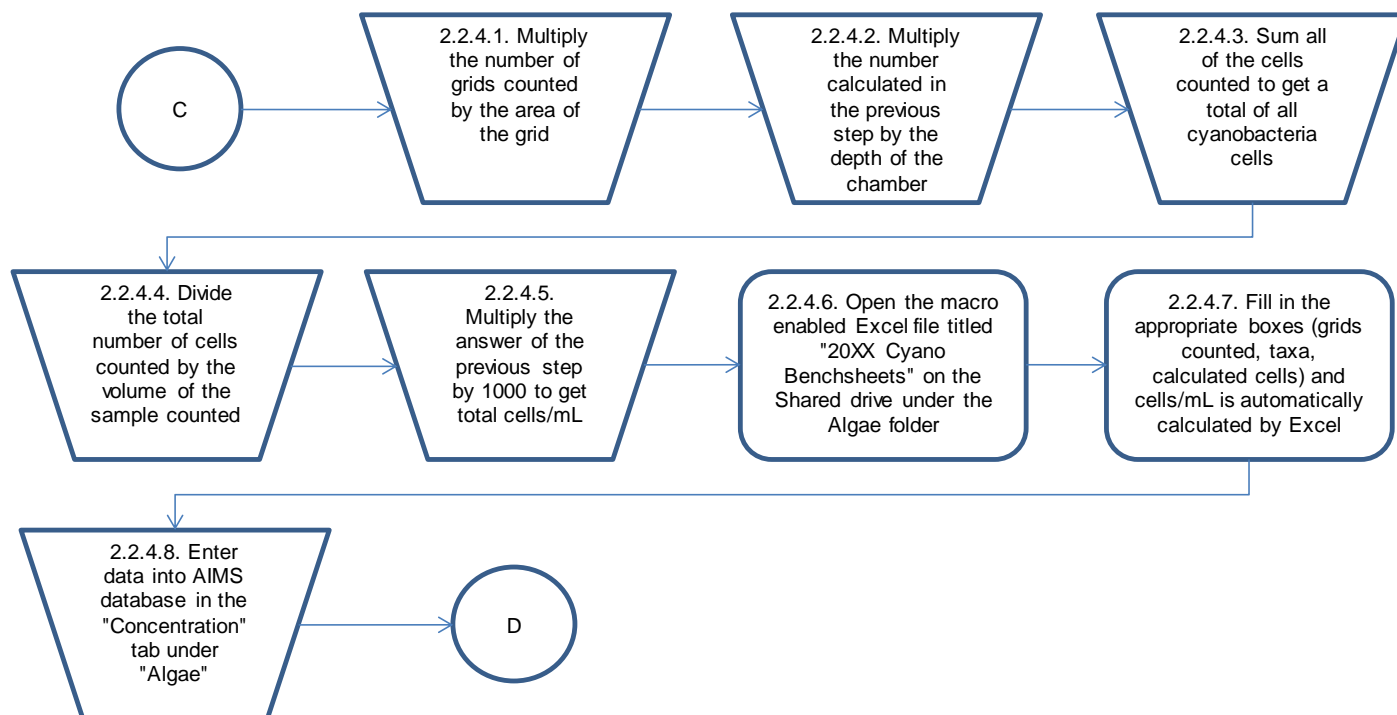
2.2.3.8. Make sure the multiple tally counter is set at “0” before counting begins. Be sure to keep track of the number of grids that are counted since this is used in the calculation to determine total cells/mL.

2.2.3.9. Choose a transect, or row, on the nanoplankton chamber to count. Using the gridded reticle in the microscope eyepiece as a boundary, identify and count the number of solitary coccoid cyanobacteria cells that occur within that grid. Only small, solitary coccoid cells, or picoplankton, that are fluorescing yellow should be counted. These cells are hard to see under DIC. Using epifluorescence causes them to glow, thus making it much easier to see and count them. It is best to perform the epifluorescent scan in a darkened room (lights off) in order to better see the dimly lit fluorescing cells. Keep track of the number of cells that are encountered in each of the grids using the multiple tally counter. The total number of cells is needed to determine total cells/mL. Also remember to record the number of grids that are used for counting in the enumeration process.

2.2.3.10. Continue the identification and enumeration process until 50 natural units or 50 grids have been counted. Again, some colonies and filaments are only partially in the grid. The rule for counting cells that fall partially outside of the boundary is to count them if they touch the upper and left boundaries. They should not be counted if they touch the bottom or right boundaries of the grid.

2.2.3.11. When scanning under both DIC and epifluorescent microscopy has been completed, thoroughly rinse and dry the nanoplankton chamber and coverslip with deionized water to avoid cross-contamination between samples.

2.2.4. Calculating total cyanobacteria concentration (cells/mL)



2.2.4.1. There are two separate sets of calculations per sample: one to determine the total cyanobacteria concentration of the discernable cells that were counted using DIC microscopy and another to determine the total cyanobacteria concentration of the picoplankton that were counted using epifluorescent microscopy. The totals from the two calculations are added together to determine the overall total cyanobacteria concentration (cells/mL) for the sample. The calculations begin with the multiplication of the number of grids counted by the area of the entire grid (0.246 mm^2 using 20X objective) to determine the area of the chamber that was enumerated. Remember that these steps are done separately for each of the counting methods. (Area = x number of grids counted x 0.246 mm^2 .)

2.2.4.2. Use the number calculated in the previous step (total area counted) and multiply it by the depth of the nanoplankton chamber to determine the volume of the sample that was counted. Each chamber has been individually calibrated so the number used in this calculation will be different depending on which chamber is being used. This calculation gives the volume in mm^3 . (Volume for chamber #715 = Area from step 2.2.4.1. x 0.373 mm.)

2.2.4.3. Sum all of the cells that were enumerated and identified to determine the total number of cyanobacteria cells that were counted.

2.2.4.4. Divide the total number of cyanobacteria cells counted by the volume of sample that was enumerated (determined in step 2.2.4.2. above). (Concentration = total number of cells counted in step 2.2.4.3. / volume calculated in step 2.2.4.2.)

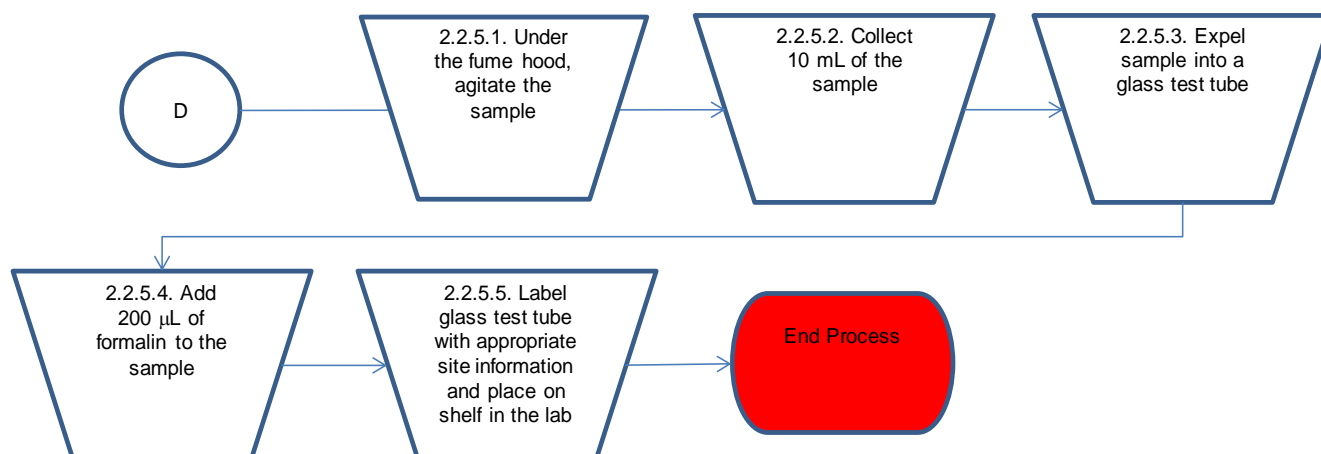
2.2.4.5. Multiply the number calculated in the previous step by 1000 to get total cyanobacteria concentration (cells/mL). (Concentration in cells/mL = concentration from step 2.2.4.4. x 1000.)

2.2.4.6. On the computer, open the macro-enabled Excel file titled “20XX Cyano Benchsheets...” on the Shared drive in the Algae folder (S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\Cyano Benchsheets). Open the correct file, which is determined by the analysis set number (also called “trip number”) and date. The trip number is shown in the upper right corner of the Cyanobacteria Benchsheet.

2.2.4.7. On the benchsheet, fill in the appropriate boxes (grids counted, taxa names, calculated cells, etc.) and the total concentration is automatically calculated in the Excel workbook. Using the Excel file with the calculations built into the spreadsheet eliminates the need to hand calculate the total cells/mL as outlined in steps 2.2.4.1. – 2.2.4.5. It is always a good idea to hand calculate a few samples throughout the season to make sure the Excel equations were not inadvertently altered.

2.2.4.8. Enter the data from the benchsheet into the Assessment Information Management System database (AIMS) in the “Concentration” tab under “Algae”.

2.2.5. Preserving and archiving samples



2.2.5.1. The procedures in this section require the use of personal protective equipment in the form of safety glasses or a safety shield and nitrile gloves. Under the fume hood in the laboratory in Building 41, agitate the sample to randomly distribute the cyanobacteria throughout the sample.

2.2.5.2. Collect 10 mL of the sample using an automatic pipettor.

2.2.5.3. Expel the sample into a glass test tube or vial with a screw top lid. The archival vial or test tube should be a type with a lined cap with integrity so the sample will not evaporate over time.

2.2.5.4. Using a 1 mL automatic pipettor, add 200 µL of formalin to the sample. This will preserve the sample for future use.

2.2.5.5. Label the glass test tube with appropriate site information such as the name of the lake, date of collection, AB number and L-site number. Place the preserved sample on the shelf in the laboratory in Building 41 with the rest of the cyanobacteria samples.

2.3. Related Technical Issues:

2.3.1. Health and Safety Warnings:

Location of safety equipment:

These procedures involve hazardous chemicals and glassware. The Safety Data Sheets (SDS) for formalin which is used in this procedure are stored in a binder in a cabinet under the fume hood in the laboratory in Building 41. An eye wash station, first aid kit, and overhead safety shower are located in the laboratory where these procedures are performed.

Broken glass:

Handle glassware such as coverslips and the nanoplankton counting chamber with care. Broken glassware should be disposed of in the “sharps” container rather than the regular trash.

Formalin hazards and safety:

- Physical hazards:
 - Thermal decomposition can lead to the release of irritating gases and vapors. Keep the product and empty container away from heat and sources of ignition.
- Health hazards:
 - May cause an allergic skin reaction.
 - Causes serious eye damage.
 - May cause drowsiness or dizziness.
 - May cause cancer.
 - Causes damage to organs.
 - May cause damage to organs through prolonged or repeated exposure.
 - May cause birth defects or other reproductive harm.
- Environmental hazards:
 - Harmful to aquatic life.
- Safety information:
 - Formalin should be handled under the fume hood using appropriate personal protective equipment (PPE) (Newhouse 1998); see “Personal Protective Equipment”, below.
 - Review the appropriate SDS before working with formalin.

Required personal protective equipment (PPE):

For the purposes of this TSOP, PPE will consist of:

- chemical resistant gloves (latex or nitrile)
- a laboratory coat; and
- protective eyewear (safety glasses, face shield, or goggles).

2.3.2. Cautions:

- Handle glassware with care.
- The nanoplankton chamber and coverslips should be stored in a protective case when not in use to eliminate the possibility of them being broken as they are very expensive.
- Be diligent about recording the correct sample number and site information on the benchsheets. Also be sure to keep track of how many grids are counted during the identification and enumeration process because this number is used in the total concentration calculation. If the number of grids counted is not recorded, enumeration and identification of the sample will need to be restarted.
- Don't let the sample dry out in the nanoplankton counting chamber. When dry patches form, identification becomes difficult.
- Make sure to thoroughly rinse the nanoplankton chamber and coverslip with deionized water and dry it after each sample to eliminate the possibility of cross-contamination between samples. Quality Assurance/Quality Control (QA/QC) field blanks help to check for this. One field blank will be analyzed each week.
- A new disposable plastic pipet should be used for each sample to eliminate the possibility of cross-contamination between samples.
- Be sure to double check calculations if completed by hand. If calculations are done in Excel, it is still important to check a couple of calculations by hand periodically throughout the sample season to ensure the equations in Excel are correct.

2.3.3. Interferences:

- Make sure to thoroughly rinse the nanoplankton chamber and coverslip with deionized water and dry it to avoid cross-contamination between samples. One field blank sample is collected each week as a QA/QC check to ensure the samples are not getting contaminated in the field or laboratory. It also serves as a way to ensure that the coverslip and counting chamber are adequately rinsed between each sample.

