

US EPA ARCHIVE DOCUMENT

# Battelle

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**Environmental Technology  
Verification Program  
Advanced Monitoring  
Systems Center**

Test/Quality Assurance Plan  
for Verification of  
Microcystin Test Kits

US EPA ARCHIVE DOCUMENT

ET ✓ ET ✓ ET ✓

**TEST/QUALITY ASSURANCE PLAN**

**for**

**Verification of  
Microcystin Test Kits**

**July 6, 2010**

**Prepared by**

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**SECTION A**  
**PROJECT MANAGEMENT**

**A1 VENDOR APPROVAL PAGE**

ETV Advanced Monitoring Systems Center

Test/Quality Assurance Plan  
for Verification of  
Microcystin Test Kits

APPROVAL:

Name \_\_\_\_\_

Company \_\_\_\_\_

Date \_\_\_\_\_

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Appendix A: Ease of Use Questionnaire

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#### A4 LIST OF ACRONYMS AND ABBREVIATIONS

AMS	Advanced Monitory Systems
ASTM	American Society for Testing and Materials
COC	chain of custody
CAS	Chemical Abstract Service
CCV	continuing calibration verification
CR	cross reactivity
DQI	data quality indicator
DQO	data quality objective
DI	deionized
DL	detection limit
EPA	Environmental Protection Agency
ETV	Environmental Technology Verification
ELISA	Enzyme-Linked Immunosorbent Assay
IDL	instrument detection limit
LFM	laboratory fortified matrix
LRB	laboratory record book
LC-MS-MS	liquid chromatography tandem mass spectrometry
MB	method blank
MDL	method detection limit
MRM	multiple reaction monitoring
NIST	National Institute of Standards and Technology
NRC	National Research Council
NDEQ	Nebraska Department of Environmental Quality
ppb	parts per billion
%D	percent different
PEA	performance evaluation audit
PT	performance test
PO	project officer
QA	quality assurance
QAM	quality assurance manger
QAO	quality assurance officer
QC	quality control
QMP	quality management plan
RMO	records management office
RW	recreational water
RPD	relative percent different
RSD	relative standard deviation
SOP	standard operating procedure
SCDHS	Suffolk County Department of Health Services
TSA	technical systems audit
WSL	Water Sciences Laboratory



## **A5 VERIFICATION TEST ORGANIZATION**

The verification test will be conducted under the U.S. Environmental Protection Agency (EPA) Environmental Technology Verification (ETV) Program. It will be performed by Battelle, which is managing the ETV Advanced Monitoring Systems (AMS) Center through a cooperative agreement with EPA. The scope of the AMS Center covers verification of monitoring technologies for contaminants and natural species in air, water, and soil.

The day to day operations of this verification test will be coordinated and performed by Battelle, with the participation of vendors who will have the performance of their technologies verified. Testing will be conducted by Battelle staff at the Battelle laboratories in Columbus, Ohio. Each vendor will provide Battelle with their respective technologies and will train the verification staff in their technologies use.

Quality Assurance (QA) oversight will be provided by the Battelle Quality Assurance Manager (QAM) and also by the EPA AMS Center Quality Manager (EPA QM), at her discretion. The organization chart in Figure 1 identifies the responsibilities of the organizations and individuals associated with the verification test. Roles and responsibilities are defined further below.

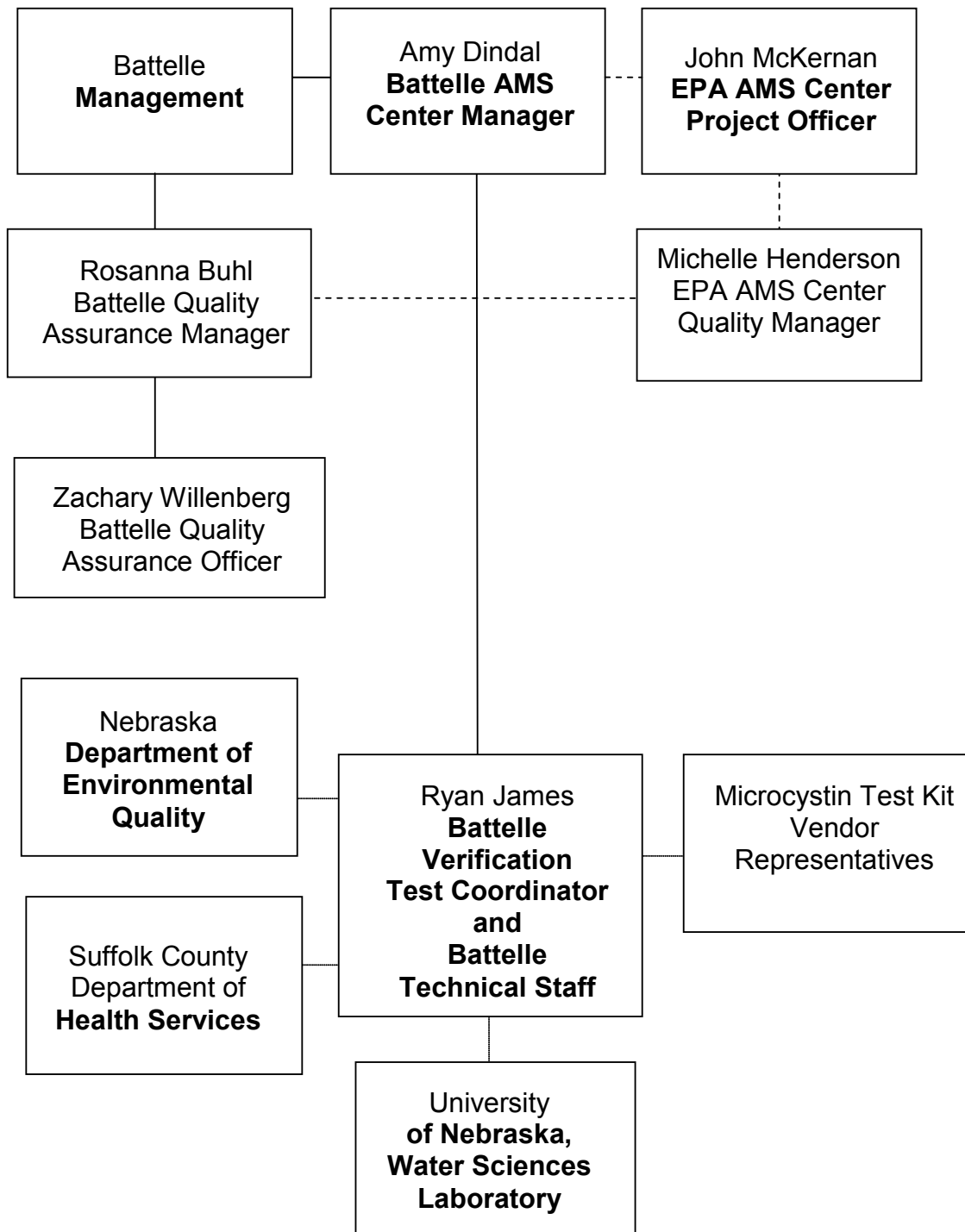


Figure 1. Organization Chart for the Verification Test

## A5.1 Battelle

Dr. Ryan James is the AMS Center's Verification Test Coordinator for this test. In this role, Dr. James will have overall responsibility for ensuring that the technical, schedule, and cost goals established for the verification test are met. Specifically, Dr. James will:

- Prepare the draft test/QA plan, verification reports, and verification statements.
- Establish a budget for the verification test and manage staff to ensure the budget is not exceeded.
- Revise the draft test/QA plan, verification reports, and verification statements in response to reviewer comments.
- Assemble a qualified technical staff to conduct the verification test.
- Direct the staff in performing the verification test in accordance with this test/QA plan.
- Hold a kick-off meeting approximately one week prior to the start of the verification test to review the critical logistical, technical, and administrative aspects of the verification test. Responsibility for each aspect of the verification test will be confirmed.
- Ensure that all quality procedures specified in this EPA Quality Level III test/QA plan and in the AMS Center Quality Management Plan<sup>1</sup> (QMP) are followed.
- Serve as the primary point of contact for vendor representatives.
- Ensure that confidentiality of sensitive vendor information is maintained.
- Assist vendors as needed during verification testing.
- Become familiar with the operation and maintenance of the technologies through instruction by the vendors.
- Respond to any issues raised in assessment reports, audits, or from verification staff observations, and institute corrective action as necessary.
- Coordinate distribution of the final test/QA plan, verification reports, and verification statements.