- The multiple tally counter should be set at “0” before counting begins. Also make sure to always record the number of grids that are encountered during the cell counting process. Not having an accurate record of the number of grids used in the counting process will result in an incorrect calculation of total cyanobacteria concentration.
- To avoid having the sample dry out while counting, measure only 20 trichomes of each species and use the average length of those trichomes as the length for all trichomes of that taxa instead of measuring each trichome individually. Measure several cells in different trichomes to come up with a standard cell size to be used for all samples. Counting would take too long if the IDEM biologist measured each trichome and/or counted all of the cells in each trichome. Using estimations where necessary ensures that samples are counted before they dry out in the chamber.
- Refer to taxonomic literature and keys and archived photographic images to resolve uncertainty regarding taxa present in the sample in order to avoid making taxonomic errors. Duplicate samples are collected at one site per week which serves as a QA/QC measure so the IDEM biologist can compare their identification and enumeration results from the same sample to ensure they are consistent with counts and identifications.

2.3.4. Calibration:

- Automatic pipettors are factory calibrated and come with a certificate of proof which is located in the laboratory.
- PhycoTech, Inc. nanoplankton counting chambers are individually calibrated by PhycoTech, Inc. Each chamber comes with its own identification number and the exact volume (in milliliters) that the chamber holds, along with instructions on how to calculate the total number of cells/mL.
- The gridded reticle in the eyepiece of the Nikon Eclipse 80i microscope was calibrated with a stage micrometer to ensure that measurements are accurate. The calibration was done before the ocular reticle was used for measurements.
- It is unknown whether the stage micrometer is traceable to a National Institute of Standards and Technology (NIST) standard.

2.3.5. Troubleshooting:

- During the identification and enumeration process, the IDEM biologist might come across trichomes and/or colonies that lie on the boundary lines of the grid on the gridded reticle. To be consistent, trichomes and colonies that extend beyond the grid

boundary should only be counted if they touch the left and upper boundary lines. Do not count cells that touch the lower and right boundary lines.

- For unidentifiable specimens, use the lowest possible classification level (e.g., if the family can be determined, but the genus is unknown record only the family name for the identification. If no classification level can be determined use “unknown cyanophyte” for the taxonomic identification.
- For trouble with the microscope, contact a customer service representative from Nikon.

3.0 Roles

3.1. Responsibilities:

A. IDEM biologist

1. Exercises proper laboratory safety – read SDS sheets and use appropriate PPE.
2. Loads the PhycoTech, Inc. nanoplankton counting chamber.
3. Identifies and enumerates cyanobacteria samples using both Nomarski DIC and epifluorescent microscopy.
4. Calculates total cyanobacteria concentration (cells/mL).
5. Preserves and archives samples.
6. Records taxonomic information in AIMS.

3.2. Training requirements:

A. Training in the process of loading the nanoplankton counting chamber.

1. IDEM biologist

B. Training in the identification and enumeration of freshwater cyanobacteria.

1. IDEM biologist

C. Training in sample preservation and archiving subsamples.

1. IDEM biologist

D. Training in AIMS database management.

1. IDEM biologist

4.0 List forms, equipment, and/or software to be used

4.1. Forms:

A. Cyanobacteria Benchsheet

- B. Field Chain of Custody Form
- C. Cyanobacteria Lab Datasheet

4.2. Equipment:

- A. Nikon Eclipse 80i microscope
- B. PhycoTech, Inc. nanoplankton counting chambers
- C. Disposable plastic pipets
- D. Kim-Wipes
- E. 22 x 22 mm² glass coverslips
- F. Epifluorescence capability on microscope
- G. Multiple tally counter
- H. Gridded reticle
- I. 10 mL automatic pipettor
- J. 10 mL pipettor tips
- K. Glass test tube with screw top lid
- L. Formalin
- M. 1 mL automatic pipettor
- N. 1 mL pipettor tips

4.3. Software:

Not applicable.

5.0 Records Management

Original copies of the field chain of custody forms and benchsheets are stored indefinitely in a file cabinet in the laboratory in Building 41. Identification and enumeration information from the benchsheets are stored in AIMS. Chain of custody forms and benchsheets are scanned and stored as attachments in AIMS.

6.0 Definitions

- 6.1. “AA/AB Number” – A number assigned to each individual watershed sampling event conducted by IDEM staff. This number is automatically generated in the Assessment Information Management System (AIMS) database.
- 6.2. “Agency staff” – Any employee or representative of the Indiana Department of Environmental Management including regular employees, temporary employees, contractors and interns.
- 6.3. “Assessment Information Management System database (AIMS database)” – IDEM database containing information related to water chemistry, aquatic habitat, macroinvertebrate, fish, and algae communities, fish tissue analyses, sediments, and *E. coli* bacteria data collected by agency staff from watershed sampling events.

- 6.4. “Benchsheet” – A paper form where data such as cyanobacteria taxa names and cell count information is recorded.
- 6.5. “Chain of Custody (COC)” – The chronological documentation or paper trail showing the custody and transfer of a sample.
- 6.6. “Cyanobacteria” – A division of microorganisms that are related to the bacteria but are capable of photosynthesis. They are also referred to as blue-green algae.
- 6.7. “Deionized water” - A type of water produced through a process called ion exchange. Water is placed in a container with a special negatively charged resin that attracts minerals, such as sodium and calcium, removing them from the water.
- 6.8. “Gridded reticle” – Clear disc marked with a grid pattern that fits in the eyepiece of a microscope.
- 6.9. “IDEM biologist” – Indiana Department of Environmental Management scientist educated and trained to understand the life of living organisms, including their structure, function, growth, origin, evolution, and distribution. In this particular case, the IDEM biologist needs to have specific knowledge of algae and the taxonomy of cyanobacteria.
- 6.10. “Nannoplankton counting chamber” – Microscope slide counting chamber that holds a known volume of sample for quantitative counting of cells with a microscope.
- 6.11. “Natural Units” – Natural grouping of algae (i.e., individual filament, colony, or isolated cell). An individual filament, colony, or isolated cell all count as only one natural unit even though the natural unit might be comprised of several cells.
- 6.12. “Nomarski differential interference contrast (DIC)” – An optical microscopy illumination technique used to enhance the contrast in unstained, transparent samples.
- 6.13. “Picoplankton” – The fraction of plankton composed of cells between 0.2 and 2 μm in size. This technical standard operating procedure (TSOP) is only concerned with cyanobacterial picoplankton that glows yellow under epifluorescence.
- 6.14. “Safety Data Sheet (SDS)” – Information intended to provide workers and emergency personnel with procedures for handling or working with that substance in a safe manner.
- 6.15. “Taxonomist” – A biologist that groups organisms into categories.
- 6.16. “Technical standard operating procedure (TSOP)” – A standard operating procedure that involves environmental data generation, manipulation or compilation of an analytical process.
- 6.17. “Trichome” – A strand or chain of cells (as in a filamentous colony of bacteria or algae).

7.0 Quality Assurance / Quality Control

QA/QC of the cyanobacteria project will be documented by checks of both field and laboratory data. A field blank is collected and analyzed each week. The field blank is kept in the cooler with the normal samples and analyzed under the microscope. This QC check ensures that the sample was not somehow contaminated in the cooler and it is a way for the IDEM biologist to assess whether the

nannoplankton chamber and coverslip are adequately rinsed between samples. If cyanobacteria cells are ever found in the field blank sample, it can be assumed the nannoplankton counting chamber and/or coverslip were not thoroughly rinsed after the last sample or that the sample got contaminated in the cooler. Duplicate cyanobacteria field samples are collected at one site every week. The duplicate sample is analyzed under the microscope in the same way as the normal samples. This helps the IDEM biologist to determine whether there is consistency between samples for both identification and enumeration purposes.

8.0 References

- 8.1. [PhycoTech nannoplankton chamber product page](#)
- 8.2. [Analysis of Cyanobacteria Toxins in Water Samples](#), S-001-OWQ-W-TM-14-T-R1, October 31, 2014.
- 8.3. Newhouse, S.A. 1998. Field and Laboratory Standard Operating Procedure (SOP) for the Transportation, Use, Handling, and Storage of Solutions Which Contain Formaldehyde (HCHO). Biological Studies Section, Assessment Branch, Office of Water Management. IDEM/32/03/006/1998.

9.0 Appendices

Appendix 1 – Example of a Cyanobacteria Benchsheet that is used for data entry


Appendix 2 – Example of a Field Chain of Custody form used when cyanobacteria were collected

Appendix 3 - Cyanobacteria Lab Datasheet

Appendix 4 – List of cyanobacteria taxonomic references (located in the laboratory)

- Clercin, N. 2010. An Identification Guide to the Cyanobacteria of Indiana, Center for Earth and Environmental Science, Indiana University Purdue University Indianapolis.
- John, D. M., Whitton, B. A. & Brook, A. J. 2002. The Freshwater Algal Flora of the British Isles, Cambridge University Press, New York, New York.
- Komarek, J. & Zapomelova, E. 2008: Planktic morphospecies of the cyanobacterial genus *Anabaena* = subg. *Dolichospermum* – 2. part: straight types. Fottea 8:1-14.
- Prescott, G. W. 1951. Algae of the Western Great Lakes Area, Cranbrook Institute of Science, Bloomfield Hills, Michigan.
- Prescott, G. W. 1970. How to Know the Freshwater Algae, Third Edition, WCB McGraw-Hill Burr Ridge, Illinois.
- St. Amand, A. & Wagner, K. J. 2012. Collection, Identification, Ecology of Freshwater Algae, PhycoTech, Inc.
- Wacklin, P., Hoffmann, L. & Komarek, J. 2009: Nomenclatural validation of the genetically revised cyanobacterial genus *Dolichospermum* (Ralfs ex Bornet et Flahault) comb. nova. Fottea 9:59-64.
- Wehr, J. D. & Sheath, R. G. 2003. Freshwater Algae of North America, Academic Press, Boston, Massachusetts.

Appendix C- Laboratory Procedure for Microcystins, Cylindrospermopsin and Anatoxin-a Analysis

| | |
|---|--|
|  | <p>Analysis of Cyanobacteria Toxins in Water Samples S-001-OWQ-WAPTGM-16-T-R2</p> <p>Technical Standard Operating Procedure Office: Office of Water Quality Branch: Watershed Assessment and Planning Branch Section: Targeted Monitoring Section</p> <p>Revised: September 30, 2016 Revision Cycle: 4 years Originally effective date: June 12, 2013</p> |
|---|--|

Scope of operations

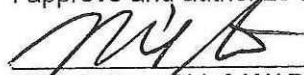
This technical standard operating procedure (TSOP) ensures that sample preparation and analysis using the enzyme-linked immunosorbent assay (ELISA) kits for microcystins, anatoxin-a, and cylindrospermopsin toxins and Anatoxin-a receptor-binding assay (RBA) kit for anatoxin-a toxin are conducted in a consistent manner in order to yield high quality data that is reproducible.

Scope of applicability

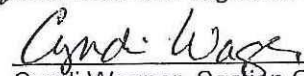
This TSOP applies to agency staff located in the Office of Water Quality (OWQ), Watershed Assessment and Planning Branch (WAPB) responsible for preparing and analyzing cyanobacteria toxins in samples collected from surface water as part of the agency's ambient water quality monitoring program.

Authorizing Signatures

I approve and authorize this technical standard operating procedure:


Mike Sutton, Chief, WAPB Quality Assurance Manager
Technical and Logistical Services Section, Office of Water Quality

8/26/16
Date


Cyndi Wagner, Section Chief
Targeted Monitoring Section, Office of Water Quality

8/29/16
Date


Marylou Renshaw, Branch Chief
Watershed Assessment and Planning Branch, Office of Water Quality

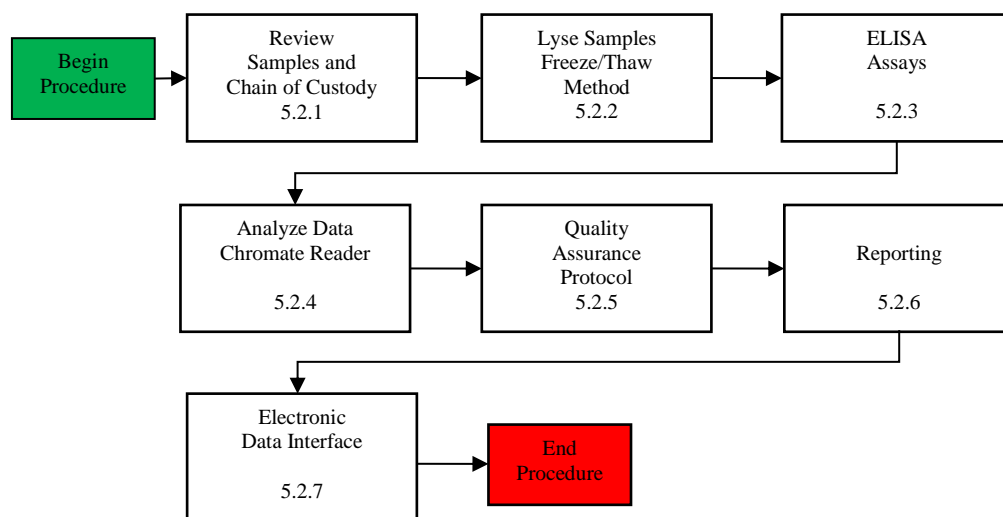
9/2/16
Date

This Technical standard operating procedure is consistent with Agency requirements.