Ms. Amy Dindal is Battelle's Manager for the AMS Center. As such, Ms. Dindal will oversee the various stages of verification testing. Ms. Dindal will:

- Review the draft and final test/QA plan.
- Review the draft and final verification reports and verification statements.
- Ensure that necessary Battelle resources, including staff and facilities, are committed to the verification test.
- Ensure that confidentiality of sensitive vendor information is maintained.
- Support Dr. James in responding to any issues raised in assessment reports and audits.
- Maintain communication with EPA's technical and quality managers.
- Issue a stop work order if Battelle or EPA QA staff discovers adverse findings that will compromise test results.

Battelle Technical Staff will support Dr. James in planning and conducting the verification test. The responsibilities of the technical staff will be to:

- Assist in planning for the test, and making arrangements for the receipt of and training on the technologies.
- Attend the verification test kick-off meeting.
- Assist vendor staff as needed during test kit receipt and training.
- Coordinate and conduct verification testing using each participating technology, following all aspects of the ETV AMS Center QMP<sup>1</sup> as well as the test/QA plan for this verification.
- Support Dr. James in the preparation of the test/QA plan and reports, as necessary.
- Support Dr. James in responding to any issues raised in assessment reports and audits related to statistics and data reduction as needed.

Ms. Rosanna Buhl is Battelle's Quality Assurance Manager (QAM) for the AMS Center. Ms. Buhl will:

- Review the draft and final test/QA plan.
- Assign a Quality Assurance Officer (QAO) for each verification test.
- Delegate to other Battelle quality staff any QAO responsibilities assigned below as needed to meet project schedules.
- Review any audit checklists prepared by the QAO for completeness and detail.

- Review draft audit reports prior to release to the Verification Test Coordinator and/or EPA for clarity and appropriate assessment of findings.
- Review audit responses for appropriateness.
- Review and approve test/QA plans, test/QA plan amendments, deviations and audit reports.
- Maintain real-time communication with the QAO on QA activities, audit results, and concerns.
- Work with the QAO, Verification Test Coordinator, and Battelle's AMS Center Manager to resolve data quality concerns and disputes.
- Recommend a stop work order if audits indicate that data quality or safety is being compromised.

Mr. Zachary Willenberg is Battelle's QAO for this test. Mr. Willenberg will:

- Attend the verification test kick-off meeting and lead the discussion of the QA elements of the kickoff meeting checklist.
- Prior to the start of verification testing, verify the presence of applicable training records, including any vendor training on test equipment.
- Conduct a technical systems audit at least once during the verification test.
- Conduct audits to verification data quality.
- Prepare and distribute an audit report for each audit.
- Verify that audit responses for each audit finding and observation are appropriate and that corrective action has been implemented effectively.
- Communicate to the Verification Test Coordinator and/or technical staff the need for immediate corrective action if an audit identifies test/QA plan deviations or practices that threaten data quality.
- Provide a summary of the QA/QC activities and results for the verification reports.
- Review the draft and final verification report(s) and verification statement(s).
- Maintain real-time communication with the Battelle QAM on QA activities, audit results, and concerns, including potential schedule and budget problems.

- Communicate data quality concerns to the Verification Test Coordinator and/or Battelle's AMS Center QAM and Manager; recommend the need for a stop work order if audits indicate that data quality or safety is being compromised.

## A5.2 Technology Vendors

The responsibilities of the technology vendors are as follows:

- Review and provide comments on the draft test/QA plan.
- Accept (by signature of a company representative) the final test/QA plan prior to test initiation.
- Provide their technology for evaluation during the verification test.
- Provide all other equipment/supplies/reagents/consumables needed to operate their technology for the duration of the verification test.
- Supply training on the use of the technology, and provide written consent and instructions for verification staff to carry out testing, including written instructions for routine operation of their technology.
- Provide maintenance and repair support for their technology, on-site if necessary, throughout the duration of the verification test.
- Review and provide comments on the draft verification report and statement for their respective technology.

## A5.3 EPA

EPA's responsibilities in the AMS Center are based on the requirements stated in the "Environmental Technology Verification Program Quality Management Plan" (ETV QMP)<sup>2</sup>.

The roles of specific EPA staff are as follows:

Ms. Michelle Henderson is EPA's AMS Center Quality Manager (EPA QM). For the verification test, Ms. Henderson will:

- Review the draft test/QA plan.
- Perform at her option one external technical systems audit during the verification test.
- Notify the EPA AMS Center Project Officer of the need for a stop work order if the external audit indicates that data quality is being compromised.

- Prepare and distribute an assessment report summarizing results of the external audit.
- Review draft verification reports and verification statements.

Dr. John McKernan is EPA's Project Officer (EPA PO) for the AMS Center. Dr. McKernan, or designee will:

- Review the draft test/QA plan.
- Approve the final test/QA plan.
- Be available during the verification test to authorize any test/QA plan deviations by phone and provide the name of a delegate to the Battelle AMS Center Manager should he not be available during the testing period.
- Review the draft verification reports and verification statements.
- Oversee the EPA review process for the test/QA plan, verification reports, and verification statements.
- Coordinate the submission of verification reports and verification statements for final EPA approval.
- Post the test/QA plan, verification reports, and verification statements on the ETV web site.

#### **A5.4 Verification Test Stakeholders**

This verification test will be conducted in collaboration with David Schumacher and his technical staff at the Nebraska Department of Environmental Quality (NDEQ). They have provided recreational water (RW) samples for verification testing. In addition, the Suffolk County Department of Health Services (SCDHS) is collaborating on this verification test by providing RW samples.

This test/QA plan and the verification report(s) and verification statement(s) based on testing described in this document will be reviewed by experts in the fields related to microcystin determination in water. The following experts have been providing input to this test/QA plan and have agreed to provide a peer review:

- Robert Waters, Suffolk County Department of Health
- Andrew Lincoff, US EPA Region 9 Laboratories

The activities responsibilities of verification test stakeholders and/or peer reviewers include:

- Participate in stakeholder discussions to provide input to the test design.
- Review and provide input to the test/QA plan
- Review and provide input to the verification report(s)/verification statement(s).

The AMS Center Water Stakeholder Committee has considered the technology category of microcystin immunoassay kits a priority area since 2005. The Battelle Verification Test Coordinator presented the fundamentals of the test design in a stakeholder committee teleconference in November 2009 to gather input from the stakeholders on the approach.

#### **A5.5 University of Nebraska Water Sciences Laboratory (WSL)**

Mr. Daniel Snow and his technical staff at the WSL will:

- Perform the reference analyses by solid phase extraction liquid chromatography tandem mass spectrometry (LC-MS-MS). This will include all necessary QC requirements, such as performance evaluation audit (PEA) samples to confirm the accuracy of the reference method prior to testing.

### **A6 BACKGROUND**

#### **A6.1 Technology Need**

The ETV Program's AMS Center conducts third-party performance testing of commercially available technologies that detect or monitor natural species or contaminants in air, water, and soil. The purpose of ETV is to provide objective and quality assured performance data on environmental technologies, so that users, developers, regulators, and consultants can make informed decisions about purchasing and applying these technologies. The ETV Water Stakeholder Committee, made up of buyers and users of such technologies recommend technology categories, and technologies within those categories, as priorities for testing. Among the technology categories recommended for testing are microcystin test kits. In particular, the use of microcystin test kits for the monitoring of recreational waters was identified as an area of interest for technology verification.

Microcystins are compounds (nonribosomal peptides) produced by cyanobacteria, also known as blue-green algae, which may pose a significant threat to human and animal health.



Exposure to microcystin could result in skin rashes, eye irritations, respiratory symptoms, and liver damage<sup>3</sup>. While alive, the toxins are contained inside the bacterial cell; however, when these cells become damaged or die (lysis), the toxins are released into the water. There are approximately 80 structural variants<sup>3</sup> (also called congeners) of microcystin that have been identified as highly toxic. The most common and most extensively studied variant is microcystin-LR. The World Health Organization (WHO) has set a provisional drinking-water guideline value of 1 microgram/liter ( $\mu\text{g/L}$ ) for microcystin-LR<sup>4</sup>. For recreational use, the WHO Guideline for Safe Recreational Water Environments is 20  $\mu\text{g/L}$ , 20 times the drinking water guideline concentration for microcystin-LR<sup>4</sup>. In addition to microcystin-LR, microcystin-LA and -RR will also be used to test the performance of the microcystin test kits. Microcystin-LA is highly toxic and microcystin-RR is more prevalent in the environment but not as toxic<sup>3</sup>. Table 1 shows the microcystin variants and their respective amino acid identifiers to be analyzed by the test kits.

**Table 1. Microcystin Variants<sup>3</sup>**

Name	Variable Amino Acids		Molecular Weight
Microcystin-LR	Leucine (L)	Arginine (R)	995.17
Microcystin-LA	Leucine (L)	Alanine (A)	910.06
Microcystin-RR	Arginine (R)	Arginine (R)	1038.2

## A6.2 Technology Description

Microcystin test kits are used to quantitatively measure total microcystin in recreational waters. These test kits are based on enzyme-linked immunosorbent assays (ELISA) with antibodies that bind specifically to microcystins or phosphate activity inhibition where the phosphatase hydrolyzes to determine the total toxicity of microcystin present in the sample. The kits report total microcystin or total toxicity in a water sample and therefore do not differentiate between the different variants of microcystin. Microcystin concentrations are indicated by a color measurement that is inversely proportional to the concentration of the total microcystins in the sample, that is, the color disappears if microcystin is present. The color change of the test kits is calibrated against microcystin-LR standards provided with the test kits. Variants bind differently to the immunosorbent resulting in different cross reactivity (CR) for the variants. The CR for specific variants are determined by the vendor and reported in the instructions manual. Kits are available in multiple formats, including 96-well microplates, tube assays, and test strips.

The 96-well microplates provide quantitative results when they are used in conjunction with a spectrophotometric plate reader set at 450 nanometers for the ELISA kits and 405 nanometers for the toxicity test kit. The tube assays can also provide quantitative results when used with a single-cell spectrophotometer. Both of those types of kits may also provide semi-quantitative results, using visual comparison of the color change. The photometer data are then reduced either by manual calculations or by a data reduction program. The test strip kits are semi-quantitative, indicating distinct colors for specified ranges of microcystin concentrations.

## **A7 VERIFICATION TEST DESCRIPTION AND SCHEDULE**

This verification test will assess the performance of the test kits relative to key verification parameters including accuracy, precision, and method detection limit. Correct preparation of test solutions will be confirmed through a comparison to reference method results. In performing the verification test, Battelle will follow the technical and QA procedures specified in this test/QA plan and will comply with the data quality requirements in the AMS Center QMP<sup>1</sup>.

### **A.7.1 Verification Test Description**

The objective of this verification test is to evaluate the microcystin test kit performance against known concentrations of microcystin in DI water, as well as against natural recreational water samples. The test will be performed in collaboration with the NDEQ and the SCDHS. Technologies undergoing verification will be used to analyze a variety of water samples for the variants: microcystin-LR, microcystin-LA, and microcystin-RR. The quantitative results from the microcystin test kits will be compared to the results from the reference method by calculating percent differences between the results. The kits provide a quantitative or semi-quantitative determination of microcystin and will be evaluated in terms of:

- Accuracy - comparison of test kit results (samples prepared in ASTM Type II deionized water (DI) as well as RW samples) to results from a reference method
- Precision – repeatability of test kit results from three sample replicates analyzed in DI water and recreational waters

- Linearity – determination of whether or not the test kit response increases in direct proportion to the known concentration of microcystin
- Method detection limit - the lowest quantity of toxin that can be distinguished from the absence of that toxin (a *blank value*) at a 99% confidence level
- Inter-kit lot reproducibility – determination of whether or not the test kit response is significantly different between two different lots of calibration standards within the kits.
- Matrix Interference – evaluation of the effect of natural recreational matrices and chlorophyll-*a* on the results of the test kits.
- Operational factors – general operation, data acquisition, set-up, consumables, etc.

Subsequent to the verification test, verification reports describing the test will be drafted.

These reports will be reviewed by the vendor and by peer reviewers, revised, and submitted to EPA for final approval. In performing the verification test, Battelle will follow the technical and QA procedures specified in this test/QA plan and will comply with the data quality requirements in the AMS Center QMP.<sup>1</sup>

#### **A.7.2 Proposed Verification Test Schedule**

Table 2 shows the proposed schedule of testing, auditing, and data analysis/reporting activities to be conducted during this verification. The performance evaluation audit (PEA) will take place before testing begins. The verification of microcystin test kits is planned to be completed over the course of a week after the PEA data are received. The verification test is expected to be conducted in July 2010. The technical systems audit (TSA) will take place during testing and the audit of data quality (ADQ) will take place after the data are reviewed by the Verification Test Coordinator, or designee.

**Table 2. Proposed Verification Test Schedule**

<b>Approximate Date(s)</b>	<b>Testing Activities</b>	<b>Data Analysis and Reporting</b>
July-August 2009	Recreational water sampling in Nebraska and New York	Not applicable
February-July 2010	Test/QA plan design and approval	Not applicable
June- July 2010	Perform Performance Evaluation Audit	Compile PEA reference method results
July 2010	Verification testing Perform Technical Systems Audit Reference analysis	Prepare report template Compile data from test kits Review and summarize testing staff observations Compile reference method results Begin draft reports Perform data analysis
July-August 2010	Perform Audit of Data Quality Prepare draft verification reports and statements	Complete draft verification reports and statements
August-September 2010	Coordinate reviews of draft verification reports and statements	Complete peer review and vendor review of draft reports
September 2010	Prepare final verification reports and statements	Revise draft verification reports and statements Submit final reports for EPA approval

**A7.3 Test Facility**

This verification test will take place in Columbus, Ohio at the Battelle laboratories. Recreational water samples were collected from nine local lakes in Nebraska by the NDEQ or in New York by the SCDHS. All samples were collected and frozen prior to testing in at least 120 milliliter volumes.

**A7.4 Health and Safety**

Battelle will conduct all verification testing following the safety and health protocols in place for the Battelle laboratory and facilities. This includes maintaining a safe work environment and a current awareness of handling potentially toxic chemicals. Exposure to potentially toxic chemicals will be minimized, personal protective equipment will be worn, and safe laboratory practices will be followed.

## A8 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

In performing the verification test, Battelle will follow the technical and QA procedures specified in this test/QA plan and will comply with the data quality requirements in the AMS Center QMP.<sup>1</sup> QA level III, Applied Research has been specified for this test by the EPA Project Officer.

To ensure that this verification test provides suitable data for a robust evaluation of performance, a variety of data quality objectives (DQOs) have been established for this test. The DQOs indicate the minimum quality of data required to meet the objectives of the verification test. The DQOs for this verification test were established to assess the performance of the microcystin test kits relative to reference measurements. In order to provide a suitable benchmark for comparison, the reference measurements must meet the DQOs. The DQOs for this verification test include specific objectives for reference method measurements and data completeness. The DQOs are quantitatively defined in Table 3 in terms of specific data quality indicators (DQIs) and their acceptance criteria.

The quality of the reference method measurements will be assured by adherence to these DQI criteria and the requirements of the reference methods, including the calibration and QA/QC requirements of the method. Blank samples will be required to generate results below the detection limit and the Laboratory Fortified Matrix (LFM) sample and PEA sample results will be required to be within 30% of the expected results. Prior to testing, Battelle's QAO will contact the reference laboratory and request submission of that laboratory's QA plan and associated records. In addition, Battelle will visit the reference laboratory and audit the QA document associated with the samples analyzed during this ETV test. More details about the QC requirements for the reference method are given in Section B5.