Quality Assurance Staff
IDEM Office of Program Support

10/3/16
Date

1.0 Overview Flowchart



2.0 Definitions

- 2.1 “AA/AB Number” – A number assigned to each individual watershed sampling event conducted by Indiana Department of Environmental Management (IDEM) staff. This number is used to identify the sampling event in the Assessment Information Management System (AIMS) database
- 2.2 “Agency staff” – Any employee or representative of IDEM including regular employees, temporary employees, contractors and interns.
- 2.3 “Assessment Information Management System (AIMS) database” – IDEM database containing information related to water chemistry, aquatic habitat, macroinvertebrate and fish communities, fish tissue analyses, sediments, algae, and *E. coli* bacteria data collected by Agency staff from watershed sampling events.
- 2.4. “Chain of Custody (COC)” – The chronological documentation or paper trail showing the custody and transfer of a sample.
- 2.5. “Deionized” – A process called ion exchange. Water is placed in a container with a special negatively charged resin that attracts minerals, such as sodium and calcium, removing them from the water.
- 2.6. “Indiana Department of Environmental Management (IDEM)” – An agency of Indiana State Government whose mission is to implement federal and state regulations to protect human health and the environment while allowing the environmentally sound operations of industrial, agricultural, commercial and government activities vital to a prosperous economy.
- 2.7. “Laboratory notebook” - A notebook used by the laboratory scientist to record analysis results, calibrations, audits, monitor checks, or any other notation necessary regarding the data. This document can be used for troubleshooting or data validation.
- 2.8. “Laboratory custodian (LC)” – The WAPB staff person responsible for checking in the sample and for the initial curation of algae, fish and macroinvertebrate samples brought into the laboratory from field sampling.
- 2.9. “Laboratory scientist”- The agency staff person responsible for preparing and analyzing water samples following a strict preparation and analysis protocol.
- 2.10. “Office of Water Quality (OWQ)” – The Office of Water Quality is within IDEM and its mission is to monitor, protect, and improve Indiana’s water quality to ensure its continued use as a drinking water source, habitat for wildlife, recreational resources and economic asset. The office achieves this by developing rules, guidance, policies, and procedures; assessing surface and ground water quality; regulating and monitoring drinking water supplies and wastewater treatment facilities; protecting watershed and wetlands and providing outreach and assistance to the regulated community and the public while supporting environmentally

responsible economic development.

- 2.11. "Portable document format (pdf)" – is an open standard for document exchange created by Adobe Systems in 1993.
- 2.12. "Probabilistic Monitoring Section (PMS)" – A section within the Watershed Assessment and Planning Branch of the Office of Water Quality.
- 2.13. "Quality control samples" – Additional samples collected in the field and prepared in the laboratory for each sampling event of 1 to 20 samples which includes: field blank, field duplicate, lab blank, and lab duplicate. For a sampling event of more than 20 samples, an additional set of quality control samples are collected. For each increment of 20 samples, quality control samples are collected.
- 2.14. "Quality assurance officer (QAO)" – An agency staff member in the program areas responsible for ensuring that quality assurance criteria are met.
- 2.15. "Receptor-Binding Assay (RBA)" – Receptor-binding assay for the detection of anatoxin-a in fresh water samples.
- 2.16. "Sample collector"-The agency staff person responsible for collecting water samples from designated beaches following assigned protocol.
- 2.17. "Sampling event" – The occasion during which environmental samples (i.e., soil, sediment, water) are collected and submitted to a laboratory for analysis.
- 2.18. "Sample set #/ Event ID"- A unique number assigned to a group of samples collected for a single sampling event. This number is used in the AIMS database to identify samples with quality control samples as a group.
- 2.19. "Targeted Monitoring Section"- A section within the WAPB of the OWQ.
- 2.20. "Technical and Logistical Services Section (TLSS)" – A section within the WAPB of the OWQ.
- 2.21. "Technical standard operating procedure (TSOP)" – A standard operating procedure that involves environmental data generation, manipulation or compilation of an analytical process.

3.0 Roles

3.1. Responsibilities:

- A. Sample collector
 - Collects water samples for the analysis of microcystins and cylindrospermopsin toxin in recreational waters.
- B. Laboratory custodian
 - Checks in algae samples brought into the laboratory from the field.
- C. Laboratory scientist
 - Extracts and quantifies microcystins and cylindrospermopsin toxin from water samples.
- D. Project leader
 - Makes final decisions on problems and changes to procedures concerning the cyanobacteria toxin sample preparation, ELISA, and reports to the section chief of the Targeted Monitoring Section.
- E. Section chief
 - Responsible for the algae project and approves results for release to the public.
- F. Quality assurance officer
 - Prepares data entry forms, validation of the quality of ELISA results in AIMS, and releases data for use in AIMS.

3.2. Training requirements:

- A. Training in collecting algae samples in the field.
 - Sample collector
- B. Training in receiving and checking algae samples into the laboratory.
 - Laboratory custodian
- C. Training in extraction, analysis, and reporting of microcystins and cylindrospermopsin toxin from water samples.
 - Laboratory scientist

4.0 List forms, equipment and/or software to be used

4.1. Forms

- A. Biological Samples Field Chain of Custody Form
- B. Microcystins ELISA Procedure Form
- C. Cylindrospermopsin ELISA Procedure Form
- D. Anatoxin-a Receptor-Binding Assay Procedure Form
- E. Anatoxin-a ELISA Procedure Form

4.2. Equipment:

- A. Microcystins ADDA Lab Book
- B. Micropipette (sizes 10-100µL, 100-1000µL)
- C. Multichannel pipette (25-300µL)
- D. Para film
- E. Vortex Mixer
- F. Centrifuge
- G. Pipette tips (1-200µL, 100-1000µL)
- H. Light Protection Cover
- I. Laptop
- J. Shell vials
- K. Nitrile gloves
- L. Incubator set at 37°C± 1°C
- M. Centrifuge tubes
- N. Borosilicate glass, Teflon-lined screw cap vials
- O. Titer-Tops Clear Adhesive Film
- P. Squirt bottle
- ~~Q.~~ Chromate Reader

4.3. Software

- A. Chromate

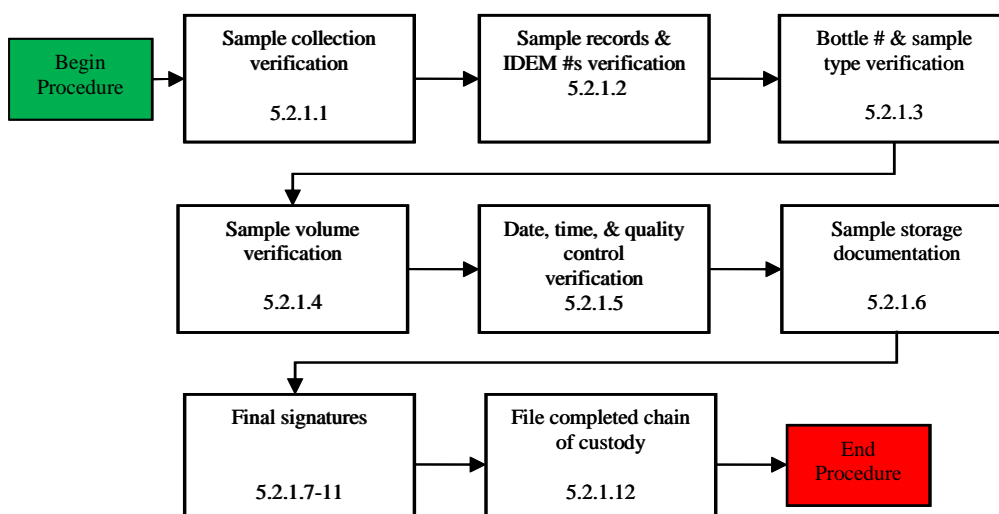
5.0 Procedure

5.1. Procedural Flowchart:

Procedural flowcharts are shown in Section 5.2 followed by a step by step description.

5.2. Procedure

5.2.1. Receiving samples and completing the Field Chain of Custody form.



- 5.2.1.1. Laboratory custodian (LC) verifies that the sample collector has signed the biological samples field COC form certifying that the sample(s) listed below was/were collected by him/her in his/her presence. Laboratory custodian also verifies the date that the samples were turned into the lab and the sample type (i.e. algae).
- 5.2.1.2. LC verifies that each sample is recorded on the field COC form. LC makes sure that the Event ID and the IDEM Sample # on the field chain of custody form matches the Event ID and the IDEM Sample # on the algae samples.
- 5.2.1.3. LC makes sure that the correct number of bottles is listed in the Volume (ml) column for type of bottle(s) on the field COC form. (Note: There should be two forty-ml bottles approximately half full (20 ml).) If Anatoxin-a analysis will be run on sample, then one of the two bottles should be amber colored.
- 5.2.1.4. LC checks that 40 is written in the "Volume (ml)" column heading. The bottles should be approximately half full. If the volume is much greater than half full it should be noted on the field COC form. (Note: If some of the sample is to be removed before placing the 40 mL bottle into freezer due to breakage during the freeze, the sample must be adequately mixed immediately prior to removing some of the liquid.)
- 5.2.1.5. LC check the date (mm/dd/yyyy) and time (24 hr) collected for each sample. Look for field duplicate (FD) and field blank (FB) samples. Identify on the field COC form with a "FD" and/or "FB." Each sample set ID/event ID should contain at least one FB and one FD. If field QC samples are missing, request an explanation from sample collector and note the explanation on COC and in lab notebook.
- 5.2.1.6. LC fills in the sample delivery building number in the final column of the COC and places a check mark for each bottle of a sample. (Note: There should be two check marks for each sample number.) If two samples were not received, request an explanation from sample collector and note the explanation on COC and lab notebook.
- 5.2.1.7. Sample collector signs next to "Relinquished by:" in order to relinquish the samples to the lab custodian.
- 5.2.1.8. LC then signs next to the "Received by:" stating that he/she has physically verified and received all the samples listed on the field chain of custody form. Fill in date and time that samples were received from sample collector.

5.2.1.9. LC signs and records the date and time at the very bottom of the field chain of custody form.

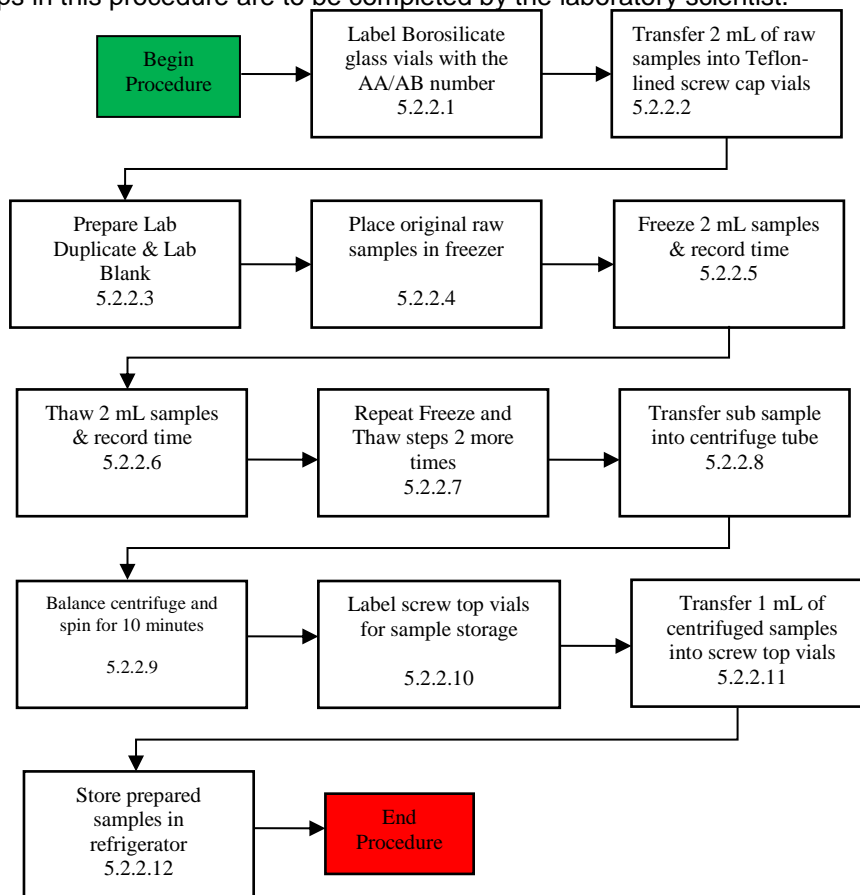
5.2.1.10. Sample collector is now finished with the sample receiving process and may leave.

5.2.1.11. The LC fills in the date and time that samples are placed in storage. The samples for algae cell enumeration are stored in the laboratory refrigerator or delivered to lab scientist in a different location. The samples for the toxin analysis are stored in the lab refrigerator until a 2- mL aliquot is removed for the lysing by freeze/thaw process (5.2.2) and then the original samples are stored on their sides in the lab freezer. The Anatoxin-a ELISA samples are received in separate amber bottles with 2 ml of 10X Buffer added to bottle before sample is collected. The 10X Buffer is supplied in each kit. The total volume in these bottles will be approximately 20 ml or half full.

5.2.1.12. Scan and create a pdf of the field chain of custody (COC) form. Save the PDF file to the proper folder on the "S" drive using the following pathway
OWQ\WSP\WSP\OWM\Algae\Cyanobacteria, and the current year of the Lab Results, and finally under the weekly folder named with the Trip number and the date of the analysis. Example pathway would be (S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\2016 Lab Results\16LAW006-20160621). Name the COC file with COC and then the trip number. Example of COC file name would be COC16LAW001.

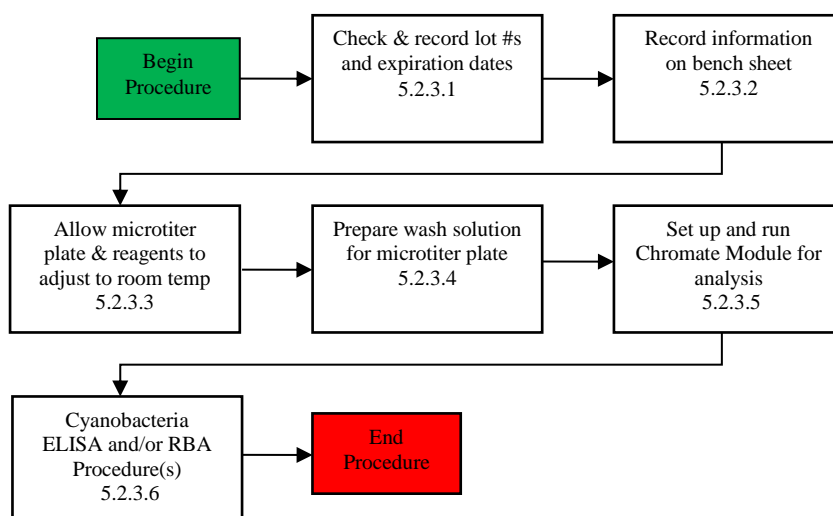
5.2.2. Lyse samples using Freeze-Thaw Method (Exclude the Anatoxin-a ELISA assay samples).

All steps in this procedure are to be completed by the laboratory scientist.



5.2.2.1. For Microcystins ELISA, Cylindrospermopsin ELISA, Anatoxin_a RBA analysis, the laboratory scientist (LS) labels borosilicate glass, screw cap vials. The AA/AB number of sample, the sample collection date, and a brief name is required for keeping track of the sample through the process. Anatoxin_a ELISA samples are pipetted directly from the collection bottle.

- 5.2.2.2. The LS, using the 100-1000 µL micropipette and corresponding tip, transfers 2 mL of well-mixed raw sample into the corresponding Borosilicate glass, screw cap vials.
 - 5.2.2.3. The LS prepares quality control samples as follows: for a lab duplicate sample, transfer an additional 2 mL aliquot from one of the field samples; and for a lab blank sample, transfer 2 mL of de-ionized water from the laboratory into another Teflon-lined screw cap vial. The lab duplicate sample will be labeled using the original sample number with LD added. The lab blank naming protocol is the current year, month, and day and add LB to the end. (Example: "20120712LB").
 - 5.2.2.4. The LS places the original raw samples in freezer for storage.
 - 5.2.2.5. The LS places 2 mL sample vials and quality control vials on their sides in freezer until completely frozen. Record the start of freeze time on the ELISA Bench Sheet form.
 - 5.2.2.6. The LS removes sample from freezer and allows it to thaw completely with no visible ice crystals remaining in samples. The sample may be placed in a room temperature or up to 37°C water bath. Record the start of thaw time on the ELISA Bench Sheet form..
 - 5.2.2.7. The LS repeats the freeze and thaw cycle two more times. Record each freeze and thaw time on Bench Sheet. (Note: each freeze step should take between 17 and 30 minutes and each thaw step should take between 17 and 30 minutes).
 - 5.2.2.8. The LS transfers thawed samples into labeled centrifuge tubes. (Suggestion: place centrifuge tubes in a test tube rack being careful in maintaining sample order.)
 - 5.2.2.9. The LS balances centrifuge with sample tubes, sets speed at 3.2 setting (3.2 x 1000 rpm) and spins for 10 minutes.
 - 5.2.2.10. The LS labels screw top shell vials for each spun-down sample with AB #, site name, preparation date, and if it is a quality control sample.
 - 5.2.2.11. The LS transfers 1 mL of sample from the top layer of centrifuge tube to corresponding Teflon-lined screw top lid shell vial. This is 1X (non-diluted) lysed sample.
 - 5.2.2.12. The LS stores covered samples in refrigerator until ready for analysis. After sample aliquot is removed for ELISA analysis, store samples in refrigerator for possible repeat analysis.
- 5.2.3. Assay Preparations and Cyanobacteria Analysis Procedures for Prepared samples.
- All the following steps are to be completed by the laboratory scientist(s).



- 5.2.3.1. The LS opens cyanobacteria toxin kit(s). With a marker or pen, write date opened and LS Initials. Check expiration date of kit. Remove metal seals from standards and control. (Note: laboratory forceps is a good instrument to use to peel off the seals quickly.)
- 5.2.3.2. The LS begins filling out bench sheet specific for the toxin analyzed. Add the start date, LC names, titer well position for samples (use duplicates), date and time sample was collected, sample AB number, location, assay name, and any additional notes. The Freeze/Thaw dates and times should already be on one of the bench sheets from an earlier step. The LS records lot numbers, expiration dates, and dates opened for all reagents and solutions. If opening a new container, write the date and initials on the container itself. Add lot #s and expiration dates for each item used in the kit onto the appropriate bench sheet (Note: bench Sheets are located in top drawer of Building 41 file cabinet in a labeled file.)
- 5.2.3.3. The LS removes from the analysis kit the predetermined microtiter plate strips/rows needed for analysis from the microtiter plate. The LS places the strips into a microtiter plate from a previous run (reused plates located in lab supply drawer). Reseal zip-lock foil bag with the remaining strips and put back into analysis kit for later use. Place the microtiter plate with the required plate strips/rows (Note: do not use a partial row), the reagents to be used early for the analysis, and the lysed samples on the bench top and allow them to adjust to room temperature.
- 5.2.3.4. The manual wash requires wash solution from the ELISA or RBA kits. The analysis kit wash solutions are the same solution for Microcystins, cylindrospermopsin, and both the Anatoxin-a analysis. The wash solution is a 5X concentrated solution that needs to be diluted with de-ionized water in a 1:5 ratio by LS. Prepare solution by adding the 5X concentrate and four equal volumes of de-ionized water into the 1L container. (Note: This solution can remain until used or until the summer sampling season is over.)
- 5.2.3.5. LS sets up the Abraxis module for running the toxin analysis on the Chromate Reader. The Chromate program is on a designated laptop stored in the Building #41. This process should take less than 30 minutes and can be started anytime during the analysis. (The analysis and storage of results are described in a later section of this SOP.)
 - A. Turn on Chromate Machine. Toggle switch is in back ("ON" position is when the top half is flat and pushed in).
 - B. Connect the lap top to the Chromate Reader (Reader must be on before connecting lap top).
 - C. Open Chromate Manager ICON on the Desk Top.
 - D. Click on "Assay Calibration" on bottom to load the assay method onto plate.

- E. From drop down menu on top left, select “Microcystin ADDA” or “Cylindrospermopsin 1X” or “Anatoxin-a 1X” or “Anatoxin-a ELISA”. Microcystin ADDA means analyzing samples for Microcystin toxin at no dilution or 1X as a freeze-thaw process.
- F. To add calibration test, go to top right and on drop down click “ALL”.
- G. Click on “Add to Plates” on bottom right. See wells are assigned.
- H. If everything is connected correctly the tray will now come out. Click “Tray In” on left.
- I. Click “Add Sample” on bottom of screen.
- J. Click “Add Sample ID” on bottom left.
- K. Click “Edit” & then add AB#s on bottom or do not click “Edit” and choose AB # from list. The AB # may already be there from a different analysis. If some are there and some of the sample #s are not, you may get in and out of the edit mode depending on whether you need to add a new AB # or select from the list.
- L. Identify sample, for example, if sample is a field duplicate put “duplicate” in 3rd column under “List Name”.
- M. Click “Done”.
- N. From Sample Database, select sample number you want to add to the analysis and in bottom of 1st column click “Choose”. Must be selected in the correct analysis order.
- O. See them on left side (top) ID column. Look to see if all are there and order is correct. Highlight all.
- P. Close the Sample ID list.
- Q. Click on Test column and highlight “Microcystins ADDA” or “Cylindrospermopsin 1X” or “Anatoxin-a 1X” or “Anatoxin-a ELISA” to right.
- R. Click “Add Test” on 3rd column at bottom of screen. See work list on right and check if highlighted.
- S. Click “Add to Plate”. If plate is not ready to be read, you can leave the program here until plate is prepared.

5.2.3.6. Cyanobacteria toxin analysis Procedures. (Note: Steps 5.2.2.1 through 5.3.2.5 are to be completed in preparation for this analysis.)

- A. LS procedures for the Microcystins ELISA (Note: approximately 3.5 hours)
 - Add 50 µL of standards, control, and samples into corresponding wells of the test strips in duplicate according to the plate layout and the Microcystins ELISA Procedure form. Use the same tip for standards 0-5 in successive order. Prime all new tips by extracting and expelling liquid at least 3 times.
 - Add 50 µL of antibody solution into each well successively using the multichannel pipette. Cover titer tray with Titer-Tops Clear Adhesive Film. Mix by moving the tray in a circular motion on the bench top for 30 seconds. Incubate for 90 minutes at room temperature.
 - Remove Titer-Tops Clear Adhesive from titer tray. Wash wells manually with squirt bottle of wash solution on countertop. Fill each well to top with Wash buffer and empty buffer in sink by shaking vigorously. Repeat 3 times. Make sure all liquid is taken out from wells and that no bubbles are present in each well. If bubbles are present, strongly tap the titer tray upside down on a paper towel on the bench top. Keep tapping until there are no bubbles present at bottom of wells. (Note: Do not wrap the plate with paper towels. Fibers from the towels can cause errors in the analysis. Bubbles left in wells can also cause erroneous results.)
 - Add 100 µL of enzyme conjugate into each well successively using the Multichannel pipette. (Note: make sure enzyme conjugate has been equilibrated to room temperature before using.) Cover titer tray with Titer-Tops Clear Adhesive Film. Mix by moving the tray in a circular motion on the bench top for 30 seconds.
 - Incubate for 30 minutes at room temperature under light-safe box/cover. Remove Titer-Tops Clear Adhesive from titer tray. Wash wells manually with squirt bottle of wash solution on countertop. Fill each well to top with wash buffer and empty buffer in sink by shaking vigorously. Repeat 3 times. Place titer tray in plate washer. Make sure all liquid is taken out from wells and that no bubbles are present in each well. If bubbles are present, strongly tap the titer tray upside down on a paper towel on the benchtop. Keep tapping till there are no bubbles present at bottom of wells.

- Add 100 µL of color substrate solution into each well successively using the multichannel pipette. (Note: make sure color substrate has been equilibrated to room temperature before using.) Cover titer tray with Titer-Tops Clear Adhesive Film.
- Incubate for 25 minutes at room temperature under light-safe box/cover.
- Add 50 µL of stop solution into each well successively using the multichannel pipette. (Note: make sure stop solution has been equilibrated to room temperature before using.) The color of the liquid in the wells will turn from blue to yellow instantly.
- Read tray using the assay named in step 5.2.4.

hours) B. LS procedure for the Cylindrospermopsin ELISA (Note: approximately two

- Add 50 µL of standards, control, and samples into corresponding wells of the test strips in duplicate according to the plate layout and the Microcystins ELISA Procedure form. Use the same tip for standards 0-5 in successive order. Prime all new tips by extracting and expelling liquid at least 3 times.
- Add 50 µL of enzyme conjugate solution into each well successively using the multichannel pipette.
- Add 50 µL of antibody solution into each well successively using the Multichannel pipette. Cover titer tray with Titer-Tops Clear Adhesive

Film. Mix by moving the tray in a circular motion on the bench top for 30 seconds.