PEA samples will be used to independently confirm the accuracy of the reference measurements. Before testing begins, standards will be diluted to a concentration within the measureable range of the reference method and sent to the reference laboratory for analysis. Currently, National Institute of Standards and Technology (NIST) traceable certified microcystin standards are not available on the market. However, the Canadian National Research Council (NRC), Institute for Marine Biosciences is in the process of certifying microcystin-LR and -RR. These standards will be obtained and diluted for the PEA. In addition, standards of microcystin-LR, RR, and -LA will also be obtained from Abraxis and sent for reference analysis.

**Table 3. DQIs and Criteria for Critical Measurements for Reference Methods.**

<b>DQI</b>	<b>Method of Assessment</b>	<b>Frequency</b>	<b>Acceptance Criteria</b>	<b>Corrective Action</b>
Performance Evaluation Audit (PEA)	PEA Samples	Once before testing begins	70% - 130% recovery of target analytes	Review data to troubleshoot results and adjust reference method as necessary, reanalyze samples.
Method contamination check	Method Blank (MB)	Once every 20 samples	Target analytes < lowest calibration standard	Review data and analysis for possible sources of contamination. Reanalyze and/or document corrective action.
Method Calibration Check	Continuing Calibration Verification (CCV)	Once every 10 samples	80% - 120% recovery of target analytes	Review data to troubleshoot results and adjust reference method as necessary, reanalyze samples.
Method precision	Laboratory Duplicates	Once every 20 samples	Target analytes < 30% Difference	Review data to assess impact of matrix. If other QC data are acceptable, then reprocess duplicate. If not possible, then flag associated reference method data.
Method accuracy	Laboratory Fortified Matrix (LFM) Spikes	Once every 20 samples	70% - 130% recovery of target analytes	Review data to assess impact of matrix. If other QC data are acceptable, then reprocess duplicate. If not possible, then flag associated reference method data.

The Battelle QAO or his designee will perform a TSA at least once during this verification test to augment these QA/QC requirements. The EPA QM also may conduct an independent TSA, at her discretion.

**A9 SPECIAL TRAINING/CERTIFICATION**

Documentation of training related to technology testing, data analysis, and reporting is maintained for all Battelle technical staff in training files at their respective Battelle location. The Battelle QAO will verify the presence of appropriate training records prior to the start of testing. The vendors will be required to train the Battelle technical staff prior to the start of testing. Battelle will document this training with a consent form, signed by the vendor, which states which Battelle technical staff have been trained to use their test kits and can train other staff. In the event that other staff members are required to use the test kits, they will be trained by the operators that were trained by the vendors. All technical staff will have a minimum of a

bachelor's degree in science/engineering or equivalent work experience (e.g., experience using ELISA test kits).

Battelle will conduct all verification testing following the safety and health protocols in place at the verification testing facilities. This includes maintaining a safe work environment and a current awareness of handling potentially toxic chemicals. Exposure to potentially toxic chemicals will be minimized, personal protective equipment will be worn, and safe laboratory practices will be followed.

#### **A10 DOCUMENTATION AND RECORDS**

The documents and records for this verification test will include the test/QA plan, laboratory record books (LRB), data collection forms, electronic files (both raw data and spreadsheets), and the final verification report. Table 4 summarizes the types of data to be recorded. Documentation of Battelle staff training by vendors and copies of other project specific training will also be included in the project files. All of these records will be maintained in the Verification Test Coordinator's office during the test and will be transferred to permanent storage at Battelle's Records Management Office (RMO) at the conclusion of the verification test. All Battelle LRBs are stored indefinitely with the project files, either by the Verification Test Coordinator or Battelle's RMO. The raw and final results from the reference measurements will be submitted to Battelle upon obtaining the results of the analyses. Section B10 further details the data recording practices and responsibilities.

**Table 4. Summary of Data Recording Process**

<b>Data to Be Recorded</b>	<b>Responsible Party</b>	<b>Where Recorded</b>	<b>How often recorded</b>	<b>Disposition of Data <sup>(a)</sup></b>
Dates, times of test events	Battelle	Laboratory record books or data collection sheets	Start/end of test, and at each change of a test parameter.	Used to organize/check test results; manually incorporated in data spreadsheets as necessary.
Test parameters	Battelle	Laboratory record books or data collection sheets	When set or changed, or as needed to document test notable details during testing	Used to organize/check test results; manually incorporated in data spreadsheets as necessary.
Field sampling data	NDEQ or SCDHS	Laboratory record books or data collection sheets	During each sampling event	Used to characterize the recreational water sample.
PEA sample records	Battelle and WSL	Laboratory record books or data collection sheets	During sample preparation and analysis	Used to verify the performance of the reference method
Reference sample data	Battelle	Laboratory record books or data collection sheets	When test samples are aliquoted for the reference analysis	Used to organize/check test results; manually incorporated in data spreadsheets as necessary.
Reference method sample analysis, chain of custody, and results	Battelle and WSL	Laboratory record books, chain of custody forms, data collection sheets, or data acquisition system, as appropriate	Throughout sample handling and analysis process	Transferred to spreadsheets/agreed upon report; project files. Retained for documentation of reference method performance.

(a) All activities subsequent to data recording are carried out by Battelle.



## SECTION B

### MEASUREMENT AND DATA ACQUISITION

#### **B1 EXPERIMENTAL DESIGN**

Technologies undergoing verification will be used to analyze a variety of water samples for the variants: microcystin-LR, microcystin-LA, and microcystin-RR. Where appropriate, the quantitative results from the microcystin test kits will be compared to the results from the reference method by calculating percent differences between the results. The kits provide a quantitative or semi-quantitative determination of microcystin and will be evaluated in terms of:

- accuracy
- precision
- linearity
- method detection limit
- inter-kit lot reproducibility
- matrix effects, and
- operational factors.

Each microcystin test kit will be operated according to the vendor's instructions. This includes kit provided calibration standards and positive and negative controls. The samples will also be tested according to the kit instructions, i.e. samples and calibration standards analyzed in duplicate and the frequency of positive and negative controls. Table 5 presents the test samples to be analyzed during this verification test.

#### **B1.1 Testing Procedures**

The ability of each microcystin test kit to determine the concentration of microcystin will be challenged using quality control (QC) samples, performance test (PT) samples and recreational water (RW) samples. These sample results will also be compared to reference method results. QC samples will include laboratory reagent blanks (RB). RB samples will be prepared from DI water and will be exposed to identical handling and analysis procedures as other prepared samples, including the addition of all reagents. These samples will be used to help ensure that no sources of contamination are introduced in the sample handling and analysis

procedures. At least 10% of all the prepared samples to be analyzed will be RBs. Other QC samples, positive and negative controls, are included in this test from the test kit procedure.

PT samples will be used to help determine the accuracy, precision, linearity, method detection limit, and inter-kit lot reproducibility of the test kits. All PT samples will be prepared at Battelle using DI water as the water source. PT samples will be individually spiked with microcystin-LR, microcystin-LA, and microcystin-RR. Additionally, solutions will be prepared to assess the linearity over a concentration range and analyzed in triplicate. The concentration levels will be 0.1, 0.5, 1.0, 2.0, and 4.0 parts-per billion (ppb) to test the dynamic range of the test kits. These concentration levels will be used for microcystin-LR. Because of estimated CR of the -LA and -RR microcystin congeners, a 7.0 ppb concentration level will also be included to evaluate the dynamic range of the test kits for these two congeners. If applicable to the test kits that participate in the verification test (i.e., a semi-quantitative test strip), a 15 ppb PT sample will also be tested in order to test the semi-quantitative capability of indicating a concentration higher than 10 ppb. To determine the detection limit of the quantitative test kits, a solution with a concentration five times the vendor's reported detection limit (DL) will be used<sup>5</sup>. Seven replicate analyses of this solution will be made individually for each variant to obtain precision data with which to determine the method detection limit<sup>5</sup>. The detection limits for the quantitative test kits being verified range from 0.1 to 0.3 ppb.

RW samples have been obtained from lakes in and around Lincoln, Nebraska and Suffolk County, New York to assess kit performance in recreational waters. The RW samples have been frozen and thawed three times to lyse the cyanobacteria followed by filtration. Then the sample will be split for verification testing and reference analysis. The procedure for collecting and preparing the samples for verification testing and reference analysis is described in Section B.2. The NDEQ staff are aware of the approximate microcystin level of the lakes from which the water samples that were collected. Using this information, the samples that will be used for testing will be selected from lakes that are expected to have both detectable and not-detectable microcystin concentrations. There will be at least nine RW samples used for this verification test. Some of the samples will have been samples from Nebraska and New York. Ideally, three of the RW samples will have microcystin concentration > 20 ppb, three RW samples will have concentrations > 10 ppb, and three RW samples will have non-detectable (ND) concentrations of microcystin. All RW samples will be tested in triplicate by the test kits.

The test kits with specific vendor recommended lysing procedures will analyze three additional RW samples in triplicate. These test samples will not undergo the three iterations of the freeze-thaw lysing procedure. They will be vigorously shaken but not mechanically homogenized. The cyanobacteria need to remain intact but also be evenly distributed to split the sample for testing and reference analysis. The reference sample aliquot for these three RW samples will be split before lysing of the samples and will follow the freeze-thaw lysing method and subsequent reference analysis.

In addition to a variety of RW samples, matrix interference samples will be tested using a RW sample that has a low level or below detection level of native microcystin concentration. This RW sample will be serial diluted by a factor of 10 with DI water to provide a less concentrated level of the RW matrix. Then, each matrix level will be fortified with 4 ppb or 2 ppb of microcystin-LR, -LA, or -RR. The spike level chosen will be within the kit detection range. The test kit results in each of the matrices will be compared in order to determine the impact of the matrix concentration on the test kit results. For example, if there is no matrix interference, the expectation would be that the test kit results would not change across the matrix dilutions. In addition, they will be compared with the PT sample in DI water of the same microcystin concentration. To evaluate the effect of chlorophyll-*a* as an interference, a DI water sample that is fortified with 10 milligram/Liter of chlorophyll-*a* will be treated in an identical fashion as the above RW sample. The solution of chlorophyll-*a* will be serial diluted by a factor of 10 to provide solutions of 10 and 1 milligram/Liter chlorophyll-*a*. Then, each of these concentration levels will be fortified with 4 or 2 ppb of microcystin-LR, -LA, or -RR. The test kit results in each of the matrices will be compared in order to determine the impact of the matrix concentration on the test kit results.

Lastly, the calibration standards provided with the microcystin test kits from different lots could cause variability in the results across test kits. Therefore, two separate lots of calibration standards will be analyzed using the kits and compared to determine the inter-kit lot reproducibility.

QC, PT, and RW samples will be prepared by Battelle technical staff. Replicate samples for the test kits will be taken from the same sample bottle. The QC, PT, and RW samples will also be prepared blindly for the operator and will be coded to ensure the results are not influenced by the operator's knowledge of the sample concentration.

Reference samples will be an aliquot of the PT or RW samples. Because the reference method is mass specific for different congeners, the PT samples for the three different congeners at each spiking concentration will be combined into a volumetric flask and brought up to a known volume with DI water. Then the calculated dilution factor will correct the reference method result to the true PT sample concentration. The RW samples will be sent for reference analysis without dilution. The results of each sample analysis by the test kits will be compared to the reference method results of the same sample. Table 5 presents a solution preparation scheme for the PT samples and Table 6 presents the test samples to be analyzed during this verification test. It assumes the stock solutions are diluted to prepare spiking solutions of the different congeners at 250 ppb.

**Table 5. Preparation of PT Samples**

Target Conc. (ppb)	Spiking Solution Conc. (ppb)	Volume Spiking Solution (mL)	Final Volume (mL)
7.0	250	3.5	125
4.0	250	2.0	125
2.0	250	1.0	125
1.5	250	0.75	125
1.0	250	0.5	125
0.5	250	0.25	125
0.1	250	0.05	125
0.0	250	0	125

**Table 6. Summary of Test Samples**

Type of Sample	Microcystin Variant	Microcystin Concentration (ppb)	Replicates	Total Number of Samples per Test Kit
QC- Laboratory Reagent Blank (RB)	none	0	3	10% of total test samples, 2
Performance Test (PT) Samples - DI Water	LR	0.1, 0.5, 1.0, 2.0, 4.0 ppb	3	15
	LA	0.5, 1.0, 2.0, 4.0, 7.0 ppb	3	15
	RR	0.5, 1.0, 2.0, 4.0, 7.0 ppb	3	15
	LR	5 times the vendor stated MDL	7	7
	LA	5 times the vendor stated MDL	7	7
	RR	5 times the vendor stated MDL	7	7
Recreational Water (RW) Samples- Lysed and Filtered	Unknown	3 samples >20 ppb, 3 samples >10 ppb, 3 samples ND	3	27
Additional RW Samples for test kits with specific lysing procedure	Unknown	3 samples at unknown concentrations	3	9
RW Matrix Interference Samples: ND RW sample and 10 serial dilution	LR	4 ppb or 2 ppb*	3	6
	LA	4 ppb or 2 ppb*	3	6
	RR	4 ppb or 2 ppb*	3	6
Chlorophyll- <i>a</i> Matrix Interference Samples: Chlorophyll- <i>a</i> sample and 10 serial dilution	LR	4 ppb or 2 ppb*	3	6
	LA	4 ppb or 2 ppb*	3	6
	RR	4 ppb or 2 ppb*	3	6
Inter-kit lot reproducibility	A second set of vendor provided calibration standards from a different lot analyzed following the vendor's procedure			

\*concentration that is within the calibration range of the test kit

## B1.2 Statistical Analysis

The microcystin test kits being verified report total microcystin and are also calibrated against microcystin-LR. Because of this, the reference method data will need to be converted to microcystin-LR equivalents to compare the test kit results to the reference method results for all

PT samples. Using cross reactivity data provided by each vendor (specific to each test kit), the microcystin-LR equivalents will be calculated as follows:

$$C_{LR\ equiv} = C_{ref\ conc} \times CR \quad (1)$$

where  $C_{ref\ conc}$  is the reference method result of the microcystin variant and  $CR$  is the mass-based cross reactivity of the variant in equivalents of microcystin-LR.<sup>6</sup>

For the RW samples, each variant identified (through analysis by the reference method) will be converted to LR-equivalents, and added together to calculate the total microcystin. The total microcystin-LR equivalents from the RW reference analyses will be compared to the total microcystin results from the test kits as described in the following sections. Because not all possible variants are monitored by the reference method, there could be a discrepancy between the test kit results and the total microcystin determined by the reference method.

#### ***B1.2.1 Accuracy***

Accuracy of the test kits being verified will be assessed relative to the results obtained from the reference analyses. The results for each set of analyses will be expressed in terms of a percent difference ( $\%D$ ) as calculated from the following equation:

$$\%D = \frac{C_T - C_R}{C_R} \times 100 \quad (2)$$

where  $C_T$  is the results from the test kits being verified and  $C_R$  is the concentration as determined by the reference method.

#### ***B1.2.2 Linearity***

Linearity will be determined by linear regression with the toxin concentration measured by the reference method as the independent variable, and the test kit result being verified as the dependent variable. Linearity will be expressed in terms of the slope, intercept, and the coefficient of determination ( $r^2$ ). In addition, plots of the observed and predicated concentration values will be constructed to depict the linearity for each variant of microcystin being tested.

### **B1.2.3 Precision**

The standard deviation ( $S$ ) of the results for the replicate samples will be calculated and used as a measure of test kit precision at each concentration.  $S$  will be calculated from the following equation:

$$S = \left[ \frac{1}{n-1} \sum_{k=1}^n (C_k - \bar{C})^2 \right]^{1/2} \quad (3)$$

where  $n$  is the number of replicate samples,  $C_k$  is the concentration measure for the  $k^{\text{th}}$  sample, and  $\bar{C}$  is the average concentration of the replicate samples. The kit precision at each concentration will be reported in terms of the relative standard deviation ( $RSD$ ) presented below as equation 4.

$$RSD = \left| \frac{S}{C} \right| \times 100 \quad (4)$$

### **B.1.2.4 Method Detection Limit**

Method detection limit ( $MDL$ ) will be determined by seven replicate analyses of a fortified sample with the toxin concentration of five times the vendor's estimated detection limit. The  $MDL$  will be calculated from the following equation:

$$MDL = t \times S \quad (5)$$

where  $t$  is the Student's value for a 95% confidence level, and  $S$  is the standard deviation of the replicate samples.