- Incubate for 45 minutes at room temperature. Remove Titer-Tops Clear Adhesive from titer tray. Place titer tray in plate washer. . Wash wells manually with squirt bottle of wash solution on countertop. Fill each well to top with wash buffer and empty buffer in sick by shaking vigorously. Repeat 4 times. Make sure all liquid is taken out from wells and that no bubbles are present in each well. If bubbles are present, strongly tap the titer tray upside down on a paper towel on the benchtop. Keep tapping till there are no bubbles present at bottom of wells.
- Add 100 µL of color substrate solution into each well successively using the multichannel pipette. (Note: make sure color substrate has been equilibrated to room temperature before using.) Cover titer tray with Titer-Tops Clear Adhesive Film.
- Incubate for 30-45 minutes at room temperature under light-safe box/cover.
- Add 100 µL of stop solution into each well successively using the multichannel pipette. (Note: make sure stop solution has been equilibrated to room temperature before using.) The color of the liquid in the wells will turn to yellow instantly.
- Insert tray into Chromate Reader and read tray within 15 minutes of adding stop solution.

hours) C. Assay procedure for the Anatoxin-a RBA (Note: approximately five

- Add 25 µL of the standard assay buffer or 25 µL of the sample assay buffer into the appropriate wells of the test strips according to the plate layout in the Anatoxin-a RBA Procedure form.
- Add 100 µL of the standard solutions and samples into the appropriate wells of the test strips, containing the appropriate assay buffer. Analysis in triplicate is recommended. 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.
- Incubate the strips in an incubator at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 2 hours.
- Add 50 µL of biotinylated alpha-bungarotoxin solution to the individual wells successively using a multichannel pipette. Cover the wells with parafilm, mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds.
- Incubate in an incubator at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 30 minutes.
- Remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times using 1X washing buffer solution. Use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
- Add 100 µL of reconstituted streptavidin-HRP conjugate solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm and mix by moving the strip holder in a circular motion on the benchtop for 60 seconds.

- Incubate in an incubator at 37 ° C for 30 minutes.
- Remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times using 1X washing buffer solution. Use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels
- Add 150 µL of substrate (color) solution to the wells using a multi-channel pipette. Cover the wells with parafilm and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds.
- Incubate for 30 minutes a room temperature. Protect the strips from direct sunlight.
- Add 100 µL of stop solution to the wells using a multi-channel pipette or a stepping pipette in the same sequence as for the substrate solution.
- Read the absorbance at 450 nm using a microplate ELISA photometer within 0 to 15 minutes after the addition of stopping solution. (Note: color will start to break down after 15 minutes.)

D. LS procedure for the Anatoxin-a ELISA (Note: approximately two and a half hours).

- Add 50 µL of the standard solutions, control, or samples into the wells of the test strips according to the working scheme given. Analyze in duplicate.
- Add 50 µL of the reconstituted enzyme conjugate solution to the individual wells successively using a multi-channel pipette.
- Add 50 µL of the reconstituted antibody solution to the individual wells successively using a multichannel 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.
- Remove the covering and decant the contents of the wells into a sink. Wash the strips four times using the 1X wash buffer solution. Use at least a volume of 250 µL of wash buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
- Add 100 µL of substrate (color) solution to the individual wells successively using a multi-channel 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.
- 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.
- Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stopping solution.

5.2.4. Analyze data in Chromate Reader using the Chromate Module Continued from section 5.2.3.5.

5.2.4.1. LS inserts prepared microtiter plate in Chromate reader. LS positions plate according to diagram on lap top screen.

5.2.4.2. Click "Read Plate".

5.2.4.3. After analysis is read (takes approximately 2 minutes), click "Select All" test results.

5.2.4.4. Click "Accept" test results. (Proceed to 5.2.4.5).

5.2.4.5. Save assay calibration and test reports on desk top of the laptop computer.

1. To observe results for standards and control, click "Assay Calibration" button on bottom of screen.
2. Click "Print Preview" to look at curve, CV%, and R² and then click "print" (pull down) to make the file for Standards. The following is an example of the naming "st2012M0614". The "st" identifies this file as a standard curve file, "2012" identifies year. "M" identifies Microcystins ELISA and, if a Cylindrospermopsin ELISA, "C", and if an Anatoxin-a RBA, "A" would be used. "0614" identifies month and day of the analysis.

3. Click "Report" to look at sample run file.
4. Click "Select ALL".
5. Click "Export" to make txt file and to make an xls file. The following are examples of both file names: "run20120901.txt and run20110901.xls."
6. Click "Print Preview" to look at the run file in a format and then click "Print" to get a file of results.
7. Save all 4 files (Standards , Sample , Sample TXT, and Sample XLS) and save to Cyanobacteria file folder on lap top under the year of analysis sub folder, and either the Microcystins, Cylindrospermopsin, or Anatoxin-a sub folders on laptop's desktop or on H drive.
8. Email through Outlook the four files to self and other staff assigned to this project. This quick and visual look at the results will provide immediate information for determining if the ELISA assay should be repeated.
9. Review using quality control decision tree specific for each assay. Copies of each quality control and decision tree are found in this TSOP in the Appendice 10.3. Results of QC elements are put into lab notebook.

5.2.5. Quality Assurance Protocol

5.2.5.1. LS will review results following Quality Assurance Protocol as detailed in the Appendice 10.3.

5.2.5.2. LS follows the Quality Assurance Process and documents in Laboratory Notebook any step that does not comply with QA limits. See checklist for QA review in appendice 10.3.

5.2.5.3. Following QC protocol and recommendations from Abraxis labs, if LS determines a critical QC element is out of specification and cannot be fixed with eliminating two or less standards, the analysis must be rerun.

5.2.6. Reporting

5.2.6.1. LS will prepare final reports for the Cyanobacteria and Cyanotoxin Program Manager or his/her designated representative and the Quality Assurance Officer assigned to the project.

5.2.6.2. LS prints out Standard file, the Run file with LS's signature on bottom, and an Excel report with sample name, sample number, collection date of samples, and results and create one file from the copier/scanner. Send this pdf and a separate Excel report to Cyanotoxin Program Manager or his/her designated representatives by Friday 10 AM through email. An example of the pdf file name is "Microcystins ELISA Results for August 8, 2012." Store pdf file in designated location. See example of final report in appendice 10.4.

5.2.6.3. LS combines COC with pdf file developed from 5.2.6.2 to be saved on the shared drive. This file will be used for the assigned Quality Assurance Officer (QAO) to review data after uploaded into Assessment Information Management System (AIMS).

5.2.7. Electronic Data Interface for Importing ELISA Analytical Results.

5.2.7.1 LS will provide an electronic folder in the Branch Share drive of all documents involved with analysis including COC and Bench Sheet for QA Officer to review and to build an Electronic Data Interface for Importing ELISA Analytical Results into the Assessment Information Management System.

5.3. Related Technical Issues:

5.3.1. Health and Safety Warnings:

- Gloves should be used whenever handling samples, standards, solutions, and reagents.
- The ELISA MSDs are stored in Algae Project information folder kept on lab benchtop and in the MSDS binder for the lab.

- The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact with skin and mucous membranes. Wash with water if contact occurs. (Abraxis users guide).
- Lab safety glasses should be worn when working in the laboratory.

5.3.2. Cautions:

- Original raw samples need to be stored in the freezer when not in use.
- Standards and solutions should be stored in refrigerator when not in use.
- Opened standards and solutions may not be as active as needed if stored over a month in refrigerator.

5.3.3. Interferences:

- Always pre-wet pipette tips before adding samples, standards, and reagents to titer wells.
- Always add reagents to the titer wells in the same order.
- Splash backs can cause errors in readings.
- Always vortex samples (a few seconds) and standards before pipetting from them.
- Bubbles left in plate wells after wash step causes analysis errors.
- Dust and fibers from outside sources such as paper towels in wells causes analysis errors.
- Standards, controls, samples, check samples should be equilibrated at room temperature before using.
- Failure to mix original sample before collecting sub sample can result in non-repeatable results.
- Do not use reagents past the expiration date.
- Prolonged exposure to light and room temperature will result in reduction of activity in reagents. If the activity is too low the Chromate program will not analyze results.
- Do not touch the top of microtiter cells containing solution with the analyst's gloves. This will ruin the cells affected.
- Do not over aerate samples by pre-wetting tips more than 5 times.

5.3.4. Calibration:

- Run check samples monthly during sampling season.
- Run controls, standards, and samples during the analysis.
- Field blanks and field duplicates are collected by sample collector and added to the analysis.
- Lab blanks and lab duplicates are added to analysis by laboratory scientist for each event.
- Relative percent differences are calculated on both field duplicates and lab duplicates.
- Regression analysis limits must be met.

5.3.5. Troubleshooting:

- Contact customer service representatives from Abraxis for advice and opinions on problems that occur with analysis.
XXXXX@abraxiskits.com
- For chromate computer module problems' contact "Awareness Technology" Model 4300 Serial Number 4300-1315. Tech Support mstandt@awaretech.com.
- Chromate computer module should be updated yearly.
- Refrigerated samples should be disposed of at end of season.
- Bubbles must be eliminated from wells before continuing with analysis.
- A second laboratory scientist may observe analyst or run analysis to identify operator error.
- When adding a new assay to Chromate Manager, the report template for the new assay should be attached to custom report format with IDEM logo. Steps on how to set the IDEM Report Template in Chromate Manager for a new assay calibration report or to reset the templates for any of the assay calibration reports are shown below. In the Chromate Manager program:
 - go to Settings -> Custom Report,
 - click to highlight the assay for which you want to set the template (i.e. Anatoxin a ELISA (2 rep)),

- click Set Template button,
- Navigate, if needed, to the .rpt template file. (It should be the RptCustom folder.),
- select IDEM Assay Calibration Report rpt. (or the template for the results if setting the result report),
- click Open button, and
- Click OK.

6.0 Records Management

- Original copies of field chain of custody forms, bench sheets, and calculations are stored in top drawer of lab file cabinet and electronic copies are found on Shared Drive. (S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\2016 Lab)
- A summary of the analysis and comments are reported in lab notebook stored in top drawer of lab file cabinet or at LS's desk.
- Final reports and QA reports are stored both electronically on the Shared Drive. (S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\2016 Lab)
- Completed field chain of custody forms are stored on the Shared Drive. (S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\2016 Lab)
- Analysis results are stored electronically on the Shared Drive. (S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\2016 Lab)
- Accepted analysis from Chromate reader are stored electronically in the Abraxis program module on designated secured laptop that is stored in laboratory 41.
- Final results are stored in AIMS.
- A copy of the Root file in Chromate program is stored electronically on the Shared Drive in case the Root file is erased during updates and re-imaging of computer. (S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\Backups of Chromate)

7.0 Quality Assurance / Quality Control

- The LC that oversees the lab will evaluate laboratory procedures for quality control and quality assurance compliance with TSOP.
- The designated overseer of the laboratory will, with recommendations from Abraxis technical support, approve minor changes to standards, samples, and quality control results on a case by case basis.

8.0 References

- 8.1 Instructions on the Building of an Electronic Data Interface for Importing Microcystin-ADDA Analytical Results into the Assessment Information Management System. S:\Algae\Cyanobacteria\Microcystins ELISA\EDI
- 8.2 Abraxis Microtiter Plate Washer Manual, S:\Algae\Cyanobacteria\Microcystins ELISA\Auto Plate Washer – Awareness Technologies.
- 8.3 Abraxis training Manual, MSDS, and step by step instructions for ELISA kits are located in black folder in the Building 41 ALGAE Lab on the bench top.

9.0 History of Revisions

The original version was approved June 2013. Second revision was approved October 2014. This third revision includes the addition of procedures for the Anatoxin-a ELISA analysis.

10.0 Appendices

- 10.1. Field Chain of Custody Form

- 10.2. Copy of Bench Sheet for Microcystins, Cylindrospermopsin, and both Anatoxin-a
- 10.3. Checklists for QA Review of Data for Microcystins, Cylindrospermopsin, and Anatoxin-a
- 10.4. Example of Final Report Formats

10.2. Copy of Bench Sheet for Microcystins, Cylindrospermopsin, and both Anatoxin-a

Microcystins ELISA Procedure Form

Start Date _____ Analysts _____
End Date _____

| Titer Tray Well Position | Sample Date | AB Number | Sample Name | Notes |
|-----------------------------|----------------|-----------|------------------------|----------------------------|
| 1A-2F | | - | Standards 0-5, control | * all samples in duplicate |
| 2G-H | | | | |
| 3A-B | | | | |
| 3C-D | | | | |
| 3E-F | | | | |
| 3G-H | | | | |
| 4A-B | | | | |
| 4C-D | | | | |
| 4E-F | | | | |
| 4G-H | | | | |
| 5A-B | | | | |
| 5C-D | | | | |
| 5E-F | | | | |
| 5G-H | | | | |

| Cycle | Freeze Date & Time | Thaw Date & Time | Notes |
|-------|--------------------|------------------|-------|
| 1 | | | |
| 2 | | | |
| 3 | | | |

Assay Name _____ Dilution _____

| Lot # | Exp. | Open | Lot # | Exp. | Open |
|---------|------|------|-----------|------|------|
| Std 0 | | | Antibody | | |
| Std 1 | | | Conjugate | | |
| Std 2 | | | Substrate | | |
| Std 3 | | | Stop | | |
| Std 4 | | | Dilutant | | |
| Std 5 | | | Wash | | |
| Control | | | Plate | | |
| | | | Kit | | |

Cylindrospermopsin ELISA Procedure Form

Start Date _____

Analysts _____

End Date _____

| Titer Tray Well Position | Sample Date | AB Number | Sample Name | Notes |
|-----------------------------|----------------|-----------|------------------------|----------------------------|
| 1A-2H | | - | Standards 0-7, control | * all samples in duplicate |
| 3A-B | | | | |
| 3C-D | | | | |
| 3E-F | | | | |
| 3G-H | | | | |
| 4A-B | | | | |
| 4C-D | | | | |
| 4E-F | | | | |
| 4G-H | | | | |
| 5A-B | | | | |
| 5C-D | | | | |
| 5E-F | | | | |
| 5G-H | | | | |

| Cycle | Freeze Date & Time | Thaw Date & Time | Notes |
|-------|--------------------|------------------|-------|
| 1 | | | |
| 2 | | | |
| 3 | | | |

Assay Name _____

Dilution _____

| Lot # | Exp. | Open | Lot # | Exp. | Open |
|---------|------|------|-----------|------|------|
| Std 0 | | | Antibody | | |
| Std 1 | | | Conjugate | | |
| Std 2 | | | Substrate | | |
| Std 3 | | | Stop | | |
| Std 4 | | | Dilutant | | |
| Std 5 | | | Wash | | |
| Std 6 | | | Plate | | |
| Control | | | Kit | | |

Anatoxin-a Receptor-Binding Assay Procedure Form

Start Date _____

Analysts _____

End Date _____

| Titer Tray Well Position | Sample Date | AB Number | Sample Name | Notes |
|-----------------------------|----------------|-----------|---------------|-----------------------------|
| 1A-2G | | - | Standards 0-4 | * all samples in triplicate |
| 2H-3B | | | | |
| 3C-E | | | | |
| 3F-H | | | | |
| 4A-C | | | | |
| 4D-F | | | | |
| 4G-5A | | | | |
| 5B-D | | | | |
| 5E-G | | | | |
| 5H-6B | | | | |
| 6C-E | | | | |
| 6F-H | | | | |
| 7A-C | | | | |
| 7D-F | | | | |

| Cycle | Freeze Date & Time | Thaw Date & Time | Notes |
|-------|--------------------|------------------|-------|
| 1 | | | |
| 2 | | | |
| 3 | | | |

Assay Name _____

Dilution _____

| Lot # | Exp. | Open | Lot # | Exp. | Open |
|-----------------------|------|------|--------------------------------|------|------|
| Std 0 | | | Biotinylated alpha-bungartoxin | | |
| Std 1 | | | Conjugate | | |
| Std 2 | | | Substrate | | |
| Std 3 | | | Stop | | |
| Std 4 | | | Diluent | | |
| | | | Wash | | |
| Standard Buffer Assay | | | Plate | | |
| Sample Buffer Assay | | | Kit | | |

Anatoxin-a ELISA Assay Procedure Form Duplicate

Start Date _____

Analysts _____

End Date _____

| Titer Tray Well Position | Sample Date | AB Number | Sample Name | Notes |
|-----------------------------|----------------|-----------|------------------------|----------------------------|
| 1A-2F | | - | Standards 0-5, control | * all samples in duplicate |
| 2G-H | | | | |
| 3A-B | | | | |
| 3C-D | | | | |
| 3E-F | | | | |
| 3G-H | | | | |
| 4A-B | | | | |
| 4C-D | | | | |
| 4E-F | | | | |
| 4G-H | | | | |
| 5A-B | | | | |
| 5C-D | | | | |
| 5E-F | | | | |
| 5G-H | | | | |
| 6A-B | | | | |
| 6C-D | | | | |
| 6E-F | | | | |
| 6G-H | | | | |

Assay Name _____

Dilution _____

| Lot # | Exp. | Open | Lot # | Exp. | Open |
|--|------|------|-------------------|------|------|
| Std 0 | | | Conjugate | | |
| Std 1 | | | Antibody | | |
| Std 2 | | | Substrate (Color) | | |
| Std 3 | | | Stop | | |
| Std 4 | | | Diluent 10X | | |
| Std 5 | | | Wash | | |
| NC 0.75 ppb ± 0.185 (0.565-0.935) | | | Plate | | |
| Conjugate Diluent | | | Kit | | |
| Antibody Diluent | | | | | |

10.3 Checklists for QA Review of Data for Microcystins, Cylindrospermopsin, and Anatoxin-a

Microcystins ADDA ELISA

Checklist for QA Review of Data Revision Date 28 November 2013

Decision Tree

1. Review mean absorbance for any overlap. If mean absorbance overlap, review individual absorbance values of the standards. Are there any overlapping individual absorbance values between standards?

Adjustments are allowed within the Chromate software to remove the individual standard absorbance value that is out of range from the calculated calibration curve.

Note: we are only allowed to remove points for valid cause such as pipetting errors, splashing, residuals, and contamination.

If mean overlap is still occurring, then the run should be rejected.

2. Is Normal Control (NC) concentration within its acceptable range 0.750 +/- 0.185 (0.665-0.935)? Vary with package direction. The run can be accepted:

- if both of the NC concentrations are within range,
- if the NC mean is within the acceptable range,
- if one NC is within the acceptable range and it is obvious that the second value was a pipetting error or a result of contamination.

If none of these conditions are met the run should be rejected.

3. Is % Coefficient of Variance (% CV) of the replicate absorbances of each standard < 10%? Strive for < 8%.

Adjustments are allowed within the Chromate software to remove the individual standard absorbance value that is out of range from the calculated calibration curve.

Note: we are only allowed to remove points for valid cause such as pipetting errors, splashing, and contamination.

After the allowed adjustments, if one % CV is ≥ 10% but < 15% the run can still be accepted. If two or more standards % CVs from the accepted values are ≥ 10% the run should be rejected.

$$CV \% = (\text{standard deviation/mean}) \times 100$$

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Microcystins ADDA ELISA

Checklist for QA Review of Data Revision Date 28 November 2013

4. For samples, Is the % CV of the replicate absorbance for each sample < 15%? If sample absorbance % CVs are > 15 but ≤ 20% this should be noted. If the sample absorbance % CV is > 20% then the sample result should be rejected.

Note: Module does not calculate the % CV for duplicate samples for you.

Website: <http://calculator.com/statistics/standard-deviation.php>

5. Is the R² of the calibration curve 0.990 or better? However this is not necessarily a reason for rejecting the run. Best professional judgment should be used. Evaluate each replicate standard where the % CV is high. For the purpose of obtaining an R² value of 0.990 or greater, individual absorbance points of a particular standard that is out of line can be removed from the calibration curve derivation as allowed by the Chromate module.

Instructions: Removal of points due to pipetting or other known errors in those wells should allow for the selection of the combination of standard that meet the R² of 0.99 and positions the Normal Control concentration closest to the known concentration of 0.750 µg/ml. This will allow for a straight calibration line in the correct vertical position resulting in the most accurate sample results.

Warning: Focus on removing only that which you know is invalid and avoid just trying to force the run into meeting the acceptability criteria when it may have other issues that need to be addressed.

If an R² value of 0.99 is not obtained and for consistency, the run could be rejected.

6. Is the field blank result < Reporting Limit (RL) / Method Detection limit (MDL)? and is the laboratory blank < RL? RL/MDL = 0.15 µg/L. (Limits vary between regular kits or enhanced kits.) If blanks are contaminated to a level that will impact the results, the run may be rejected. Example of non rejected sample results: if sample result is biased high and the results are still below the detection limits then results can be accepted. IDEM QA flags will be applied in the EDI.

7. Is Relative Percent Difference (RPD) of the mean concentration between sample and its field duplicate ≤ 30% (not calculated yet)? If one or more values are below the RL, the RPD cannot be measured or QC flags applied in the AIMS EDI.

$$RPD = \frac{(S-D)}{(S+D)} \times 200$$

Where: S = sample concentration
D = duplicate sample concentration

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Microcystins ADDA ELISA

Checklist for QA Review of Data Revision Date 28 November 2013

8. Is RPD of mean concentration between sample and its laboratory duplicate ≤ 30%? If one or more values are below the RL, the RPD cannot be measured or QC flags applied in the AIMS EDI.

9. If result is > the calibration range (5.0 ppb), the sample should be diluted 5x and rerun to obtain a result within the calibration range. Results above calibration range will not be reported. (From the diluted run, a mean value of 6 replicate results will be the accepted result.)

Observational Checklist

10. Was the titor tray read in the spectrophotometer within 15 minutes of introducing the Stop Solution to the filter wells?

Note: Abraxis recommends reading within 15 minutes as absorbance begins to attenuate after this time frame and the chances of meeting QA criteria decreases after this time period due to decrease in binding and the % CV rises.

11. Is the mean absorbance of standard D > 1.000? (> 1.400 preferred).

12. Calculate percent recovery of Normal Control

$$\%R = \frac{(A-B)}{C} \times 100$$

Record calculation and record in log book for later assessment.

Where: A = Analyte concentration determined experimentally with known quantity of reference material added.
B = Background concentration determined by separate field blank.
C = True value of reference standard added (x dilution factor)

13. Calculate each B_i/B₀

- a. Each B_i/B₀ should be less than 0.4 (B₀ is the mean absorbance of the replicates for standard 5 and B₀ is the mean absorbance of the replicates of standard 0).

- b. Each B_i/B₀ should decrease by about 0.150 with each successive standard (each B_i is the mean absorbance for each x standard).

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Microcystins ADDA ELISA

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14. The concentration of each standard (0.00, 0.15, 0.40, 1.0, 2.0, and 5.0 µg/l) should be within +/- 20% of the expected value. Adjustments will be necessary to account for dilutions for the run. Ranges are listed next.

| | |
|------|-------------------|
| 0.15 | range 0.12 - 0.18 |
| 0.40 | range 0.32 - 0.48 |
| 1.0 | range 0.80 - 1.2 |
| 2.0 | range 1.6 - 2.4 |
| 5.0 | range 4.0 - 6.0 |

15. Check Samples Concentrations for 2013 Spring & Summer

- Check Sample A expected value < 0.15 ppb
- Check Sample B expected value 2.0 +/- 0.5 ppb
- Check Sample C expected value 20 +/- 5 ppb

Note: All QA Checklist results will be noted in Laboratory Notebook.

For technical assistance for determining the acceptance of a run call Dave Deardorff or other technical support staff at Abraxis 215-357-3911. Email a copy of run to Abraxis (Lisa Kamp or Dave Deardorff) so they can make an accurate determination. lkamp@abraxiskits.com or ddeardorff@abraxiskits.com

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Cylindrospermopsin ELISA

Checklist for QA Review of Data

Revision Date 13 November 2013

O:\Algae\Cyanobacteria\QA Procedures\Toxins & Pigments\Cylindrospermopsin ADDA ELISA QA Analysis criteria 11-28-2013.docx

Decision Tree

1. Review mean absorbance for any overlap. If mean absorbance overlap, review individual absorbance values of the standards. Are there any overlapping individual absorbance values between standards?

Adjustments are allowed within the Chromate software to remove the individual standard absorbance value that is out of range from the calculated calibration curve.

Note: we are only allowed to remove points for valid cause such as pipetting errors, splashing, residuals, and contamination.

If mean overlap is still occurring, then the run should be rejected.

2. Is Normal Control (NC) concentration within its acceptable range 0.750 +/- 0.150 (0.600-0.900)? The run can be accepted:

- if both of the NC concentrations are within range,
- if the NC mean is within the acceptable range,
- if one NC is within the acceptable range and it is obvious that the second value was a pipetting error or a result of contamination.

If none of these conditions are met the run should be rejected.

3. Is % Coefficient of Variance (% CV) of the replicate absorbances of each standard < 10%? Strive for < 6%.

Adjustments are allowed within the Chromate software to remove the individual standard absorbance value that is out of range from the calculated calibration curve.

Note: we are only allowed to remove points for valid cause such as pipetting errors, splashing, and contamination.

After the allowed adjustments, if one % CV is ≥ 10% but < 15% the run can still be accepted. If two or more standards % CVs from the accepted values are ≥ 10% the run should be rejected.

$$CV = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

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Cylindrospermopsin ELISA

Checklist for QA Review of Data

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Where: S = sample concentration
D = duplicate sample concentration

8. Is RPD of mean concentration between sample and its laboratory duplicate ≤ 30%? If one or more values are below the RL, the RPD cannot be measured or QC flags applied in the AIMS EDI.
9. If result is > the calibration range (2.0 ppb), the sample should be diluted 5x and rerun to obtain a result within the calibration range. Results above calibration range will not be reported. (From the diluted run, a mean value of 3 samples or 6 replicate results will be the accepted result.)

Observational Checklist

10. Was the filter tray read in the spectrophotometer within 15 minutes of introducing the Stop Solution to the filter wells?

Note: Abraxis recommends reading within 15 minutes as absorbance begins to attenuate after this time frame and the chances of meeting QA criteria decreases after this time period due to decrease in binding and the % CV rises.

11. Is the mean absorbance of standard 0 > 1.000? (>1.400 preferred).

12. Calculate percent recovery of Normal Control:

$$\%R = \frac{(A-B) \times 100}{C}$$

Record calculation and record in log book for later assessment.

Where: A = Analyte concentration determined experimentally with known quantity of reference material added.
B = Background concentration determined by separate field blank.
C = True value of reference standard added (x dilution factor)

13. Calculate each B_1/B_0

- a. Each B_1/B_0 should be less than 0.4 (B_1 is the mean absorbance of the replicates for standard 5 and B_0 is the mean absorbance of the replicates of standard 0).

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Cylindrospermopsin ELISA

Checklist for QA Review of Data

Revision Date 13 November 2013

O:\Algae\Cyanobacteria\QA Procedures\Toxins & Pigments\Cylindrospermopsin ADDA ELISA QA Analysis criteria 11-28-2013.docx

4. For samples, is the % CV of the replicate absorbance for each sample < 15%? If sample absorbance % CVs are > 15 but ≤ 20% this should be noted. If the sample absorbance % CV is > 20% then the sample result should be rejected.

Note: Module does not calculate the % CV for duplicate samples for you.

Website: <http://easyvcalculation.com/statistics/standard-deviation.php>

5. Is the R^2 of the calibration curve 0.990 or better? However this is not necessarily a reason for rejecting the run. Best professional judgment should be used. Evaluate each replicate standard where the % CV is high. For the purpose of obtaining an R^2 value of 0.990 or greater, individual absorbance points of a particular standard that is out of line can be removed from the calibration curve derivation as allowed by the Chromate module.

Instructions: Removal of points due to pipetting or other known errors in those wells should allow for the selection of the combination of standard that meet the R^2 of 0.99 and positions the Normal Control concentration closest to the known concentration of 0.750 µg/ml. This will allow for a straight calibration line in the correct vertical position resulting in the most accurate sample results.

Warning: Focus on removing only that which you know is invalid and avoid just trying to force the run into meeting the acceptability criteria when it may have other issues that need to be addressed.

If an R^2 value of 0.98 is not obtained and for consistency, the run could be rejected.

6. Is the field blank result < Reporting Limit (RL) / Method Detection Limit (MDL)? and is the laboratory blank < RL? $RL/MDL = 0.15 \mu\text{g/L}$ (Limits vary between regular kits or enhanced kits.) If blanks are contaminated to a level that will impact the results, the run may be rejected. Example of non rejected sample results: If sample result is biased high and the results are still below the detection limits then results can be accepted. IDEM QA flags will be applied in the EDI.

7. Is Relative Percent Difference (RPD) of the mean concentration between sample and its field duplicate ≤ 30% (not calculated yet)? If one or more values are below the RL, the RPD cannot be measured or QC flags applied in the AIMS EDI.

$$RPD = \frac{(S-D)}{(S+D)} \times 200$$

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Cylindrospermopsin ELISA

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O:\Algae\Cyanobacteria\QA Procedures\Toxins & Pigments\Cylindrospermopsin ADDA ELISA QA Analysis criteria 11-28-2013.docx

- b. Each B_1/B_0 should decrease by about 0.150 with each successive standard (each B_1 is the mean absorbance for each x standard).

14. The concentration of each standard (0.00, 0.05, 0.25, 0.50, 1.0, and 2.0 ng/l) should be within +/- 20% of the expected value. Adjustments will be necessary to account for dilutions for the run. Ranges are listed next.

| | |
|------|-------------------|
| 0.05 | range 0.04 - 0.06 |
| 0.10 | range 0.08 - 0.12 |
| 0.25 | range 0.20 - 0.30 |
| 0.50 | range 0.40 - 0.60 |
| 1.0 | range 0.80 - 1.20 |
| 2.0 | range 1.60 - 2.40 |

Note: All QA Checklist results will be notated in Laboratory Notebook.

For technical assistance for determining the acceptance of a run call Dave Deardorff or other technical support staff at Abraxis 215-357-3911. Email a copy of run to lkamp@abraxiskits.com or ddeardorff@abraxiskits.com

Warning: Gloves must be tight fitting. Touching the solution in cells before the first wash with gloves can ruin that sample. The cells along the edges that obviously show low results must be rejected from analysis.

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Anatoxin-a RBA

Checklist for QA Review of Data

Revision Date 27 April 2014

Decision Tree

1. Review mean absorbance for any overlap. If mean absorbance overlap, review individual absorbance values of the standards. Are there any overlapping individual absorbance values between standards?

Adjustments are allowed within the Chromate software to remove the individual standard absorbance value that is out of range from the calculated calibration curve.

Note: we are only allowed to remove points for valid cause such as pipetting errors, splashing, residuals, and contamination.

If mean overlap is still occurring, then the run should be rejected.

2. Is % Coefficient of Variance (% CV) of the replicate absorbances of each standard < 10%? Strive for < 6%.

Adjustments are allowed within the Chromate software to remove the individual standard absorbance value that is out of range from the calculated calibration curve.

Note: we are only allowed to remove points for valid cause such as pipetting errors, splashing, and contamination.

After the allowed adjustments, if one % CV is $\geq 10\%$ but < 15% the run can still be accepted. If two or more standards % CVs from the accepted values are $\geq 10\%$ the run should be rejected.

$$CV \% = (\text{standard deviation/mean}) \times 100$$

3. For samples, is the % CV of the replicate absorbance for each sample < 15%? If sample absorbance % CVs are > 15 but < 20% this should be noted. If the sample absorbance % CV is > 20% then the sample result should be rejected.

Note: Module does not calculate the % CV for duplicate samples for you.

Website: <http://easycalculation.com/statistics/standard-deviation.php>

4. Is the R^2 of the calibration curve 0.990 or better? However this is not necessarily a reason for rejecting the run. Best professional judgment should be used. Evaluate each replicate standard where the % CV is high. For the purpose of obtaining an R^2 value of 0.990 or greater, individual absorbance points of a particular standard that is

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Anatoxin-a RBA

Checklist for QA Review of Data

Revision Date 27 April 2014

out of line can be removed from the calibration curve derivation as allowed by the Chromate module.

Instructions: Removal of points due to pipetting or other known errors in those wells should allow for the selection of the combination of standard that meet the R^2 of 0.99 and positions the Normal Control concentration closest to the known concentration of 0.750 ng/mL. This will allow for a straight calibration line in the correct vertical position resulting in the most accurate sample results.

Warning: Focus on removing only that which you know is invalid and avoid just trying to force the run into meeting the acceptability criteria when it may have other issues that need to be addressed.

If an R^2 value of 0.98 is not obtained and for consistency, the run could be rejected.

5. Is the field blank result < Reporting Limit (RL) / Method Detection limit (MDL)? and is the laboratory blank < RL? RL/MDL = 10 µg/L. (Limits vary between regular kits or enhanced kits.) If blanks are contaminated to a level that will impact the results, the run may be rejected. Example of non-rejected sample results: if sample result is biased high and the results are still below the detection limits then results can be accepted. IDEM QA flags will be applied in the IDEM.

6. Is Relative Percent Difference (RPD) of the mean concentration between sample and its field duplicate $\leq 30\%$ (not calculated yet)? If one or more values are below the RL, the RPD cannot be measured or QC flags applied in the IDEM EDI.

$$RPD = \frac{(S-D)}{(S+D)} \times 200$$

Where: S = sample concentration
D = duplicate sample concentration

7. Is RPD of mean concentration between sample and its laboratory duplicate $\leq 30\%$? If one or more values are below the RL, the RPD cannot be measured or QC flags applied in the IDEM EDI.

8. If result is > the calibration range (500 ppb), the sample should be diluted 5x and re-run to obtain a result within the calibration range. Results above calibration range will not be reported. (From the diluted run, a mean value of 6 replicate results will be the accepted result.)

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Anatoxin-a RBA

Checklist for QA Review of Data

Revision Date 27 April 2014

Observations Checklist

9. Was the filter tray read in the spectrophotometer within 15 minutes of introducing the Stop Solution to the filter wells?

Note: Abraxis recommends reading within 15 minutes as absorbance begins to attenuate after this time frame and the chances of meeting QA criteria decreases after this time period due to decrease in binding and the % CV rises.

10. Is the mean absorbance of standard 0 > 1.000? (> 1.400 preferred).

11. Calculate each B_1/B_0 :

a. Each B_1/B_0 should be less than 0.4 (B_1 is the mean absorbance of the replicates for standard 4 and B_0 is the mean absorbance of the replicates of standard 0).

b. Each B_1/B_0 should decrease by about 0.150 with each successive standard (each B_1 is the mean absorbance for each x standard).

12. The concentration of each standard (0.00, 10, 35, 125, and 500 µg/L) should be within $\pm 20\%$ of the expected value. Adjustments will be necessary to account for dilutions for the run. Ranges are listed next.

| | |
|-----|-----------------|
| 10 | range 8 - 12 |
| 35 | range 28 - 42 |
| 125 | range 100 - 150 |
| 500 | range 400 - 600 |

Note: All QA Checklist results will be notated in Laboratory Notebook.

For technical assistance for determining the acceptance of a run call Dava Doardorf or other technical support staff at Abraxis 216-357-3911. Email a copy of run to Abraxis (Lisa Kaemp or Dava Doardorf) so they can make an accurate determination. lkaemp@abraxiskits.com or ddea'dorf@abraxiskits.com

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Anatoxin-a ELISA

Checklist for QA Review of Data

Revision Date 8 July 2016

Decision Tree

1. Review mean absorbance for any overlap. If mean absorbance overlap, review individual absorbance values of the standards. Are there any overlapping individual absorbance values between standards?

Adjustments are allowed within the Chromate software to remove the individual standard absorbance value that is out of range from the calculated calibration curve.

Note: we are only allowed to remove points for valid cause such as pipetting errors, splashing, residuals, and contamination.

If mean overlap is still occurring, then the run should be rejected.

2. Is Normal Control (NC) concentration within its acceptable range 0.750 +/- 0.185 (0.565-0.935)? Vary with package direction. The run can be accepted:

- If both of the NC concentrations are within range,
- If the NC mean is within the acceptable range,
- If one NC is within the acceptable range and it is obvious that the second value was a pipetting error or a result of contamination.

If none of these conditions are met the run should be rejected.

3. Is % Coefficient of Variance (% CV) of the replicate absorbances of each standard < 10%? Strive for < 5%.

Adjustments are allowed within the Chromate software to remove the individual standard absorbance value that is out of range from the calculated calibration curve.

Note: we are only allowed to remove points for valid cause such as pipetting errors, splashing, and contamination.

After the allowed adjustments, if one % CV is $\geq 10\%$ but $< 15\%$ the run can still be accepted. If two or more standards % CVs from the accepted values are $\geq 10\%$ the run should be rejected.

$$CV \% = (\text{standard deviation/mean}) \times 100$$

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Anatoxin-a ELISA

Checklist for QA Review of Data

Revision Date 8 July 2016

8. Is RPD of mean concentration between sample and its laboratory duplicate $\leq 30\%$? If one or more values are below the RL, the RPD cannot be measured or QC flags applied in the AIMS EDI.

9. If result is > the calibration range (5.0 ppb), the sample should be diluted 5x and rerun to obtain a result within the calibration range. Results above calibration range will not be reported. (From the diluted run, a mean value of 5 replicate results will be the accepted result.)

Observational Checklist

10. Was the filter tray read in the spectrophotometer within 15 minutes of introducing the Stop Solution to the filter wells?

Note: Abraxis recommends reading within 15 minutes as absorbance begins to attenuate after this time frame and the chances of meeting QA criteria decreases after this time period due to decrease in binding and the % CV rises.

11. Is the mean absorbance of standard 0 > 1.000? (>1.400 preferred).

12. Calculate percent recovery of Normal Control:

$$\%R = [(A) \times 100] / B.$$

Record calculation and record in log book for later assessment.

Where: A = Analyte concentration determined experimentally with known quantity of reference material added.

B = True value of reference standard added (x dilution factor)

13. Calculate each B_1/B_0

- a. Each B_1/B_0 should be less than 0.4 (B_0 is the mean absorbance of the replicates for standard 5 and B_1 is the mean absorbance of the replicates of standard 0).

- b. Each B_1/B_0 should decrease by about 0.150 with each successive standard (each B_1 is the mean absorbance for each x standard).

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Anatoxin-a ELISA

Checklist for QA Review of Data

Revision Date 8 July 2016

4. For samples, is the % CV of the replicate absorbance for each sample < 15%? If sample absorbance % CVs are > 15 but $\leq 20\%$ this should be noted. If the sample absorbance % CV is > 20% then the sample result should be rejected.

Note: Module does not calculate the % CV for duplicate samples for you.

Website: <http://easycalculation.com/statistics/standard-deviation.php>

5. Is the R^2 of the calibration curve 0.990 or better? However this is not necessarily a reason for rejecting the run. Best professional judgment should be used. Evaluate each replicate standard where the % CV is high. For the purpose of obtaining an R^2 value of 0.990 or greater, individual absorbance points of a particular standard that is out of line can be removed from the calibration curve derivation as allowed by the Chromate module.

Instructions: Removal of points due to pipetting or other known errors in those wells should allow for the selection of the combination of standard that meet the R^2 of 0.99 and positions the Normal Control concentration closest to the known concentration of 0.750 $\mu\text{g/ml}$. This will allow for a straight calibration line in the correct vertical position resulting in the most accurate sample results.

Warning: Focus on removing only that which you know is invalid and avoid just trying to force the run into meeting the acceptability criteria when it may have other issues that need to be addressed.

If an R^2 value of 0.98 is not obtained and for consistency, the run could be rejected.

6. Is the field blank result < Reporting Limit (RL) / Method Detection limit (MDL)? and is the laboratory blank < RL? $RL/MDL = 0.15 \mu\text{g/L}$. (Limits vary between regular kits or enhanced kits.) If blanks are contaminated to a level that will impact the results, the run may be rejected. Example of non rejected sample results: if sample result is biased high and the results are still below the detection limits then results can be accepted. IDEM QA flags will be applied in the EDI.

7. Is Relative Percent Difference (RPD) of the mean concentration between sample and its field duplicate $\leq 30\%$ (not calculated yet)? If one or more values are below the RL, the RPD cannot be measured or QC flags applied in the AIMS EDI.

$$RPD = (S-D) \times 200 / (S+D)$$

Where: S = sample concentration
D = duplicate sample concentration

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Anatoxin-a ELISA

Checklist for QA Review of Data

Revision Date 8 July 2016

14. The concentration of each standard (0.00, 0.15, 0.40, 1.0, 2.0, and 5.0 $\mu\text{g/l}$) should be within $\pm 20\%$ of the expected value. Adjustments will be necessary to account for dilutions for the run. Ranges are listed next.

| | |
|------|-------------------|
| 0.15 | range 0.12 - 0.18 |
| 0.40 | range 0.32 - 0.48 |
| 1.0 | range 0.80 - 1.2 |
| 2.5 | range 2.0 - 3.0 |
| 5.0 | range 4.0 - 6.0 |

Note: All QA Checklist results will be noted in Laboratory Notebook.

For technical assistance for determining the acceptance of a run call Dave Deardoff or other technical support staff at Abraxis 215-367-3911. Email a copy of run to Abraxis (Lisa Kemp or Dave Deardoff) so they can make an accurate determination. kemp@abraxiskits.com or ddeardoff@abraxiskits.com

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10.4 Examples of Final Reports



Assay Calibration Report

Assay Information

Assay Name: Microcystins ADDA Units: ng/mL
Assay Mode: 4-Parameter Logistic # of decimals: 4
Normal: 0.1500 - 5.0000 Assay Description:

Controls:

Normal Control

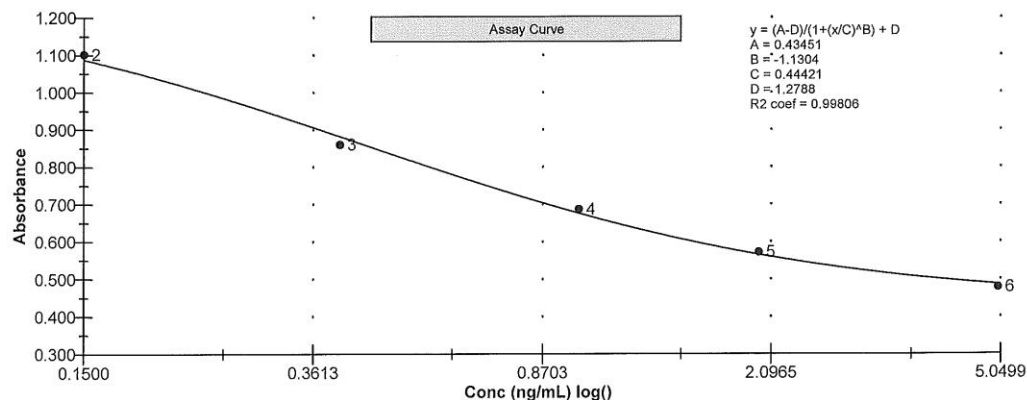
Standards:

Std1, Concentration = 0.0000, Minimum number to use: 2
Std2, Concentration = 0.1500, Minimum number to use: 2
Std3, Concentration = 0.4000, Minimum number to use: 2
Std4, Concentration = 1.0000, Minimum number to use: 2
Std5, Concentration = 2.0000, Minimum number to use: 2
Std6, Concentration = 5.0000, Minimum number to use: 2
Curve valid interval: 7 days 0 hours
Axis Mode: Y = Abs, X = Log(Conc)

Assay Calibration and Statistics

| Name | Absorbance | Concentration | Position |
|-----------------------|------------|----------------|----------|
| 8/22/2012 10:37:57 AM | | | |
| Std1 | 1.289 Abs | < 0.0000 ng/mL | A01 |
| Std1 | 1.260 Abs | 0.0157 ng/mL | B01 |
| Std2 | 1.133 Abs | 0.1110 ng/mL | C01 |
| Std2 | 1.072 Abs | 0.1640 ng/mL | D01 |
| Std3 | 0.909 Abs | 0.3584 ng/mL | E01 |
| Std3 | 0.812 Abs | 0.5360 ng/mL | F01 |
| Std4 | 0.657 Abs | 1.1020 ng/mL | G01 |
| Std4 | 0.716 Abs | 0.8200 ng/mL | H01 |
| Std5 | 0.568 Abs | 1.9500 ng/mL | A02 |
| Std5 | 0.576 Abs | 1.8350 ng/mL | B02 |
| Std6 | 0.460 Abs | > 5.0000 ng/mL | C02 |
| Std6 | 0.495 Abs | 4.2850 ng/mL | D02 |
| 8/22/2012 10:37:57 AM | | | |
| Normal Control | 0.702 Abs | 0.8765 ng/mL | E02 |
| Normal Control | 0.691 Abs | 0.9250 ng/mL | F02 |

| Name | Mean Abs | SD Abs | CV Abs | Mean Conc | SD Conc | CV Conc | Diff Conc |
|----------------|----------|--------|--------|-----------|---------|---------|-----------|
| Std1 | 1.275 | 0.021 | 1.61 | | | | |
| Std2 | 1.102 | 0.043 | 3.91 | 0.138 | 0.037 | 27.26 | -8.00 |
| Std3 | 0.860 | 0.069 | 7.97 | 0.446 | 0.127 | 28.46 | 11.50 |
| Std4 | 0.687 | 0.042 | 6.08 | 0.961 | 0.199 | 20.75 | -3.90 |
| Std5 | 0.572 | 0.006 | 0.99 | 1.893 | 0.081 | 4.30 | -5.35 |
| Std6 | 0.478 | 0.025 | 5.18 | | | | -100.00 |
| Normal Control | 0.697 | 0.008 | 1.12 | 0.901 | 0.034 | 3.81 | |





Test Report

Test Information

| Name/ID | Assay | Absorbance | Concentration | Interpretation | Reference | Position |
|-----------------------|-------------------|--------------------|---------------------------|-------------------|-----------------|----------|
| 8/22/2012 10:37:57 AM | | | | | | |
| Std1 | Microcystins ADDA | 1.289 Abs | < 0.0000 ng/mL | | | A01 |
| Std1 | Microcystins ADDA | 1.260 Abs | 0.0157 ng/mL | | | B01 |
| Std2 | Microcystins ADDA | 1.133 Abs | 0.1110 ng/mL | | | C01 |
| Std2 | Microcystins ADDA | 1.072 Abs | 0.1640 ng/mL | | | D01 |
| Std3 | Microcystins ADDA | 0.909 Abs | 0.3564 ng/mL | | | E01 |
| Std3 | Microcystins ADDA | 0.812 Abs | 0.5360 ng/mL | | | F01 |
| Std4 | Microcystins ADDA | 0.657 Abs | 1.1020 ng/mL | | | G01 |
| Std4 | Microcystins ADDA | 0.716 Abs | 0.8200 ng/mL | | | H01 |
| Std5 | Microcystins ADDA | 0.568 Abs | 1.9500 ng/mL | | | A02 |
| Std5 | Microcystins ADDA | 0.576 Abs | 1.8350 ng/mL | | | B02 |
| Std6 | Microcystins ADDA | 0.460 Abs | > 5.0000 ng/mL | | | C02 |
| Std6 | Microcystins ADDA | 0.495 Abs | 4.2850 ng/mL | | | D02 |
| Std6 | Microcystins ADDA | 0.495 Abs | 0.8765 ng/mL | | | E02 |
| Normal Control | Microcystins ADDA | 0.702 Abs | | | | F02 |
| Normal Control | Microcystins ADDA | 0.691 Abs | 0.9250 ng/mL | | | G02 |
| AB09103 | Microcystins ADDA | 0.615 Abs | 1.4050 ng/mL | | 0.1500 - 5.0000 | H02 |
| AB09103 | Microcystins ADDA | 0.584 Abs [0.5995] | 1.7300 ng/mL [1.5525] | | 0.1500 - 5.0000 | A03 |
| AB09104 | Microcystins ADDA | 1.162 Abs | 0.0880 ng/mL | LOW | 0.1500 - 5.0000 | B03 |
| AB09104 | Microcystins ADDA | 1.109 Abs [1.1355] | 0.1310 ng/mL [0.1090] | Low [Low] | 0.1500 - 5.0000 | C03 |
| AB09105 | Microcystins ADDA | 1.248 Abs | 0.0245 ng/mL | LOW | 0.1500 - 5.0000 | D03 |
| AB09105 | Microcystins ADDA | 1.276 Abs [1.2620] | 0.0000 ng/mL [0.0142] | Low [Low] | 0.1500 - 5.0000 | E03 |
| AB09109 | Microcystins ADDA | 1.083 Abs | 0.1540 ng/mL | | 0.1500 - 5.0000 | F03 |
| AB09109 | Microcystins ADDA | 1.146 Abs [1.1145] | 0.1006 ng/mL [0.1265] | Low [Low] | 0.1500 - 5.0000 | G03 |
| AB09843 | Microcystins ADDA | 0.621 Abs | 1.3550 ng/mL | | 0.1500 - 5.0000 | H03 |
| AB09843 | Microcystins ADDA | 0.638 Abs [0.6295] | 1.2250 ng/mL [1.2870] | | 0.1500 - 5.0000 | A04 |
| 20120820LB | Microcystins ADDA | 1.334 Abs | < 0.0000 ng/mL | Out(LR) | 0.1500 - 5.0000 | B04 |
| 20120820LB | Microcystins ADDA | 1.338 Abs [1.3360] | < 0.0000 ng/mL [< 0.0000] | Out(LR) [Out(LR)] | 0.1500 - 5.0000 | C04 |
| AB09843LD | Microcystins ADDA | 0.661 Abs | 1.0790 ng/mL | | 0.1500 - 5.0000 | D04 |
| AB09843LD | Microcystins ADDA | 0.657 Abs [0.6590] | 1.1020 ng/mL [1.0905] | | 0.1500 - 5.0000 | E04 |
| AB09106 | Microcystins ADDA | 1.038 Abs | 0.1970 ng/mL | | 0.1500 - 5.0000 | F04 |
| AB09106 | Microcystins ADDA | 1.053 Abs [1.0455] | 0.1822 ng/mL [0.1895] | | 0.1500 - 5.0000 | G04 |
| AB09107 | Microcystins ADDA | 0.860 Abs | 0.4380 ng/mL | | 0.1500 - 5.0000 | H04 |
| AB09107 | Microcystins ADDA | 0.798 Abs [0.8290] | 0.5690 ng/mL [0.4990] | | 0.1500 - 5.0000 | A05 |
| AB09108 | Microcystins ADDA | 1.323 Abs | < 0.0000 ng/mL | Out(LR) | 0.1500 - 5.0000 | B05 |
| AB09108 | Microcystins ADDA | 1.268 Abs [1.2955] | 0.0095 ng/mL [< 0.0000] | Low [Out(LR)] | 0.1500 - 5.0000 | C05 |
| Check A | Microcystins ADDA | 1.206 Abs | 0.0550 ng/mL | LOW | 0.1500 - 5.0000 | D05 |
| Check A | Microcystins ADDA | 1.201 Abs [1.2035] | 0.0587 ng/mL [0.0569] | Low [Low] | 0.1500 - 5.0000 | E05 |
| Check B | Microcystins ADDA | 0.583 Abs | 1.7405 ng/mL | | 0.1500 - 5.0000 | F05 |
| Check B | Microcystins ADDA | 0.555 Abs [0.5690] | 2.1700 ng/mL [1.9350] | | 0.1500 - 5.0000 | G05 |
| Check C | Microcystins ADDA | 0.412 Abs | > 5.0000 ng/mL | Out(LR) | 0.1500 - 5.0000 | H05 |
| Check C | Microcystins ADDA | 0.444 Abs [0.4280] | > 5.0000 ng/mL [> 5.0000] | Out(LR) [Out(LR)] | 0.1500 - 5.0000 | |

Notes

Rebecca Carliff
8/22/2012

Signature

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