### **B.1.2.5 Inter-Kit Lot Reproducibility**

Inter-kit lot reproducibility will be assessed by performing a linear regression of sample results generated by kits using calibration solutions from two different lots. The slope, intercept, and  $r^2$  will be used to evaluate the degree of inter-lot reproducibility. A paired t-test will also be conducted to evaluate whether the two sets of sample results were significantly different at a 95% confidence level.

### **B.1.2.6 Matrix Effects**

The effect of a natural matrix and of chlorophyll- $a$  in DI water will be evaluated by comparing the response of the test kits of the samples with matrix to the 4 or 2 ppb microcystin

PT sample in DI water without matrix. The percent difference between the test kit results from 4 or 2 ppb microcystin in DI water and the test kit result of 4 or 2 ppb in each RW and chlorophyll-*a* matrix interference sample will be calculated. If there is no matrix interference, the percent difference should be negligible in all cases.

### ***B1.2.7 Operational Factors***

Operational factors such as maintenance needs, calibration frequency, data output, consumables used, ease of use, repair requirements, waste production, and sample throughput will be documented based on operator and Verification Test Coordinator observations. An example of an ease of use questionnaire is provided in Appendix A.

## **B1.3 Reporting**

Separate verification reports will be prepared for each vendor that is participating in the verification testing. The statistical comparisons described above will be conducted separately for each of the test kits being tested, and information on the operational factors will also be compiled and reported separately for each test kit. The verification report will present the test procedures, test data as statistical evaluation of those data, and discuss any deviations from the approved test/QA plan.

Operational aspects of the monitoring systems will be recorded by the testing staff at the time of observation during the verification test, and summarized in the verification report. The verification report will briefly describe the ETV program, the AMS Center, and the procedures used in verification testing. The results of the verification test regarding microcystin test kit performance will be stated quantitatively. Each draft verification report will be subjected to review by the vendor, EPA, and peer reviewers. The resulting review comments will be addressed in a subsequent revision of the report, and the peer review comments and responses will be tabulated to document the peer review process, and submitted to EPA. The reporting and review process will be conducted according to the requirements of the AMS Center QMP.<sup>1</sup>

## **B2 SAMPLING METHOD REQUIREMENTS**

As described above, multiple recreational water samples were collected for this verification test. The samples were collected according to the sample collection and handling



instructions included in the NDEQ standard operating procedure for microcystin analysis (SOP# SWS-2320.1A)<sup>7</sup>. In brief, recreational water samples were collected in either plastic or glass amber collection containers. The sampling staff collected the samples throughout the summer of 2009 from locations that are representative of where human exposure would be expected (e.g., a swim area in knee deep water). In addition, when algae were present, the sampling staff collected the “worst case” sample by agitating the scum layer and collecting the sample six to eight inches below the surface. A small amount of head space was left to allow for proper shaking and mixing prior to analysis. Samples were frozen immediately after collection and stored at < -10 °C until testing takes place. Samples may be kept at 4 °C +/- 3 °C for up to one week after collection or frozen (at < -10 °C) if held for longer periods (e.g., more than one week)<sup>7</sup>. Temperatures for the refrigerator and freezer will be logged on a monthly basis. If found to be outside of the specified range above, the samples will be transferred to an acceptable refrigerator or freezer and the deviation will be noted in the LRB, in a deviation report, and in the final verification reports. In cases where there is not enough sample volume for testing and reference analysis, RW samples may be combined. They will not be diluted with DI water.

The same SOP for microcystin analysis also contains a procedure for lysing the cyanobacteria to release the microcystin into the water sample for analysis<sup>7</sup>. A procedure will be necessary to perform on all of the RW samples. This procedure goes through three iterations of completely freezing and thawing in order to breakdown the cell walls of the bacteria<sup>7</sup>. Then the samples will be filtered. Once the RW samples are lysed and filtered, the sample will be transferred, handled, and stored in glass containers to minimize any potential absorption of microcystin by plastic. Then more than 100 milliliters of the samples will be aliquoted into individual glass vials and transported to the reference laboratory on ice.

If the test kits require approaches other than freezing and thawing to lysing the cyanobacteria, that approach will be used for the applicable test kits. For the toxicity test kit, three additional RW samples will be included in testing that will not undergo freeze-thaw before being analyzed by the kit. For these three samples, the reference will be split before lysing.

### **B3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS**

Sample custody will be documented throughout collection and analysis of the test samples following the Battelle SOP for Chain of Custody<sup>8</sup>. A chain-of-custody (COC) form will

include details about the sample such as the time, date, location, and person collecting the sample. The COC form will track sample release from the sampling location to the analysis laboratory. Each COC form will be signed by the person relinquishing samples once that person has verified that the COC form is accurate. Upon arrival at the analysis laboratory, COC forms will be signed by the person receiving the samples (if different from the sample collector) once that person has verified that all samples identified on the COC forms are present. Copies of all COC forms will be delivered to the Verification Test Coordinator and maintained with the test records. When samples are delivered to a reference laboratory, a second COC form will be completed as described above.<sup>8</sup>

#### **B4 LABORATORY REFERENCE METHOD**

Technology verification will involve comparison of the results from each test kit being verified to the results obtained from an appropriate reference method. The reference method chosen for this verification test is proven liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis method for the determination of algal toxins<sup>9</sup>. This method has been slightly modified from the publication but follows the scientific approach of utilizing a reversed-phase separation column, electrospray ionization, and multiple reaction monitoring (MRM) in positive ion mode to detect the specific mass-to-charge (m/z) precursor and product ions associated with the variants of microcystin. By monitoring specific m/z values of precursor and product ions, the method is specific to the different variants of microcystin. It utilizes an internal standard, Nodularin, to minimize any matrix effects from the water samples and a surrogate recovery standard, Enkephalin to normalize the extraction efficiency of the extraction method. Table 4 shows the Chemical Abstract Service (CAS) number, MRM reaction monitored by the reference laboratory on each microcystin of interest for this verification test. Because to the IDLs of the reference method are higher than some of the PT samples, samples sent for analysis will go through solid-phase extraction (SPE) and concentration steps<sup>10</sup>. The reference laboratory will receive all samples blindly.

The reference laboratory, University of Nebraska Water Center in Lincoln, Nebraska, will perform the analysis following the QA/QC procedures described in Section B5. In addition, prior to testing, Battelle's QAO will contact the reference laboratory and request submission of that laboratory's QA plan and associated records. In addition, Battelle will visit the reference

laboratory and audit the QA document associated with the samples analyzed during this ETV test.

**Table 7. Microcystin Reference Method Information<sup>6</sup>**

Name	CAS number	Positive Ion MRM Reaction
Microcystin-LR	101043-37-2	995.35>135.1
Microcystin-LA	96180-79-9	910.2>135.1
Microcystin-RR	111755-37-4	520.0>135.0
Nodularin (Internal Standard)	118399-22-7	825.1>135.1
Enkephalin (Surrogate Recovery Standard)	58822-25-6	556.1>278.0

## B5 QUALITY CONTROL REQUIREMENTS

Quality control steps will follow the vendor specified frequency and levels for the microcystin test kits. All of the test kits require a positive and negative control and the quantitative test kits also include multiple concentrations of calibration standards.

The reference method requires the analysis of a method blanks (MB), Laboratory Fortified Matrix (LFM) samples and duplicate samples. One MB, LFM, and duplicate sample analysis will be performed during reference analysis for every 20 samples analyzed. The MB should be rejected if the microcystin concentration is above the reporting limit. The LFM is acceptable if within 30% of the expected concentration. The relative percent difference (*RPD*) of the duplicate measurements will be required to be less than 30%. *RPD* will be calculated as in Equation 6 below, where *d* will be the absolute difference between the duplicate samples and  $\bar{C}$  will be the average of the duplicate sample results. A continuing calibration verification (CCV) standard will be analyzed every 10 samples to ensure that the calibration is still valid. It will be a mid-level standard and must be within 20% of the expected value. The reporting limits for the three congeners used in this verification test must be < 0.1 ppb. See Table 3 for a summary of these requirements.

$$RPD = \frac{|d|}{\bar{C}} \times 100 \quad (6)$$

Sample sets producing results not meeting these requirements may be reanalyzed by the reference method. If the results are still outside the required tolerance, the reference instrument will be recalibrated (if applicable) and/or the reference samples reanalyzed. If the outlying

results persist, the repeat of the appropriate parts of the verification test or use of a different reference instrument may be considered.

## **B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE**

The instruments used for the reference analyses will be tested and inspected as per the instrument manuals, the standard operating procedures of the analysis laboratory, or the methods being used to make each measurement. Operation of the test kits during the verification test will be performed by Battelle technical staff as directed by the vendor.

## **B7 CALIBRATION VERIFICATION**

The instruments used for the reference analyses will be calibrated per the instrument manual, the methods being used to make each measurement or the standard operating procedures of the analysis laboratory. The vendor will provide the Battelle verification staff with the necessary training/information to properly maintain each test kit. All calibrations performed will be documented by the verification staff in the project LRB or data collection forms.

Calibration of the test kit will be done as often as suggested by the vendor. Vendors will be required to supply the necessary calibration solutions and devices specific to the test kits being verified. Balances and pipettes used during test solution preparation will be maintained and calibrated per the manufacturer's procedures which will be reviewed by the Battelle QAO prior to the verification test.

## **B8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES**

All materials, supplies, and consumables will be ordered by the Verification Test Coordinator or designee. Where possible, Battelle will rely on sources of materials and consumables that have been used previously as part of ETV verification testing without problems. Battelle will also rely on previous experience or recommendations from NDEQ technical staff and the vendors to guide selection of manufacturers and materials. The manufacturer's criteria for acceptance/purity will be required to be met. Microcystin certified standards are not available; therefore, the source used for this verification test will be purchased and verified by the reference method before use.

## **B9 NON-DIRECT MEASUREMENTS**

Data published previously in the scientific literature will not be used during this verification test.

## **B10 DATA MANAGEMENT**

Records received by or generated by any of the verification staff during the verification test will be reviewed by the Verification Test Coordinator or designee. Test data will be reviewed at intervals sufficient to ensure that test data are meeting the DQOs. The Verification Test Coordinator or delegate will review 100% of the first data batch within one week of delivery. Given the short duration of this test, all of the test samples and subsequent aliquots for reference method analysis will be prepared the day before testing begins. The reference samples will be shipped to the reference laboratory for analysis. Therefore, the first batch of reference method data is defined as all of the reference method data. However, the first batch of testing data is defined as the testing data collected on the first day of testing. The Verification Test Coordinator's review will verify that:

- All data are reported as required in the test/QA plan.
- Calibration and QC results are reported and are acceptable.
- Data are reasonable (within expected ranges).
- Technologies appear to be generating data as expected.

Records will be reviewed and verified prior to use to calculate, evaluate, or report verification results. These checks will include:

- QC samples and calibration standards were analyzed according to the test/QA plan.
- Calibration and QC sample results are reported and the acceptance criteria were met.
- Corrective action for exceedances was implemented.
- 100% hand-entered and/or manually calculated data were checked for accuracy.
- Calculations performed by software are verified at a frequency sufficient to ensure that the formulas are correct, appropriate, and consistent.

- For each cut and paste function, the first and last data value was verified vs. the source data.
- Data are reported in the units specified in the test/QA plan.

Records reviews will be documented as the dated initials of the reviewer. Any issues identified during the data review will be addressed with the testing staff in real time (i.e.,  $\leq 5$  few days of discovery) so that corrective action can be implemented and testing stopped, if needed, to ensure that data of sufficient quality are collected to meet the DQOs.

## SECTION C

### ASSESSMENT AND OVERSIGHT

#### C1 ASSESSMENTS AND RESPONSE ACTIONS

Every effort will be made in this verification test to anticipate and resolve potential problems before the quality of performance is compromised. One of the major objectives of the test/QA plan is to establish mechanisms necessary to ensure this. Internal quality control measures described in this test/QA plan, which is peer reviewed by external experts, implemented by the technical staff and monitored by the Verification Test Coordinator, will give information on data quality on a day-to-day basis. The responsibility for interpreting the results of these checks and resolving any potential problems resides with the Verification Test Coordinator. Technical staff have the responsibility to identify problems that could affect data quality or the ability to use the data. Any problems that are identified will be reported to the Verification Test Coordinator, who will work with the Battelle QAO to resolve any issues. Action will be taken to control the problem, identify a solution to the problem, and minimize losses and correct data, where possible. Battelle will be responsible for ensuring that the following audits are conducted as part of this verification test. See Table 2 for the proposed verification test schedule of audits.

Any changes to the approved test/QA plan must be reported within 24 hours and documented in a formal deviation submitted to the Battelle AMS Center Manager, EPA PO, and EPA QM. If approval by EPA or its designee is not received within 24 hours of notification, testing will be halted until a suitable resolution has been achieved.

##### C1.1 Performance Evaluation Audits

A PEA will be conducted to assess the quality of the reference measurements made in this verification test. Before testing begins, blind samples prepared from independent standards will be submitted to the WSL for analysis. As NIST standards are not available, Microcystin-LR and -RR will be obtained from the Canadian NRC (with draft certificates of analyses) and microcystin-LR, -RR, and -LA will be obtained from Abraxis. A dilution of these standards will be sent for reference analysis. The NRC standards will be diluted together in a volumetric flask

into one PEA sample, and the Abraxis standards will also be diluted together into a second PEA sample. The results of the PEA samples must be within the acceptable tolerance of 30%. If the results do not meet this requirement, they will be repeated. If the outlying results persist, the Verification Test Coordinator, or designee, and the reference laboratory representative will discuss corrective actions, and a repeat of the PEA will have to be performed. Testing will not take place unless the PEA samples are within the acceptable range. The results from the PEA will be sent to the EPA PO and EPA QM within 10 days of receipt from the reference laboratory. The PEA report will include the raw data, draft certificate from the Canadian NRC, calculations of the comparison to the expected concentration and a discussion of corrective action, if applicable.

### **C1.2 Technical Systems Audits**

Battelle QAO or delegate will perform a technical systems audit (TSA) at least once during this verification test. The purpose of this audit is to ensure that the verification test is being performed in accordance with the AMS Center QMP<sup>1</sup> and this test/QA plan. The primary focus of the audit will be operation of the technologies being verified. The audit will compare actual test procedures to those specified or referenced in the test/QA Plan, and will review data acquisition and handling procedures. The audit of the technologies will include verification that:

- The technologies are calibrated and operated as defined in the test/QA plan.
- Any test/QA plan specifications and QC are implemented.
- The data generated by the technologies are 'reasonable' based on the vendor specifications.
- Documentation and sample labeling are sufficient to ensure data traceability.

The audit of the reference method laboratory may include:

- A review of the testing facility and equipment (instrument/equipment calibration, maintenance, and operation).
- Sample handling procedures.
- Comparison of test procedures to the reference method specifications.



- Verification that calibration and QC procedures conform to the method and that the results meet the acceptance criteria.
- Review of documentation procedures.

Based on available time, the focus of the reference method audit will be on ensuring that the method is fully implemented and that instrument calibration and QC results are acceptable. The auditor will confer with the reference laboratory staff and Battelle technical staff during the audit, as needed. The TSA will be guided by a project-specific checklist based on the test/QA plan and reference method. A TSA debriefing will be conducted with the testing staff at the conclusion of the audit. The EPA PO and EPA QM will be invited to the debriefing.

A TSA report will be prepared as a memo to the Verification Test Coordinator within 10 business days after completion of the audit; the completed checklist will be attached. The Battelle AMS Center Manager, EPA PO, and EPA QM will be copied on the memo. The Verification Test Coordinator will respond to the audit within 10 business days. The Battelle QAO or designate will verify that all audit Findings and Observations have been addressed and that corrective actions are appropriately implemented. A copy of the complete TSA report with corrective actions will be provided to the EPA PO and EPA QM within 10 business days after receipt of the audit memo. At EPA's discretion, EPA QA staff may also conduct an independent on-site TSA during the verification test. The TSA findings will be communicated to technical staff at the time of the audit and documented in a TSA report.

### **C1.3 Data Quality Audits**

The Battelle QAO or designee will perform a data quality audit (DQA) on at least 10% of the sample results acquired in the verification test and 100% of the calibration and QC data vs. the test/QA plan requirements. The exact percentage of data results audited is less critical than the overall reasonableness of the data. If data quality errors are detected the auditor will track the data to identify upstream causes and downstream impacts. A checklist based on the test/QA plan will guide the audit. The primary focus of the audit will be the reference method data although the testing data will also be audited.

An initial data quality audit will be conducted on the first batch of test data within 3 business days of when data were posted on the project SharePoint site to identify errors early in the data reduction process. Given the short duration of this test, all of the test samples and

subsequent aliquots for reference method analysis will be prepared the day before testing begins. The reference samples will be shipped to the reference laboratory for analysis. Therefore, the first batch of reference method data is defined as all of the reference method data. However, the first batch of testing data is defined as the testing data collected on the first day of testing. The remaining data will be audited once all data for the technologies have been posted on the project SharePoint site and once all statistical analyses are complete. The Battelle QAO, or designee, will trace the data from initial acquisition, through reduction and statistical comparisons, to final reporting. The audit will reproduce the reported results based the raw data and any calculations and data reduction procedures performed on the data to ensure that the reported results are traceable. The review of testing data will be limited to ensuring that calibrations were performed as defined in the test/QA Plan and will review 10% of the data calculations and transcriptions to identify errors and verify that the reported data are traceable to the raw data.

A DQA audit report will be prepared as a memo to the Verification Test Coordinator within 15 business days after the data are posted; the completed checklist will be attached. The Battelle AMS Center Manager, EPA PO, and EPA QM will be copied on the memo. The Verification Test Coordinator will respond to the audit within 10 business days. The Battelle QAO or designate will verify that all audit Findings and Observations have been addressed and that corrective actions are appropriately implemented. A copy of the complete DQA report with corrective actions will be provided to the EPA PO and EPA QM within 10 business days after receipt of the audit memo. At EPA's discretion, EPA QA staff may also conduct an independent audit of data quality.

#### **C1.4 QA/QC Reporting**

Each assessment and audit will be documented in accordance with Section 3.3.4 of the AMS Center QMP<sup>1</sup>. The results of the TSA and DQA will be submitted to EPA. Assessment reports will include the following:

- Identification of Findings and Observations.
- Recommendations for resolving problems.
- Response to adverse findings or potential problems.

- Confirmation that solutions have been implemented and are effective.
- Citation of any noteworthy practices that may be of use to others.

## **C2 REPORTS TO MANAGEMENT**

The Battelle QAO, during the course of any assessment or audit, will identify to the technical staff performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, the Battelle QAO is authorized to notify the Battelle AMS Center Manager who will issue the stop work. Once the assessment report has been prepared, the Verification Test Coordinator will ensure that a response is provided for each adverse finding or potential problem and will implement any necessary follow-up corrective action. The Battelle QAO will ensure that follow-up corrective action has been taken.

In addition to this test/QA plan, a final report and a verification statement for each vendor will be prepared and reviewed, with test kit data presented separately for each technology. The final report is a comprehensive document describing the verification test. The verification statement is a two-to-three page summary of the technology, the test procedures, and the test results. Each draft report and verification statement will be submitted to the respective vendor for review. They are then reviewed by EPA QM and the EPA PO. Upon approval by EPA, each verification statement will be signed by a senior manager of Battelle and by an EPA laboratory director. Original signed verification statements will be provided to the respective vendors for use in marketing their technology. Upon final review and approval, the documents will then be posted on the ETV website ([www.epa.gov/etv](http://www.epa.gov/etv)).

## **SECTION D**

### **DATA VALIDATION AND USABILITY**

#### **D1 DATA REVIEW, VALIDATION, AND VERIFICATION REQUIREMENTS**

The key data review requirements for the verification test are the collection of QC samples as outlined in the test/QA plan, a comparison of data sheet comments against final data to flag any suspect data, and a review of final data to resolve any questions about apparent outliers. The QA audits, as described within this document are designed to assure the quality of this data.

#### **D2 VALIDATION AND VERIFICATION METHODS**

Section C of this test/QA plan provides a description of the validation safeguards employed for this verification test. Data validation and verification efforts include the analysis of QC samples as required in this document, and the performance of the TSA and PEA as described in Section C.

#### **D3 RECONCILIATION WITH USER REQUIREMENTS**

This test/QA plan and the resulting ETV verification report(s) will be subjected to review by the microcystin test kit vendors, EPA, and expert peer reviewers. These reviews will assure that this test/QA plan and the resulting verification report(s) meet the needs of potential users of the microcystin test kits. Performance data for the microcystin test kits, collected under conditions where the quality control requirements for the duplicate and PEA samples were met, will be presented in the final verification report without any further comment. Performance data and reference measurements that do not meet these criteria will be noted and a discussion of the possible impact of the failed requirements on the performance evaluation will be presented in the final verification report. The final verification report(s) will be submitted to EPA in MS Word and Adobe portable document format (PDF) and subsequently posted on the ETV website ([www.epa.gov/etv](http://www.epa.gov/etv)).

## SECTION E

### REFERENCES

#### E1 REFERENCES

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4. *Guidelines for Safe Recreational Water Environments, Volume 1, Coastal and Fresh Waters.* 2003: World Health Organization.
5. "Guidelines Establishing Test Procedures for the Analysis of Pollutants.", USEPA, Editor. 2000, U.S. Code of Federal Regulations.
6. Loftin, K.A., et al., *Comparison of Two Cell Lysis Procedures for Recovery of Microcystins in Water Samples from Silver Lake in Dover, Delaware, with Microcystin Producing Cyanobacterial Accumulations,* in *USGS Open-File Report 2008 -1341.* 2008. p. 9.
7. *SOP# SWS-2320.1A: Microcystin Analysis Using the Abraxis ELISA (Enzyme-Linked Immuno-Sorbent Assay) Method.* Nebraska Department of Environmental Quality.
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9. Hollrah, M., *Standard Operating Procedure (SOP) Determination of algaltoxin residues in water extracts by liquid chromatography (LC)- atmospheric pressure electrospray ionization tandem mass spectrometry (MS/MS).* December, 2005, Water Sciences Laboratory, University of Nebraska.
10. Cong, L.H., B.; Chen, Q.; Lu, B.; Zhang, J.; Ren, Y., *Determination of trace amount of microcystins in water samples using liquid chromatography coupled with triple quadrupole mass spectrometry.* *Anal. Chim. Acta*, 2006. **569** (1-2): p. 157-168.

## APPENDIX A EASE OF USE QUESTIONNAIRE

Technology Evaluated:

Operator:

Date:

Kit-

- 1) Clarity of instruction manual:
- 2) Solutions/reagents easily identifiable?
- 3) Storage conditions of solutions/reagents readily marked/easily available?
- 4) Number of samples that can be processed per kit?
- 5) All containers/packaging easy to open?

Reagents-

- 1) Ease of reagent preparation:
- 2) Reagent storage requirements:
- 3) Shelf life of reagents as received in kit:
- 4) Shelf life of reagents once prepared for analysis:
- 5) Equipment/materials required for reagent prep (i.e., balances, pipettes, etc)? Anything specialized?

Equipment-

- 1) User-friendliness of software or electronic readout:

- 2) Data endpoint or reaction easy to visually observe?
- 3) Does equipment require any special preparation before use?
- 4) Is equipment easy to clean off?
- 5) Does equipment require any routine maintenance?

Overall-

- 1) Comments on general convenience of product:
- 2) Estimate of training/education required to carry out testing with this technology?
- 3) Does this product generate a lot of solvent or solid waste?
- 4) Are the wastes generated hazardous (i.e., need special disposal)?
- 5) Does the vendor provide support (phone or otherwise?)
- 6) If you encountered any problems with the technology was it easy to remedy?

Other Comments: