May 2011

Environmental Technology Verification Report

BEACON MICROCYSTIN TEST KITS

TUBE KIT
PLATE KIT

Prepared by
Battelle

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ETV ETV ETV

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ETV Advanced Monitoring Systems Center

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MICROCYSTIN TEST KITS
TUBE KIT
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by
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Notice

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Foreword

The EPA is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center1.html.

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List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
CCV	continuing calibration verification
^{o}C	degrees Celsius
CR	cross reactivity
CV	coefficient of variation
DQI	data quality indicator
DQO	data quality objective
DI	Deionized
EPA	Environmental Protection Agency
ETV	Environmental Technology Verification
ELISA	Enzyme-Linked Immunosorbent Assay
LFM	laboratory fortified matrix
LC-MS-MS	liquid chromatography tandem mass spectrometry
LOQ	Limit of quantification
MB	method blank
MDL	method detection limit
mg/L	milligram per liter
mL	Milliliter
Nm	Nanometer
NDEQ	Nebraska Department of Environmental Quality
NRC	National Research Council
OD	optical density
Ppb	parts per billion
%D	percent different
PEA	performance evaluation audit
PT	performance test
QA	quality assurance
QAO	quality assurance officer
QC	quality control
QMP	quality management plan
r^2	coefficient of determination
RB	reagent blank
RW	recreational water
RSD	relative standard deviation
S	standard deviation
SOP	standard operating procedure
SPE	solid phase extraction
TQAP	Test/Quality Assurance Plan
TSA	technical systems audit
µg/L	Microgram per linter
WSL	Water Sciences Laboratory

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible. The definition of ETV verification is to establish or prove the truth of the performance of a technology under specific, pre-determined criteria or protocols and a strong quality management system. The highest-quality data are assured through implementation of the ETV Quality Management Plan. ETV does not endorse, certify, or approve technologies.

The EPA's National Risk Management Research Laboratory (NRMRL) and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of two technologies offered by Beacon Analytical Systems, Inc.: Microcystin Plate Kit and Microcystin Tube Kit.

Chapter 2
Technology Description

This verification report provides results for the verification testing of two Beacon Analytical Systems Microcystin Test Kits. Following are descriptions of the Microcystin Plate and Tube Test Kits based on information provided by the vendor. The information provided below was not verified in this test.

2.1 Microcystins Tube and Plate Kit

Beacon Microcystin Tube Kit, Figure 1, is an immunological laboratory test for the quantification of microcystins in water. The tube kit uses a polyclonal antibody that binds both microcystins and a microcystin-enzyme conjugate. Microcystins in the sample compete with the microcystin-enzyme conjugate for a limited number of antibody binding sites. The assay procedure includes the following steps:

- Add microcystin-enzyme conjugate and a sample for analysis of microcystins to a test tube, followed by antibody solution. The conjugate competes with any microcystins in the sample for the same antibody binding sites. The test tube is coated with anti-rabbit immunoglobulin G (IgG) to capture the rabbit anti-microcystin added.
- Wash away any unbound molecules, after incubating this mixture for 20 minutes.
- Add clear substrate solution to each tube. In the presence of bound microcystin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites is available in every tube, and each tube receives the same number of microcystin-enzyme conjugate molecules, a sample containing a low concentration of microcystins allows the antibody to bind many microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of microcystins allows fewer microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution. The color is analyzed using a colorimeter or spectrophotometer to obtain optical density (OD) values at 450 nanometers (nm). Reader software or a spreadsheet is used to generate a standard curve and interpolate the sample values from that curve.

There are approximately 80 different variants of microcystins present in the environment and the kits tested are not able to detect the difference between microcystin variants. Results from the kits tested are calibrated with respect to the microcystin-LR variant. However, other microcystin variants are known (based on information provided by Beacon) to react to different extents with the antibodies used for detection. Cross reactivity values provided by Beacon are used to quantify results for different variants based on the LR calibration.

The Beacon Microcystin Plate Kit (Figure 2) is also an immunological laboratory test for the quantification of microcystins in water. The plate kit uses the same principles as the tube kit but

employs the use of a 96 well assay plate to allow for processing a larger number of samples. The plate is a break-apart strip design allowing for assays of any number of wells to be employed from 1 to 96. The manufacturer recommends significant familiarity with the technology, incorporation of additional assay controls and the use of multichannel pipettes for "whole plate" (96 well) assays.

The price of the tube kit at the time of this verification test is $200 for a 40-tube kit (24 samples). The plate kit costs $275 per plate to analyze a maximum of 84 samples. Both kits measure 6.25 x 5.125 x 3.75 inches (15.9 x 13.0 x 9.5 centimeters), with the plate kit weighing 11ounces (312 grams) and the tube kit is 17 ounces (483 grams).

Figure 1. Beacon Microcystin Tube Kit

Figure 2. Beacon Microcystin Plate Kit

Chapter 3
Test Design and Procedures

3.1 Test Overview

This verification test was conducted according to procedures specified in the Test/Quality Assurance Plan for Verification of Microcystin Test Kits[1] (TQAP) and adhered to the quality system defined in the ETV AMS Center Quality Management Plan (QMP)[2]. It assessed the performance of microcystin test kits relative to key verification parameters including accuracy, precision, and method detection limit. This verification test took place from July 26 through August 12, 2010. The reference analysis was performed the week of August 16th, 2010.

This verification report has been reviewed by experts in the field related to microcystin detection. The following experts have provided input to the TQAP that guided this testing as well as the verification report and verification statement.

- Andy Lincoff, U.S. EPA Region 9
- Keith Loftin, U.S. Geological Survey
- Daniel Snow, University of Nebraska

The responsibilities of verification test stakeholders include:

- Participate in technical discussions as a part of the test designing process,
- Review and provide input to the TQAP, and
- Review and provide input to the verification report and verification statements.

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.

3.2 Experimental Design

The objective of this verification test was to evaluate the performance of the microcystins test kits against known concentrations of microcystin in ASTM International Type II deionized (DI) water, as well as in natural recreational water samples. Battelle conducted this verification test with recreational samples provided from the Nebraska Department of Environmental Quality (NDEQ), document review by the Suffolk County Department of Health Services (SCDHS), with the University of Nebraska Water Sciences Laboratory (WSL) providing reference analyses. The technologies were used to analyze a variety of water samples spiked with the variants microcystin-LR, microcystin-LA, and microcystin-RR. Because none of the three technologies can specify between the different variants, the samples were spiked with individual variants. The quantitative results from the microcystin test kits were compared to the results from the reference method by calculating percent differences between the results. The reference method for microcystin was a liquid chromatography tandem mass spectrometry (LC-MS-MS) method for the determination of microcystins[3]. To attain lower levels of detection, a sample preparation

method[4] was used to extract the microcystins from the water samples and concentrate the samples using solid phase extraction (SPE). The enzyme-linked immunosorbent assay (ELISA) tube kit and plate kits were evaluated in terms of:

- Accuracy - comparison of test kit results (samples prepared in DI) to results from a reference method;
- Precision - repeatability of test kit results from three sample replicates analyzed in DI water, matrix interference, and recreational water samples;
- 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 95% 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; and
- Operational and Sustainability factors such as general operation, data acquisition, set-up, and consumables.

Test kits were operated according to the vendor's instructions by a vendor-trained Battelle technician. Water samples were tested according to the kit instructions and in compliance with the TQAP.

3.3 Test Procedures

The ability of each microcystin test kit to determine the concentration of microcystin was challenged using quality control (QC) samples, performance test (PT) samples and recreational water (RW) samples. These sample results were also compared to reference method results. Table 1 presents the test samples analyzed during this verification test.

QC, PT, and RW samples were prepared by Battelle technical staff the day before testing began. The test samples were prepared in glass volumetric flasks and stored in amber glass vials at 4 degrees Celsius (^{o}C) ± 3 ^{o}C. The reference samples that were aliquotted from the test samples were stored in amber glass bottles at < -10^{o}C until analysis approximately two weeks later. Replicate samples for the test kits were taken from the same sample bottle. The QC, PT, and RW samples were prepared blindly for the operator by coding the sample labels to ensure the results were not influenced by the operator's knowledge of the sample concentration and variant.

Because the reference method is mass specific for different variants, the PT samples for the three different variants at each spiking concentration were combined into a volumetric flask and brought up to a known volume with DI water before being sent to the reference laboratory. Then the calculated dilution factor was applied to the reference method result to determine the PT sample concentration of each variant. The RW samples were sent for reference analysis without dilution.

3.3.1 QC Samples

Reagent Blank (RB) samples were prepared from DI water and were exposed to identical handling and analysis procedures as other prepared samples, including the addition of all reagents. These samples were used to ensure that no sources of contamination were introduced in the sample handling and analysis procedures. At least 10% of all the prepared samples were RBs. As specified in the test kit procedure, at least one positive was analyzed with each ELISA plate.

3.3.2 PT Samples

PT samples were used to verify the accuracy, precision, linearity, method detection limit, and inter-kit lot reproducibility of the test kits. All PT samples were prepared at Battelle using DI water as the water source. PT samples were individually spiked with microcystin-LR, microcystin-LA, and microcystin-RR and analyzed in triplicate. The concentration levels were 0.10, 0.50, 1.0, 2.0, and 4.0 parts per billion (ppb). These concentration levels were used for microcystin-LR, and because of the cross reactivity (CR) of the LA and RR microcystin variants, a 7.0 ppb concentration level was also included to evaluate the dynamic range of the test kits for these two variants. EPA Guidelines[5] were followed to determine the method detection limit (MDL) of the quantitative test kits. In doing so, a solution with a concentration five times the vendor's reported detection limit was used. Seven replicate analyses of this solution were made individually for each variant to obtain precision data with which to determine the method detection limit.

Additional performance testing was performed to verify the impact of possible interferences on the performance of the test kits. Two types of possible interferences were tested, the possible interference of RW water and chlorophyll. Testing was performed using a RW sample with a low level of native microcystin concentration (based on information from NDEQ). This RW sample was serial diluted by a factor of 10 with DI water to provide a less concentrated level of the RW matrix. Then both the original RW sample and diluted RW samples were fortified with 4.0 ppb (tube test kit) or 2.0 ppb (plate test kit) of microcystin LR, LA, or RR. The spike level chosen was dependent on the detection range of each kit. The test kit results in each of the matrices were compared to determine the impact of the matrix concentration on the test kit results. In addition, the results from the matrix samples were compared with the PT sample in DI water of the same microcystin concentration.

To evaluate the effect of chlorophyll-*a* as a possible interferent, a DI water sample that was fortified with 10 milligram/Liter (mg/L) of chlorophyll-*a* (Sigma Aldrich, Cat # C5753-5MG Chlorophyll-*a* from spinach) was prepared by adding known amount of chlorophyll-*a* into a volumetric flask and diluting to volume. The chlorophyll was insoluble. Therefore the resulting solutions were clear solutions containing small black pieces of solid chlorophyll-*a*. These solutions were then treated in an identical fashion as the above RW sample. The solution of chlorophyll-*a* was serial diluted by a factor of 10 to provide solutions of 10 and 1 mg/L chlorophyll-*a*. Then, each of these concentration levels were fortified with 4.0 or 2.0 ppb of microcystin-LR, -LA, or -RR. The test kit results in each of the matrices were compared to determine the impact of the chlorophyll-*a* on the test kit results.

Table 1. Summary of Test Samples

Type of Sample	Microcystin Variant	Microcystin Concentration (ppb)	Replicates	Total Number of Samples per Test Kit
QC Samples – Kit Positive Controls	LR	1.0	1	1
QC Samples- Laboratory Reagent Blank (RB)	none	0	3	10% of total test samples, 2
PT Samples - DI Water	LR	0.10, 0.50,1.0, 2.0, 4.0	3	15
	LA	0.50, 1.0, 2.0, 4.0, 7.0	3	15
	RR	0.50, 1.0, 2.0, 4.0, 7.0	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
PT Samples - RW Matrix Interference Samples: ND RW sample and tenfold dilution	LR	4.0 or 2.0*	3	6
	LA	4.0 or 2.0*	3	6
	RR	4.0 or 2.0*	3	6
PT Samples - Chlorophyll-*a* Matrix Interference Samples: Chlorophyll-*a* sample and tenfold dilution	LR	4.0 or 2.0*	3	6
	LA	4.0 or 2.0*	3	6
	RR	4.0 or 2.0*	3	6
PT Samples - Inter-kit lot reproducibility	A second set of vendor provided calibration standards from a different lot analyzed following the vendor's procedure			
RW Samples- Through freeze-thaw lysing procedure	Unknown	3 samples >20 ppb, 3 samples >10 ppb, 3 samples ND	3	27
RW Samples- Through the vendor recommended procedure	Unknown	3 samples at unknown concentrations	3	9

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

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 were analyzed using the kits and compared to determine the inter-kit lot reproducibility.

3.3.3 RW Samples

RW samples were obtained from lakes in and around Lincoln, Nebraska to assess kit performance in recreational waters. The procedure for collecting and preparing the samples for verification testing and reference analysis is described in the NDEQ standard operating procedure for microcystin analysis (SOP# SWS-2320.1A)[6]. In summary, staff from NDEQ collected the water samples from lakes where there is a potential for human exposure to microcystins. The RW samples were collected in brown plastic bottles with head space remaining and returned to the laboratory where they were frozen and thawed three times to lyse the cyanobacteria and free the microcystin into solution, making it available for analysis. Then the samples were split for verification testing and reference analysis. Using analytical data generated by NDEQ, samples used for ETV testing were selected from lakes that had both detectable and not-detectable microcystin concentrations. 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.

Chapter 4
Quality Assurance/Quality Control

QA/quality control (QC) procedures were performed in accordance with the AMS Center QMP and the TQAP for this verification test. QA level III, Applied Research was specified for this test by the EPA Project Officer. These procedures and results are described in the following subchapters.

4.1 Reference Method Quality Control

To ensure that this verification test provided suitable data for a robust evaluation of performance, a variety of data quality objectives (DQOs) were established for this test. The DQOs indicated the minimum quality of data required to meet the objectives of the verification test. The DQOs were quantitatively defined in terms of specific data quality indicators (DQIs) and their acceptance criteria. The quality of the reference method measurements were 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 were required to generate results below the detection limit and the Laboratory Fortified Matrix (LFM), duplicate, and Performance evaluation audit (PEA) sample results were required to be within 30% of the expected results. Continuing calibration verification (CCV) samples were required to be within 20% of the expected result. Battelle visited the reference laboratory prior to initiation of the reference analysis and audited the data package provided by the reference laboratory following analysis. More details about the audits are provided in Section 4.2. Table 2 presents these DQIs and the reference method QC sample results. A total of 22 samples were analyzed by the reference method, so in cases where the frequency required was one per 20 samples, only one sample was analyzed to assess the DQI.

The calibration of the LC-MS/MS method was verified by the analysis of a CCV every 10 samples. All of the calibration standards were used as CCVs and were interspersed throughout the run every five samples. The percent recoveries (*%R*) of CCVs were calculated from the following equation:

$$\%R = \frac{C_s}{s} \times 100 \qquad (1)$$

where C_s is the measured concentration of the CCV, s is the spiked concentration. If the CCV analysis differed by more than 20% from the true value of the standard (i.e., % R values outside of the acceptance window of 80-120%), the instrument was recalibrated before continuing the analysis. As shown in Table 3, all reference CCV analyses were within the required range.

Spiked samples were analyzed to assess the efficiency of the extraction method. There was a laboratory fortified matrix (LFM) spike performed every 20 samples; it was assessed by calculating the spike percent recovery (*%Rs*) as shown below.

$$\%Rs = \frac{C_s - C}{s} \times 100 \quad (2)$$

C_s is the measured concentration of the spiked sample, C is the measured concentration of the unspiked samples, and s is the spiked concentration. The spike percent recovery was required to be within 30% of the spiked amount. The LFM sample results were within this range.

Duplicate samples were analyzed to assess the precision of the reference analysis. There was an analytical duplicate performed at least every 20 samples and these were expected to be within 30% of each other. The relative percent difference (*RPD*) of the duplicate sample analysis was calculated from the following equation:

$$RPD = \frac{|C - C_D|}{(C + C_D)/2} \times 100 \quad (3)$$

Where C is the concentration of the sample analysis, and C_D is the concentration of the duplicate sample analysis. If the *RPD* was greater than 30%, then the extraction method and the analytical methods were investigated. As shown in Table 3, the *RPD* for the duplicate sample analyses was calculated from the duplicate 30 and 60 ppb CCVs. All *RPD* were within the acceptable range for duplicate analysis.

Table 2. DQIs and Summary of Reference Method QC Results

DQI	Method of Assessment (Frequency)	Acceptance Criteria for Microcystins	Results
Performance Evaluation Audit (PEA)	PEA Samples (Once before testing begins)	70% - 130% Recovery	See Tables 4 and 5 in Section 4.2.1
Method contamination check	Method Blank (MB) (Once every 20 samples)	< Lowest Calibration Standard	ND for all three variants
Method Calibration Check	Continuing Calibration Verification (CCV) (Once every 5 samples)	80% - 120% Recovery	See Table 3
Method precision	Laboratory Duplicates (Once every 20 samples)	< 30% RPD	See Table 3
Method accuracy	Laboratory Fortified Matrix (LFM) Spikes (Once every 20 samples)	70% - 130% Recovery	93% LR 79% LA 97% RR

Table 3. Summary of Reference Method CCV Percent Recoveries and Method Precision

CCV Conc. (ppb)	% Recovery			Duplicate CCV Sample Results (*RPD*)		
	LR	LA	RR	LR	LA	RR
10	99.5	98.2	96.1	NA	NA	NA
30	109	104	112	12%	7%	13%
30	96.5	97.1	98.7			
60	97.6	94.2	93.5	5%	14%	14%
60	103	109	108			
75	98.7	91.8	101	NA	NA	NA

NA-not applicable, no duplicate measurements made

4.2 Audits

Three types of audits were performed during the verification test, a performance evaluation audit (PEA), a technical systems audit (TSA) of the verification test procedures, and an audit of data quality audit (ADQ). Audit procedures and results are described further below.

4.2.1 Performance Evaluation Audit

A PEA was conducted to assess the quality of the reference measurements made in this verification test. National Institute of Standards and Technology (NIST) traceable standards of microcystin are not available; however, the Canadian National Research Council (NRC) offers standards that have gone through the most validation of any commercially available standards and were recognized by the vendors and stakeholders reviewing the TQAP as the most reliable standards. The microcystin-LA variant was not available through the Canadian NRC and therefore was obtained from Abraxis. The standards from Abraxis also undergo a high level of scrutiny and are considered a reliable source. The approach of using the microcystin-LA variant standard from Abraxis was approved by all participating vendors prior to use. The standards obtained from both sources were prepared at 50 ppb in DI and sent blindly to the reference laboratory for analysis. These PEA samples were analyzed directly (i.e., without additional preparation) and were in the mid-level of the calibration range of the reference method. The standards used to prepare the calibration standards by the reference laboratory were obtained from EMD Biosciences (microcystin-LR), Sigma Aldrich (microcystin-LA), and ENZO Life Sciences (microcystin-RR). The results from the analysis are presented in Table 4.

Table 4. PEA Results: Analytical Comparison of Microcystin Standards

Standard Source	# of Replicates	Analysis Date	MC-LR	MC-LA	MC-RR
NRC Canada	2	27-May	150% ± 3%	Not available	192% ± 1%
	8	9-Jun	135% ± 7%	Not available	194% ± 12%
Abraxis	2	27-May	129% ± 2%	86% ± 2%	144% ± 0%
	8	9-Jun	121% ± 6%	86% ± 5%	153% ± 10%

Shading indicates results outside acceptable 30% tolerance based on TQAP

The over-recoveries of these standards revealed that the reference laboratory method (using the standards from alternate sources) did not determine either the NRC or Abraxis standards within ±30%. Therefore it was discussed with the stakeholders, and accepted by the vendors and the EPA Project Officer, that the reference laboratory would use the two available NRC standards (LR and RR) as well as LA from Abraxis for their calibration solutions. Therefore, the same standards were used for test solutions and reference calibration. It is not a common practice for calibration standards and test solutions to be generated from the same source, but since the objective was to generate comparable vendor and reference data, it was deemed appropriate for this verification test due to the difficulties in obtaining certified microcystin standards.

To achieve the low detection limits required to analyze the test samples, an SPE extraction method was used by the reference laboratory for samples expected to be below 5 ppb. The MDL of this method was determined using eight solutions of LR, LA, and RR at 0.38 ppb which were extracted and analyzed. The reference method MDLs for LR, LA, and RR were determined to be 0.10 ppb, 0.14 ppb, and 0.13 ppb, respectively. Appendix A is the memo from the reference laboratory presenting the MDL data.

A second PEA was performed to evaluate the extraction method efficiency and the analytical method at a lower concentration relevant for this verification test. Battelle provided WSL with a blind spiked DI sample at 0.25 ppb that was extracted in triplicate. The results from the second PEA are presented in Table 5.

Table 5. PEA Results: Evaluation of Extracted Low Level Water Sample

	LR		LA		RR	
0.25 ppb Spiked Sample	**Conc. (ppb)**	**% Recovery**	**Conc. (ppb)**	**% Recovery**	**Conc. (ppb)**	**% Recovery**
Replicate 1	0.23	92%	0.21	84%	0.24	96%
Replicate 2	0.25	100%	0.23	92%	0.22	88%
Replicate 3	0.23	92%	0.22	88%	0.26	104%
Average	0.24	95%	0.22	88%	0.24	96%
Standard Deviation	0.01	5%	0.01	4%	0.02	8%

4.2.2 Technical Systems Audit

Battelle's Quality Assurance Officer (QAO) conducted a TSA to ensure that the verification test was being conducted in accordance with the TQAP and the AMS Center QMP. As part of the TSA, test procedures were compared to those specified in the TQAP, and data acquisition and handling procedures as well as the reference method procedures were reviewed. Two observations on storage of test records and sample handling and custody were documented and submitted to the Battelle Verification Test Coordinator for response. None of the observations from the TSA required corrective action. TSA records are permanently stored with the QAO.

4.2.3 Data Quality Audit

Two Audits of Data Quality (ADQ) were performed for this verification test. The first was for the data collected on the first day of testing and the second was on the complete data package

generated during verification test preparation and execution. During the audits, test kit data were reviewed and verified for completeness, accuracy and traceability.

Because the EPA Project Officer designated this as an EPA Category III verification test, at least 10% of the data acquired were audited. The QAO traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

Observations and findings (mostly related to test record documentation) were reported and submitted to the Battelle Verification Test Coordinator for response.

Chapter 5
Statistical Methods

The statistical methods used to evaluate the quantitative performance factors listed in Section 3.2 are presented in this chapter. Qualitative observations were also used to evaluate verification test data.

The microcystin test kits being verified report total microcystin and are also calibrated against microcystin-LR. Because of this, the kit data was converted from microcystin-LR equivalents to compare the test kit results to the reference method results for all PT samples. Using cross reactivity data provided by Beacon, the microcystin-LR equivalents were converted to microcystin concentration by variant as follows:

$$C_{by\ \mathrm{var}\,iant} = \frac{C_{LR\,equiv}}{CR} \tag{4}$$

where $C_{LR\,equiv}$ is the test kit result in equivalents of microcystin-LR and CR is the mass-based cross reactivity of the variant.[7]

For the RW samples, each variant identified through analysis by the reference method was converted to LR-equivalents, and added together to calculate the total microcystins. The total microcystin-LR equivalents from the RW reference analyses were compared to the total microcystin results from the test kits. 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.

5.1 Accuracy

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

$$\%D = \frac{C_T - C_R}{C_R} \times 100 \tag{5}$$

where C_T is the microcystin-LR equivalent results from the test kits being verified and C_R is the concentration as determined by the reference method.

5.2 Linearity

Linearity was 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 was expressed in terms of the slope, intercept, and the coefficient of determination (r^2). In addition, plots of the observed and predicated concentration values were constructed to depict the linearity for each variant of microcystin being tested.

5.3 Precision

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

$$S = \left[\frac{1}{n-1} \sum_{k=1}^{n} (C_k - \overline{C})^2 \right]^{1/2} \quad (6)$$

where n is the number of replicate samples, C_k is the concentration measure for the kth sample, and $\overline{C}$ is the average concentration of the replicate samples. The kit precision at each concentration was reported in terms of the relative standard deviation (*RSD*) presented below as equation 7.

$$RSD = \left| \frac{S}{\overline{C}} \right| \times 100 \quad (7)$$

5.4 Method Detection Limit

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

$$MDL = t \times S \quad (8)$$

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

5.5 Inter-Kit Lot Reproducibility

Inter-kit lot reproducibility was assessed by calculating the RPD (Equation 3) between OD results from two lots of calibration standards.

5.6 Matrix Effects

Matrix interference effects also were assessed by using a t-test to compare the microcystin test kit results generated from samples made by spiking undiluted and diluted interference matrices with the PT sample results at the same spiked concentration (either 2 or 4 ppb spike concentration). Each paired t-test was performed using the replicate data from each type of sample. The null hypothesis is that there is no difference between the two sets of data. Therefore, the resulting probability (p)-value gives the likelihood of observing a difference as large as is seen in the data, or a larger difference, if the null hypothesis were true. Therefore, at the 95% confidence level, p-values less than 0.05 will indicate there is evidence against the null hypothesis being true and therefore a significant difference between the two sets of data.

Chapter 6
Test Results for the Beacon Microcystin Tube Kit

The following sections provide the results of the quantitative and qualitative evaluations of this verification test for the Beacon microcystin tube kit.

6.1 Beacon Test Kit Summary

As discussed in Chapter 2, the tube kit quantifies total microcystins in water based on an LR calibration. Other variants of microcystins bind differently to the immunosorbent (i.e., cross reactivity). Therefore, the relative ability for other microcystins to bind has been experimentally determined by the vendor. For the tube kit, the CR of microcystin LA is 2% and the CR of microcystin RR is 73%. In this report, the test kit data have been reported in both test kit results as LR equivalents and in CR corrected results by variant, based on Equation 4.

Each tube kit contains five calibration solutions including a blank (0 ppb) standard. Following the analysis method, the tube reader measured the absorbance containing the calibration solutions at 450 nm wavelengths and the calibration curve was generated based on the OD of each standard. These results were plotted against concentrations using a vendor-provided spreadsheet that generated a four parameter curve to quantify the samples. The data from a batch of samples was considered acceptable when the positive control was recovered within 80% and 130% of 1.0 ppb. According to Beacon, if the data result of a sample was out of range it was determined to be either above or below the calibration range and either diluted into the linear range or reported as less than limit of quantification (< LOQ) or non-detectable (ND). The results below the calibration curve were reported as < LOQ when the OD value was greater than the lowest standard OD value but less than the negative control sample OD value. A sample was reported as a ND when the OD value was greater than the negative control sample OD value.

6.2 Test Kit QC Sample

As described in Section 3.3.1, the QC samples analyzed with the tube kit included RB samples and the positive controls included in the test kit. Ten percent of all samples analyzed were RB samples, and the results were used to verify that no contamination was introduced during sample handling. All RB sample results were reported as ND or < LOQ for the tube kit, as presented in Table 6. Two RB samples were analyzed by the reference method and were determined to be < LOQ for all three variants.

Table 6. RB Sample Results for the Beacon Tube Kit

QC Sample ID	Plate	Mean Concentration (ppb)
RB 1	1	ND
RB 2	1	ND
RB 3	1	< LOQ
RB 4	3	ND
RB 5	3	ND
RB 6	3	ND

The positive controls for the tube kit are presented in Table 7. The vendor stated acceptable range for recovery of the positive control is between 80% and 130%. At least one positive control was analyzed at the end of each batch of 20. The positive control for Batch 2 was a 5.0 ppb calibration solution rather than the 1.0 ppb sample used for the rest of the plates. As shown in Table 7, all tube kit batches used for testing produced a positive control result within the acceptable range.

Table 7. Positive Control Sample Results for the Beacon Tube Kit

QC Sample ID	Plate	Mean Concentration (ppb)	Percent Recovery (%)
1	1	0.91	91%
2[a]	2	5.8	120%
3	3	0.94	94%
4	4	0.98	98%
5	5	0.98	98%
6	6	1.1	110%
7	7	0.91	91%
8	8	0.82	82%
9	9	1.1	110%
10	10	1.1	110%
11	11	1.1	110%
12	12	0.98	98%

[a] 5.0 ppb positive control standard from a different lot

6.3 PT Samples

Tables 8, 9, and 10 present the results for the PT samples for the three variants of microcystin used during this verification test. In addition, the tables present the sample concentration corrected for the microcystin cross reactivity, the reference method results and the accuracy results by variant for the PT samples prepared in DI water. All samples were analyzed in triplicate.

6.3.1 Accuracy

Tables 8, 9, and 10 present the accuracy results for the tube kit, expressed as percent difference (%D) between the tube kit concentrations and reference concentrations. As shown in Equation 5 (Section 5.1), the reference method value was used for calculation of accuracy. For LR, the reference method was within 10% of the spike concentration. For LA and RR, the reference value was 5-45% lower than the spike concentration depending upon the sample. All data are

provided so that the calculation of % D can be calculated relative to the spike value as well as the reference method if desired by the reader.

Table 8. Beacon Tube Kit Sample Results and Reference Method Results for LR

Sample Description	Kit Results: LR Equivalents (ppb)	CR Corrected Conc. by Variant (ppb)	Accuracy by Variant (% Difference)	Reference Concentration (ppb)
0.1 LR	<LOQ	<LOQ	<LOQ	0.10
	<LOQ	<LOQ	<LOQ	
	<LOQ	<LOQ	<LOQ	
Avg ± SD	<LOQ	<LOQ	<LOQ	
0.5 LR	<LOQ	<LOQ	<LOQ	0.42
	0.10	0.10	-76%	
	<LOQ	<LOQ	<LOQ	
Avg ± SD	0.10	0.10	-76%	
1.0 LR	1.0	1.0	21%	0.83
	0.87	0.87	5%	
	0.96	0.96	16%	
Avg ± SD	0.95 ± 0.07	0.95 ± 0.07	14% ± 8%	
2.0 LR	2.2	2.2	15%	1.9
	2.2	2.2	15%	
	2.3	2.3	21%	
Avg ± SD	2.2 ± 0.10	2.2 ± 0.10	17% ±3%	
4.0 LR	4.3	4.3	16%	3.7
	4.5	4.5	21%	
	3.7	3.7	-1%	
Avg ± SD	4.2± 0.40	4.2± 0.40	12% ± 11%	

Table 9. Beacon Tube Kit Sample Results and Reference Method Results for LA

Sample Description	Kit Results: LR Equivalents (ppb)	CR Corrected Conc. by Variant (ppb)	Accuracy by Variant (% Difference)	Reference Concentration (ppb)
0.5 LA	0.23	12	28	0.40
	0.28	14	34	
	0.24	12	29	
Avg ± SD	0.25 ± 0.03	13± 1.3	3000% ± 310%	
1.0 LA	0.28	14	19	0.70
	0.36	18	25	
	0.35	17	24	
Avg ± SD	0.33 ± 0.04	16 ± 2.0	2200% ± 310%	
2.0 LA	0.40	20	11	1.7
	0.40	20	11	
	0.37	19	9.9	
Avg ± SD	0.39 ± 0.02	20± 1.0	1100% ± 51%	
4.0 LA	0.46	23	6.7	3.0
	0.46	23	6.6	
	0.49	24	7.1	
Avg ± SD	0.47 ± 0.02	23 ± 0.77	680% ± 26%	
7.0 LA	0.53	27	4.6	4.7
	0.57	28	5.0	
	0.52	26	4.5	
Avg ± SD	0.54± 0.02	27 ± 1.2	470% ± 25%	

Table 10. Beacon Tube Kit Sample Results and Reference Method Results for RR

Sample Description	Kit Results: LR Equivalents (ppb)	CR Corrected Conc. by Variant (ppb)	Accuracy by Variant (% Difference)	Reference Concentration (ppb)
0.5 RR	0.38	0.52	36%	0.38
	0.39	0.53	40%	
	0.45	0.62	63%	
Avg ± SD	0.41 ± 0.04	0.56 ± 0.06	46% ± 15%	
1.0 RR	1.2	1.6	190%	0.54
	0.88	1.2	120%	
	1.1	1.6	190%	
Avg ± SD	1.1 ± 0.15	1.5 ± 0.21	170% ± 38%	
2.0 RR	1.7	2.3	47%	1.6
	1.7	2.3	45%	
	2.4	3.3	110%	
Avg ± SD	1.9 ± 0.40	2.7 ± 0.60	67% ± 36%	
4.0 RR	2.7	3.7	17%	3.2
	2.5	3.5	8%	
	2.6	3.6	13%	
Avg ± SD	2.6 ± 0.10	3.6 ± 0.10	13% ± 4%	
7.0 RR	3.9	5.4	20%	4.5
	4.0	5.4	22%	
	4.2	5.7	28%	
Avg ± SD	4.0± 0.10	5.5 ± 0.10	23% ± 4%	

For the LR spiked samples, the reference method results were approximately 17% lower than the spike value. For LR, the percent difference between the tube kit results and the reference method results ranged from -76% to 21%, with overall average percent difference values ranging from -76% to 17%. The 0.10 ppb samples were determined as being < LOQ so no %D was calculated. One sample was greater than the LOQ for the 0.5 ppb samples with a %D of -76%. For the 1.0 ppb samples, the %D ranged from 5% to 21%, corresponding to a maximum absolute difference from the reference concentration of 0.17 ppb. Similarly, for the 2.0 ppb samples, the %D ranged from 15% to 21% and the maximum absolute difference from the reference concentration was 0.40 ppb. For the 4.0 ppb samples, the %D ranged from -1% to 21% and the maximum absolute difference from the reference concentration was 0.80 ppb.

For the LA spiked samples, the reference method results were approximately 15% to 33% lower than the spike value. For LA, the percent difference between the tube kit results and the reference method results ranged from 450% to 3400%. These %Ds were calculated based on the concentration being corrected for the CR of LA of 2%. The concentrations in LR equivalents more closely tracked the spiked concentrations, suggesting that the CR for LA may have a different value than was provided by Beacon.

For the RR spiked samples, the reference method results were approximately 20% to 45% lower than the spike value. For RR, the percent difference between the tube kit results and the reference method results ranged from 8% to 190%, with overall average percent difference values ranging

from 13% to 168%. For the 0.50 ppb samples, the %D ranged from 36% to 63%, corresponding to an absolute maximum difference from the reference concentration of 0.24 ppb. For the 1.0 ppb samples, the %D ranged from 120% to 190% and the maximum absolute difference from the reference concentration was 1.1 ppb. The reference result for the 1.0 ppb PT sample was 53% of the target concentration; the lowest recovered reference measurement. For the 2.0 ppb samples, the %D ranged from 45% to 110% corresponding to a maximum absolute difference from the reference concentration of 1.7 ppb. For the 4 ppb samples, the %D ranged from 8% to 17%, corresponding to a maximum absolute difference from the reference concentration of 0.50 ppb. For the 7 ppb samples, the %D ranged from 20% to 28%, corresponding to a maximum absolute difference from the reference concentration of 1.2 ppb.

6.3.2 Precision

Precision results for the tube kit are presented in Table 11. The RSD was determined as a percentage according to Equation 7 (Section 5.3) for all DI water, matrix interferent and recreational water samples. The RSDs ranged from 3% to 10% for the LR variant. For the LA variant, the RSDs ranged from 0% to 18% and from 3% to 22% for the RR variant. The precision for the RW sample sets ranged from 2% to 99%, however, all except two samples sets had RSDs less than 14% and within these two sample sets two replicates were very similar to one another and the third replicate was somewhat different, thus causing the high standard deviations.

Table 11. Beacon Tube Kit Precision Results

Variant	Sample Concentration in DI	Precision (%RSD)
LR	0.10 ppb	NA
	0.50 ppb	NA
	1.0 ppb	7%
	2.0 ppb	3%
	4.0 ppb	10%
	4.0 ppb LR in 1 mg/L Chlorophyll-*a* DI	7%
	4.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	5%
	4.0 ppb LR in 10x dilution of RW Matrix	9%
	4.0 ppb LR in RW Matrix	9%
LA	0.50 ppb	10%
	1.0 ppb	13%
	2.0 ppb	4%
	4.0 ppb	3%
	7.0 ppb	4%
	4.0 ppb LA in 1 mg/L Chlorophyll-*a* DI	7%
	4.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	0%
	4.0 ppb LA in 10x dilution of RW Matrix	14%
	4.0 ppb LA in RW Matrix	18%
RR	0.50 ppb	10%
	1.0 ppb	14%
	2.0 ppb	22%
	4.0 ppb	4%
	7.0 ppb	3%
	4.0 ppb RR in 1 mg/L Chlorophyll-*a* DI	7%
	4.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	9%
	4.0 ppb RR in 10x dilution of RW Matrix	17%
	4.0 ppb RR in RW Matrix	17%
Unknown	RW 1	14%
	RW 2	2%
	RW 3	45%
	RW 4	4%
	RW 5	5%
	RW 6	7%
	RW 7	NA
	RW 8	11%
	RW 9	99%

NA - Result was < LOQ so no calculation of RSD

6.3.3 Linearity

The linearity of the tube kit measurements was assessed by performing a linear regression of the tube kit results against the reference method results for the five PT samples ranging from 0.10 ppb to 4.0 ppb of microcystin LR in DI water and four PT samples ranging from 0.50 ppb to 4.0 ppb for microcystin LA and RR in DI water. Figures 3, 4, and 5 present the results of the linear regressions for LR, LA, and RR respectively. The slope, intercept, and coefficient of determination (r^2) for each regression equation are shown on the charts. The linear regressions compared to the reference method results had coefficients of determination of 0.98 for LR, 0.90 for LA and RR.

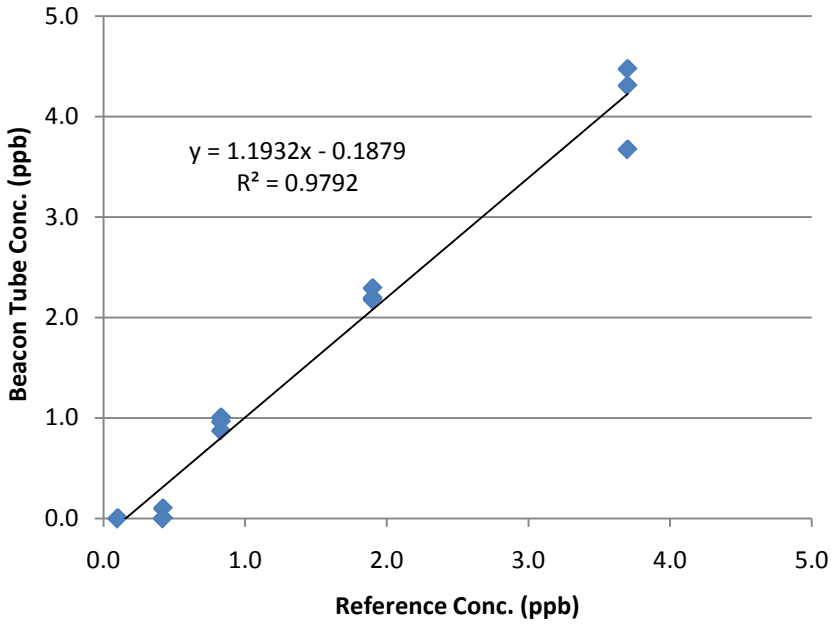

Figure 3. Linearity for the Beacon Tube Kit for LR

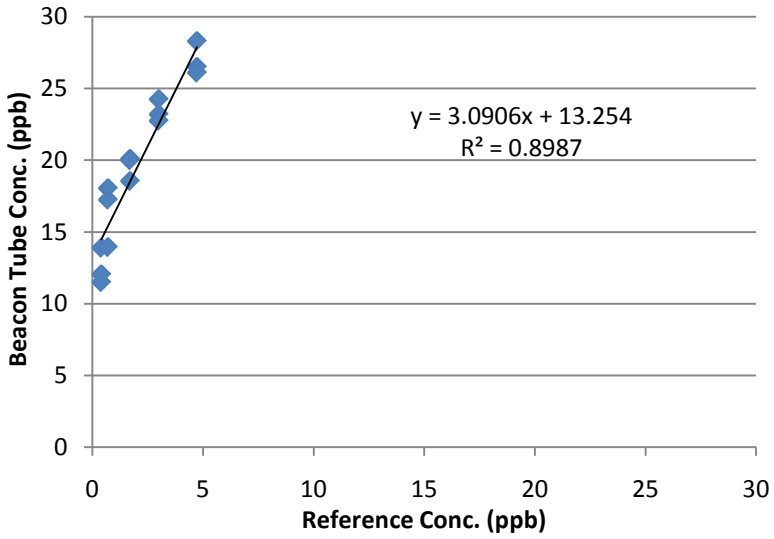

Figure 4. Linearity for the Beacon Tube Kit for LA

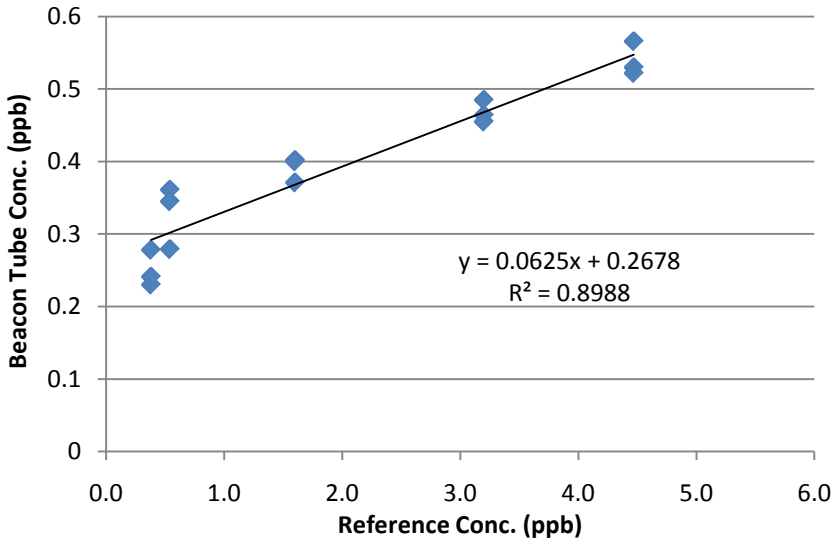

Figure 5. Linearity for the Beacon Tube Kit for RR

6.3.4 Method Detection Limit

The MDL was assessed by analyzing at least seven replicates of a sample spiked at approximately five times the vendor-stated detection limit for the microcystin test kit (which was 0.30 ppb). Table 12 lists the replicate results, the standard deviations for the replicate results, and shows the calculated MDLs for the three variants. The calculated MDL values were 0.18, 0.34, and 0.52 ppb for LR, LA, and RR respectively.

Table 12. Detection Limit Results for the Beacon Tube Kit

Variant	LR	LA	RR
Sample Concentration (ppb)	**Mean Conc. (ppb)**	**Mean Conc. (ppb LR equivalents)**	**Mean Conc. (ppb LR equivalents)**
1.5	1.5	0.27	1.3
1.5	1.6	0.56	1.2
1.5	1.7	0.26	1.4
1.5	1.5	0.21	1.5
1.5	1.6	0.66	1.1
1.5	1.7	0.24	1.9
1.5	1.5	0.28	1.6
Standard Deviation	0.09	0.18	0.27
t (n=7)[5]	1.9	1.9	1.9
MDL	**0.18**	**0.34**	**0.52**

6.3.5 Inter-Kit Lot Reproducibility

Two sets of kit calibration standards were analyzed on the sample plate to compare whether or not the calibration standards from different lots were similar. The data are presented in Table 13. The OD values were compared by calculation of the RPD between each pair of OD measurements. In addition, the RPD for each pair of OD results are shown. All RPDs were less than 14%.

Table 13. Inter-kit lot Comparison of Kit Calibration Standards for the Beacon Tube Kit

	OD Values		
Standard (ppb)	Set A	Set B	RPD
0	1.21	1.11	14%
0.30	0.820	0.735	11%
0.80	0.591	0.658	-11%
2.0	0.407	0.370	10%
5.0	0.275	0.261	5%

6.3.6 Matrix Effect

Matrix interference effects were assessed by using a t-test to compare the tube kit results generated from samples made by spiking undiluted and diluted interference matrices with the PT sample results at the same concentration. The two possible interfering matrices included a RW sample both undiluted and after undergoing a tenfold dilution and addition of chlorophyll-*a* at 10 mg/L and 1.0 mg/L. Tables 14 and 15 provide the tube kit sample results for the RW matrix interference samples and chlorophyll-*a* interference samples, respectively, including the average and SD for each sample. Because this comparison is made to evaluate only the impact of the matrix on the sample result, LR equivalents are used.

Each paired t-test was performed using the replicate data from each type of sample. The null hypothesis is that there is no difference between the two sets of data. The resulting probability (p)-value gives the likelihood of observing a difference as large as is seen in the data, or a larger difference, if the null hypothesis were true. Therefore, at the 95% confidence level, p-values less than 0.05 indicate there is evidence against the null hypothesis being true and therefore a significant difference between the two sets of data.

Table 16 summarizes the results of a paired t-test for both sets of interference data by showing the p-values associated with each of the applicable comparisons across both types of possible interfering matrices. Across both the chlorophyll-*a* and RW results, two out of 18 comparisons resulted in statistically significant differences. The first statistically significant difference was between the diluted RW 4.0 ppb LR spikes and 4.0 ppb DI water with a p-value of 0.016. Table 14 shows that the 4.0 ppb spike into DI water generated an average result of 4.2 ppb compared with an average result of 3.5 ppb. In addition, the 4.0 ppb undiluted RW LR spikes were very close to also being significantly different (p=0.054). The other statistically significant difference was between the LA DI spikes and LA spikes into diluted RW (p=0.041). None of the comparisons of the chlorophyll-*a* samples had significant differences.

There are p-values from 18 tests reported in Table 16 and only two of them is smaller than 0.05. At a significance level of 5%, we would expect one test out of every 20 to have a p-value below 0.05 just by chance, even if the null hypothesis were true in each case. A formal multiple comparisons adjustment is not needed here because a performance standard is not being evaluated as this is more of an exploratory test to determine if there is any difference caused by the matrix. However, a conservative Bonferroni correction, for example, would set the p-value associated with a significant result at 0.05 divided by 18, corresponding to a p-value of 0.0028 for the individual tests.

Table 14. RW Matrix Interferent Sample Results for the Beacon Tube Kit

Variant	Sample Description	Mean Kit Results: LR Equivalents (ppb)	Average Result (ppb)	SD	CR Corrected Conc. By Variant (ppb)
Unknown	Unspiked RW Matrix (RW 9)	0.21	0.48	0.47	
		1.0			
		0.19			
LR	4.0 ppb LR in DI	4.3	4.2	0.42	4.2
		4.5			
		3.7			
	4.0 ppb LR in 10x dilution of RW Matrix	3.6	3.5	0.33	3.6
		3.9			3.9
		3.2			3.2
	4.0 ppb LR in RW Matrix	3.7	3.3	0.31	3.7
		3.2			3.2
		3.1			3.1
LA	4.0 ppb LA in DI	0.46	0.47	0.02	23
		0.46			
		0.49			
	4.0 ppb LA in 10x dilution of RW Matrix	0.66	0.75	0.24	33
		1.0			51
		0.56			28
	4.0 ppb LA in RW Matrix	0.77	0.97	0.18	39
		1.1			56
		1.0			51
RR	4.0 ppb RR in DI	2.7	2.6	0.10	3.6
		2.5			
		2.6			
	4.0 ppb RR in 10x dilution of RW Matrix	4.3	3.8	0.64	5.9
		3.8			5.3
		3.1			4.2
	4.0 ppb RR in RW Matrix	2.7	3.0	0.51	3.7
		3.6			4.9
		2.7			3.8

Table 15. Chlorophyll-*a* Interferent Sample Results for the Beacon Tube Kit

Variant	Sample Description	Mean Kit Results: LR Equivalents (ppb)	Average Result (ppb)	SD	CR Corrected Conc. By Variant (ppb)
LR	4.0 ppb LR in DI	4.3	4.2	0.42	4.2
		4.5			
		3.7			
	4.0 ppb LR in 1.0 mg/L Chlorophyll-*a* DI	3.9	3.9	0.29	3.9
		4.2			4.2
		3.6			3.6
	4.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	3.4	3.6	0.17	3.4
		3.7			3.7
		3.6			3.6
LA	4.0 ppb LA in DI	0.46	0.47	0.02	23
		0.46			
		0.49			
	4.0 ppb LA in 1.0 mg/L Chlorophyll-*a* DI	0.42	0.45	0.03	21
		0.44			22
		0.47			24
	4.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	0.50	0.50	0	25
		0.50			25
		0.50			25
RR	4.0 ppb RR in DI	2.7	2.6	0.10	3.6
		2.5			
		2.6			
	4.0 ppb RR in 1.0 mg/L Chlorophyll-*a* DI	3.3	3.1	0.23	4.6
		3.2			4.4
		2.9			4.0
	4.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	2.8	3.1	0.28	3.8
		3.3			4.5
		3.1			4.3

Table 16. Statistical Comparisons between Interference Samples

Description of Comparison	p-value (D-different, ND-not different) LR	LA	RR
4.0 ppb in DI compared with 4.0 ppb in 10x dilution of RW	0.016 (D)	0.202 (ND)	0.088 (ND)
4.0 ppb in DI compared with 4.0 ppb in undiluted RW	0.054 (ND)	0.041 (D)	0.399 (ND)
4.0 ppb in undiluted RW compared with 10x dilution of RW	0.404 (ND)	0.201 (ND)	0.244 (ND)
4.0 ppb in DI compared with 4.0 ppb in 1.0 mg/L Chlorophyll-*a* DI	0.150 (ND)	0.199 (ND)	0.060 (ND)
4.0 ppb in DI compared with 4.0 ppb in 10 mg/L Chlorophyll-*a* DI	0.168 (ND)	0.078 (ND)	0.194 (ND)
4.0 ppb in 1.0 mg/L Chlorophyll-*a* DI compared with 4.0 ppb in 10 mg/L Chlorophyll-*a* DI	0.184 (ND)	0.086 (ND)	0.772 (ND)

Shading indicates a statistically significant difference

6.4 RW Sample Results

Table 17 presents the RW results for the tube kit and the reference analysis. The concentrations were determined by the reference method for only three of the approximately 80 variants that are naturally occurring in recreational waters. The total microcystins measured by the tube kit may have other variants present that would not have been detected by the reference method. Therefore, no quantitative comparison was made between the tube kit and the reference method results. The reference data were converted into LR-equivalents according to the tube kit cross reactivity for the variants. In general, the samples were in agreement when comparing the tube kit to the reference method. In particular, results from RW 1, RW 3, and RW 6 were within 1 ppb of the reference method result. This indicates that the LR, LA, and RR variants make up a considerable proportion of the microcystins that are measurable by the tube kit.

Table 17. Recreational Water Sample Results for the Beacon Tube Kit

	Test Kit Results					Reference Results (LR Equivalents ppb)			
Sample Description	Kit Results: LR Equivalents (ppb)	Dilution Factor	CR Corrected Conc. (ppb)	Average Conc. (ppb)	Standard Deviation (ppb)	LR	LA	RR	Total
RW 1 (10x dilution)	2.4	10	24	23	3.2	9.6	0.036	14	23
	2.0	10	20						
	2.6	10	26						
RW 2 (10x dilution)	0.49	10	4.9	5.0	0.13	7.2	0	4.6	12
	0.50	10	5.0						
	0.52	10	5.2						
RW 3 (10x dilution)	1.5	10	15	9.6	4.3	7.6	0	1.9	9.6
	0.70	10	7.0						
	0.72	10	7.2						
RW 4	0.62	1	0.62	0.65	0.026	0.0	0.070	0	0.070
	0.67	1	0.67						
	0.66	1	0.66						
RW 5 (4x dilution)	0.10	4	0.42	0.42	0.022	3.1	0.0	0.29	3.3
	0.11	4	0.44						
	0.10	4	0.40						
RW 6	1.6	1	1.6	1.7	0.12	1.8	0	0.19	1.9
	1.6	1	1.6						
	1.9	1	1.9						
RW 6 (2x dilution)	0.86	2	1.7						
	0.90	2	1.8						
	0.82	2	1.6						
RW 7	< LOQ	1	< LOQ	NA	NA	0	0	0	0
	< LOQ	1	< LOQ						
	< LOQ	1	< LOQ						
RW 8	0.57	1	0.57	0.53	0.059	0.27	0.0	0.089	0.36
	0.56	1	0.56						
	0.46	1	0.46						
RW 9 (RW Matrix)	0.21	1	0.21	0.48	0.47	0.18	0	0	0.18
	1.0	1	1.0						
	0.19	1	0.19						

NA - Result was < LOQ so no calculation of Average or Standard Deviation

6.5 Operational Factors

During testing activities, the technical operators were instructed to fill out an Ease of Use Questionnaire. This section summarizes these observations as well as other operational considerations about the technology.

6.5.1 Ease of Use

The test kit operator reported that the tube kit was easy to use. The brochure is clear and easy to follow. Solution and sample preparation are minimal, involving diluting the wash solution or the samples that are above the quantification range. The procedure includes two incubation periods that are 20 minutes each. A spectrophotometer tube reader is necessary for obtaining the spectrophotometric readings that are then analyzed using any commercial ELISA evaluation program (for example, 4-parameters, Logit/Log or alternatively point to point).

6.5.2 Cost and Consumables

According to the vendor, once the analysis is complete, the remaining solutions and tube contents may be flushed down the drain with no hazardous waste being generated for disposal. Since waste disposal requirements vary from state-to-state, the reader is encouraged to consult with the appropriate state government agency for proper waste disposal requirements.

The listed price for the tube kit at the time of the verification test was $200 for a 40 tube kit that will analyze 24 samples. The kit has a 6-month shelf life as received and should be stored at 4 – 8 ^{o}C. Other consumables not included in the kit are pipettes, pipette tips, and distilled or DI water.

Chapter 7
Test Results for the Beacon Microcystin Plate Kit

The following sections provide the results of the quantitative and qualitative evaluations of this verification test for the Beacon microcystin plate kit.

7.1 Beacon Microcystin Plate Kit Summary

As discussed in Chapter 2, the plate kit quantifies total microcystins in water based on an LR calibration. Other variants of microcystins bind differently to the immunosorbent. Therefore, the relative ability for other microcystins to bind has been experimentally determined by the vendor and is published in the vendor literature as the cross reactivity (CR) of the microcystin. For the plate kit, the CR of microcystin LA is 2% and the CR of microcystin RR is 73%. The published CR value was determined using a different source of LA than was used for this study and CR values can vary with microcystin concentration which can impact the quantitative results. In this report, the test kit data have been reported in both test kit results as LR equivalents and in CR corrected results by variant, based on Equation 4.

The plate kit requires that each standard and sample be analyzed in duplicate, and the raw data output from the plate reader software reports a mean concentration of the duplicate analyses. Therefore, a sample indicated in Table 1 would have three replicates that corresponded to six wells being filled as part of the plate kit. Each plate kit plate contains five calibration solutions, including a blank (0 ppb) standard. Following the analysis method, the plate reader measured the absorbance of the wells containing the calibration solutions at 450 nm wavelengths and the calibration curve was generated based on the OD of each well. These results were plotted against concentrations using a 4-parameter curve to quantify the rest of the samples. The results below the calibration curve were reported as < LOQ when the OD value was greater than the lowest standard OD value but less than the negative control sample OD value. A sample was reported as a ND when the OD value was greater than the negative control sample OD value. The coefficient of variation (CV) of the duplicate analyses was reported as a gauge for accurate quantification of microcystins. According to Beacon, the plate was acceptable when the positive control was recovered within 80% and 130% of a 1.0 ppb positive control and the calibration standard %CVs were less than 10%.

7.2 Test Kit QC Sample

As described in Section 3.3.1, the QC samples analyzed with the plate kit included RB samples and the positive control included in the test kit. Ten percent of all samples analyzed were RB samples, and the results were used to verify that no contamination was introduced during sample handling. All RB sample results were < LOQ for the plate kit and are presented in Table 18. Two RB samples were analyzed by the reference method and determined to be < LOQ for all three variants.

Table 18. RB Sample Results for the Beacon Plate Kit

QC Sample ID	Plate	Mean Concentration (ppb)
RB 1	1	< LOQ
RB 2	1	< LOQ
RB 3	1	< LOQ
RB 4	3	< LOQ
RB 5	3	< LOQ
RB 6	3	< LOQ

The positive controls for the plate kit are presented in Table 19. The vendor stated that the acceptable range for recovery of the positive control was between 80% and 130%. At least one positive control was analyzed at the end of each plate, and in some instances when space allowed, there were additional positive controls analyzed. All plate kit plates used for testing produced a positive control result within the acceptable range. In addition, it was required by Beacon, that the %CV for the calibration standards be less than 10% for the calibration standards. During verification testing of the plate kit, all plates passed that acceptance criteria for the calibration standards.

Table 19. Positive Control Sample Results for the Beacon Plate Kit

QC Sample ID	Plate	Mean Concentration (ppb)	CV%	Percent Recovery (%)
1	1	0.98	0.50	98%
2a	2	1.1	8.5	110%
2b	2	1.3	0.50	130%
3	3	1.2	25	120%
4	4	1.1	0.30	110%
5a	5	1.3	10	130%
6	6	0.97	14	97%
6a	6	1.1	11	110%
6b	6	1.3	2.2	130%

7.3 PT Samples

Tables 20, 21, and 22 present the results for the PT samples for the three variants of microcystin used during this verification test. In addition, the tables present the sample concentration corrected for the microcystin cross reactivity, the reference method results and the accuracy results by variant for the PT samples prepared in DI water. All samples were analyzed in triplicate. Table 21 for the LA variant contains twice as many data points as detailed in the test design because the DI PT samples for LA were inadvertently analyzed twice. Also, the 0.50 ppb solutions include the seven replicates from the MDL determination data in addition to the triplicate analyses of these samples.

7.3.1 Accuracy

Tables 20, 21, and 22 present the accuracy results for the plate kit, expressed as percent difference (%D). As shown in Equation 5 (Section 5.1), the reference method value was used for calculation of accuracy. For LR, the reference method was within 10% of the spike concentration. For LA and RR, the reference value was 5-45% lower than the spike concentration depending upon the sample. All data are provided so that the calculation of %D

can be calculated relative to the spike value as well as the reference method if desired by the reader.

Table 20. Beacon Plate Kit Sample Results and Reference Method Results for LR

Sample Description	Kit Results: LR Equivalents (ppb)	CR Corrected Conc. by Variant (ppb)	Accuracy by Variant (% Difference)	Reference Concentration (ppb)
0.1 LR	0.13	0.13	34%	0.10
	0.15	0.15	51%	
	0.18	0.18	81%	
Avg ± SD	0.16 ± 0.02	0.16 ± 0.02	55% ± 24%	
0.5 LR	0.60	0.60	42%	0.42
	0.50	0.50	20%	
	0.49	0.49	16%	
	0.60	0.60	43%	
	0.62	0.62	48%	
	0.72	0.72	72%	
	0.67	0.67	59%	
	0.53	0.53	26%	
	0.49	0.49	18%	
	0.50	0.50	19%	
Avg ± SD	0.57 ± 0.08	0.57 ± 0.08	36% ± 19%	
1.0 LR	1.1	1.1	27%	0.83
	1.2	1.2	47%	
	1.0	1.0	26%	
Avg ± SD	1.1 ± 0.10	1.1 ± 0.10	33% ± 12%	
2.0 LR	2.6	2.6	38%	1.9
	2.3	2.3	21%	
	2.3	2.3	22%	
Avg ± SD	2.4 ± 0.20	2.4 ± 0.20	27% ± 9%	

Table 21. Beacon Plate Kit Sample Results and Reference Method Results for LA

Sample Description	Kit Results:LR Equivalents (ppb)	CR Corrected Conc. By Variant (ppb)	Accuracy by Variant (% Difference)	Reference Concentration (ppb)
0.5 LA	0.18	8.8	2100%	0.40
	0.19	9.3	2200%	
	0.18	9.2	2200%	
	0.19	10	2300%	
	0.17	8.6	2000%	
	0.18	9.0	2100%	
	0.19	9.4	2200%	
	0.19	9.3	2200%	
	0.21	10	2500%	
	0.24	12	2900%	
	0.24	12	2900%	
Avg ± SD	0.20 ± 0.02	9.8 ± 1.2	2300% ± 290%	
1.0 LA	0.21	11	1400%	0.70
	0.21	11	1400%	
	0.29	15	2000%	
	0.30	15	2000%	
	0.31	15	2100%	
	0.27	13	1800%	
Avg ± SD	0.26 ± 0.04	13 ± 2.0	1800% ± 300%	
2.0 LA	0.28	14	720%	1.7
	0.22	11	560%	
	0.33	16	870%	
	0.32	16	840%	
	0.34	17	900%	
	0.36	18	970%	
Avg ± SD	0.31 ± 0.05	16 ± 2.5	810% ± 150%	
4.0 LA	0.38	19	530%	3.0
	0.30	15	410%	
	0.34	17	470%	
	0.46	23	660%	
	0.44	22	640%	
	0.44	22	630%	
Avg ± SD	0.39 ± 0.06	20 ± 3.0	560% ± 100%	
7.0 LA	0.39	20	320%	4.7
	0.42	21	350%	
	0.35	18	270%	
	0.55	28	490%	
	0.52	26	450%	
	0.45	22	370%	
Avg ± SD	0.45 ± 0.08	22 ± 4.0	370% ± 81%	

Table 22. Beacon Plate Kit Sample Results and Reference Method Results for RR

Sample Description	Kit Results: LR Equivalents (ppb)	CR Corrected Conc. By Variant (ppb)	Accuracy by Variant (% Difference)	Reference Concentration (ppb)
0.5 RR	0.42	0.58	51%	0.38
	0.41	0.56	49%	
	0.57	0.78	100%	
	0.71	0.97	160%	
	0.66	0.90	140%	
	0.75	1.0	170%	
	0.64	0.88	130%	
	0.62	0.85	120%	
	0.64	0.87	130%	
	0.57	0.77	100%	
Avg ± SD	0.60 ± 0.11	0.82 ± 0.15	120% ± 40%	
1.0 RR	1.1	1.5	180%	0.54
	1.1	1.4	170%	
	1.2	1.6	190%	
Avg ± SD	1.1 ± 0.10	1.5 ± 0.10	180% ±12%	
2.0 RR	2.0	2.8	74%	1.6
	2.3	3.2	100%	
	1.9	2.5	59%	
Avg ± SD	2.1 ± 0.20	2.8 ± 0.30	77% ± 21%	

For the LR spiked samples, the reference method results ranged from 0% – 17% less than the target concentration. For LR, the percent difference ranged from 16% to 81%, with overall average percent difference values ranging from 27% to 55% between the plate kit and the reference method. For the 0.1 ppb samples, the %D ranged from 34% to 81%, corresponding to an absolute maximum difference from the reference concentration of 0.08 ppb. For the 0.50 ppb samples, the %D ranged from 16% to 72%, but the absolute difference from the reference concentration was no more than 0.30 ppb. For the 1.0 ppb samples, the %D ranged from 26% to 47%, corresponding to a maximum absolute difference from the reference concentration was 0.37 ppb. Similarly, for the 2.0 ppb samples, the %D ranged from 21% to 38% and the maximum absolute difference from the reference concentration was 0.70 ppb. No replicates are given for the 4.0 ppb samples as the samples were above the calibration range of the plate kit. The sample was not diluted because lower concentrations had already been analyzed.

For the LA spiked samples, the reference method results were 15% - 33% less than the target concentration. For LA, the percent difference ranged from 270% to 2900%. These %Ds were calculated based on the concentration being corrected for the CR of LA. The LR equivalents were closer to the spiked concentration, suggesting that the actual CR for LA may have a different value than was published in Beacon's instruction booklet. The published CR value was determined using a different source of LA than was used for this study. Also, CR values can vary with microcystin concentration which may have contributed to the large %Ds observed here. Calculation of a range of concentrations based on a CR determined at a single point of the dose response curve (50% preferential binding of microcystin) used to generate the published CR is not recommended by the vendor.

For the RR spiked samples, the reference method results were 20% – 46% less than the target concentration. For RR, the percent difference ranged from 49% to 181%, with overall average percent difference values ranging from 77% to 180%. For the 0.5 ppb samples, the %D ranged from 49% to 170%, corresponding to an absolute maximum difference from the reference concentration of 0.62 ppb. For the 1 ppb samples, the %D ranged from 168% to 192% and the maximum absolute difference from the reference concentration was 1.1 ppb. The reference result for the 1.0 ppb PT sample was only 53% of the target concentration, the lowest recovered reference measurement. For the 2.0 ppb samples, the %D ranged from 59% to 100% corresponding to a maximum absolute difference from the reference concentration of 1.6 ppb. No replicates are given for the 4.0 and 7.0 ppb samples as the samples were above the calibration range of the plate kit. The sample was not diluted because lower concentrations had already been analyzed.

7.3.2 Precision

Precision results for the plate kit are presented in Table 23. The RSD was determined as a percentage according to Equation 7 (Section 5.3) for all DI water, matrix interferent and recreational water samples. The RSDs ranged from 1% to 15% for the LR variant. For LA, the RSDs ranged from 3% to 16%, and from 4% to18% for the RR variant. The precision for the RW samples sets ranged from 3% to 59%. The highest RSD at 59% is from RW 4; however, all other RW RSDs were below 9%.

Table 23. Beacon Plate Kit Precision Results

Variant	Sample Concentration in DI	Precision (%RSD)
LR	0.10 ppb	15%
	0.50 ppb	14%
	1.0 ppb	9%
	2.0 ppb	7%
	2.0 ppb LR in 1.0 mg/L Chlorophyll-*a* DI	13%
	2.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	1%
	2.0 ppb LR in 10x dilution of RW Matrix	9%
	2.0 ppb LR in RW Matrix	6%
LA	0.50 ppb	12%
	1.0 ppb	16%
	2.0 ppb	16%
	4.0 ppb	15%
	7.0 ppb	16%
	2.0 ppb LA in 1.0 mg/L Chlorophyll-*a* DI	5%
	2.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	10%
	2.0 ppb LA in 10x dilution of RW Matrix	3%
	2.0 ppb LA in RW Matrix	5%
RR	0.50 ppb	18%
	1.0 ppb	4%
	2.0 ppb	12%
	2.0 ppb RR in 1.0 mg/L Chlorophyll-*a* DI	7%
	2.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	9%
	2.0 ppb RR in 10x dilution of RW Matrix	17%
	2.0 ppb RR in RW Matrix	17%
Unknown	RW 1	4%
	RW 2	2%
	RW 3	2%
	RW 4	7%
	RW 4 (4x dilution)	18%
	RW 5	3%
	RW 6	7%
	RW 7	NA
	RW 8	3%
	RW 9	8%

NA - Result was < LOQ so no calculation of RSD

7.3.3 Linearity

The linearity of the plate kit measurements was assessed by performing a linear regression of the plate kit results against the reference method results for the five PT samples ranging from 0.10 ppb to 4.0 ppb of microcystin LR in DI water and four PT samples ranging from 0.50 ppb to 4.0 ppb for microcystin LA and RR in DI water. Figures 6, 7, and 8 present the results of the linear regressions for LR, LA, and RR respectively. The slope, intercept, and coefficient of determination (r^2) for each regression equation are shown on the charts. The linear regressions compared to the reference method results had coefficients of determination of 0.99, 0.76, and 0.91 for LR, LA, and RR respectively.

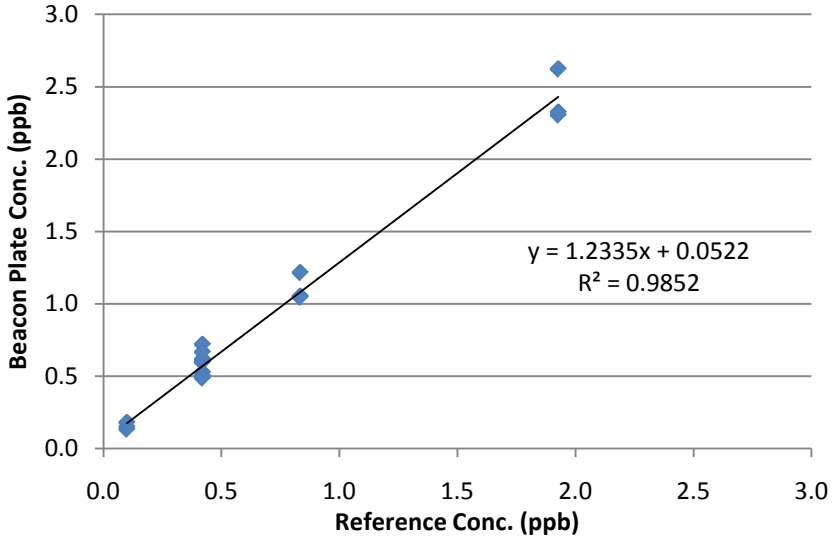

Figure 6. Linearity for the Beacon Plate Kit for LR

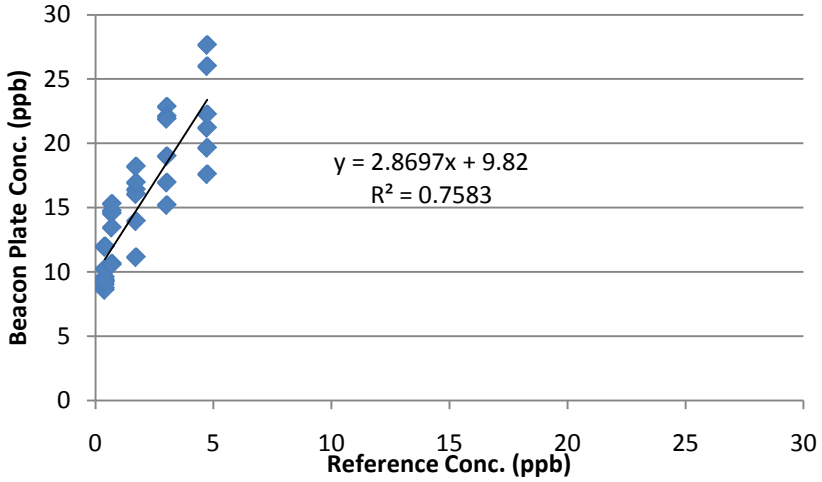

Figure 7. Linearity for the Beacon Plate Kit for LA

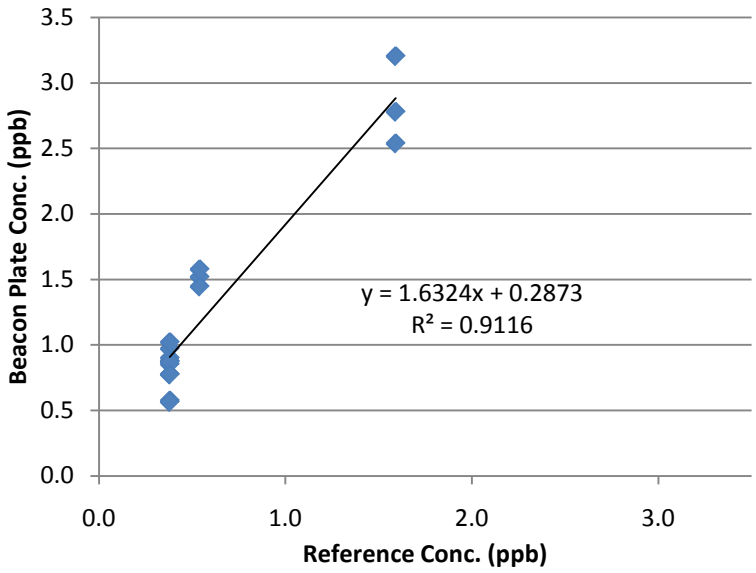

Figure 8. Linearity for the Beacon Plate Kit for RR

7.3.4 Method Detection Limit

The MDL was assessed by analyzing at least seven replicates of a sample spiked at approximately five times the vendor-stated detection limit for the microcystin test kit (which was 0.10 ppb). Table 24 lists the replicate results, the %CV of the duplicate plate kit analysis for each individual replicate, the standard deviations for the replicate results, and shows the calculated MDLs for the three variants. The calculated MDL values were 0.15, 0.04, and 0.20 ppb for LR, LA, and RR respectively.

Table 24. Detection Limit Results for the Beacon Plate Kit

Variant	LR		LA		RR	
Sample Concentration (ppb)	**Mean Conc. (ppb)**	**%CV**	**Mean Conc. (ppb LR Equivalents)**	**%CV**	**Mean Conc. (ppb LR Equivalents)**	**%CV**
0.5	0.60	7.7	0.18	13	0.42	4.9
0.5	0.50	5.7	0.19	10	0.41	2.5
0.5	0.49	3.1	0.18	4.0	0.57	2.7
0.5	0.60	8.4	0.19	3.7	0.71	9.3
0.5	0.62	1.0	0.17	1.0	0.66	14
0.5	0.72	1.1	0.18	7.8	0.75	6.1
0.5	0.67	15	0.19	11	0.64	2.8
0.5	0.53	7.0	0.19	1.4	0.62	16
0.5	0.49	13	0.21	5.7	0.64	11
0.5	0.50	2.4	0.24	14	0.57	5.4
0.5	NA	NA	0.24	1.5	NA	NA
Standard Deviation	0.082		0.024		0.11	
t value	1.8		1.8		1.8	
n	10		11		10	
MDL	**0.15**		**0.043**		**0.20**	

7.3.5 Inter-Kit Lot Reproducibility

Two sets of kit calibration standards were analyzed on the sample plate to compare whether or not the calibration standards from different lots were similar. The OD values were compared by calculation of the RPD between each pair of OD measurements. The RPD for each pair of OD results are shown along with the OD data in Table 25. The RPDs were less than 14%.

Table 25. Inter-kit lot Comparison of Kit Calibration Standards for the Beacon Plate Kit

	OD Values		
Standard	**Set A**	**Set B**	**RPD**
Std 0 ppb	1.35	1.31	3%
	1.43	1.37	4%
Std 0.1	1.15	1.01	13%
	1.13	1.02	10%
Std 0.3 ppb	0.830	0.738	12%
	0.830	0.747	11%
Std 0.8 ppb	0.546	0.527	4%
	0.555	0.495	11%
Std 2.0 ppb	0.398	0.362	9%
	0.390	0.340	14%

7.3.6 Matrix Effects

Matrix interference effects were assessed by using a t-test to compare the plate kit results generated from samples made by spiking undiluted and diluted interference matrices with the PT sample results at the same concentration. The two possible interfering matrices included a RW sample both undiluted and after undergoing a tenfold dilution and addition of chlorophyll-*a* at 10 mg/L and 1.0 mg/L. Tables 26 and 27 give the plate kit sample results for the RW matrix interference samples and chlorophyll-*a* interference samples, respectively, including the average and SD for each sample. Because this comparison is made to evaluate only the impact of the matrix on the sample result, LR equivalents are used.

Each paired t-test was performed using the replicate data from each type of sample. The null hypothesis is that there is no difference between the two sets of data. The resulting probability (p)-value gives the likelihood of observing a difference as large as is seen in the data, or a larger difference, if the null hypothesis were true. Therefore, at the 95% confidence level, p-values less than 0.05 will indicate there is evidence against the null hypothesis being true and therefore a significant difference between the two sets of data.

Table 28 summarizes the results of a paired t-test for both sets of interference data by showing the p-values associated with each of the applicable comparisons across both types of possible interfering matrices. Across both the RW and chlorophyll-*a* results, five out of 18 comparisons resulted in statistically significant differences. The 2.0 ppb LA spike into DI water was significantly different from the 2.0 ppb LA spike into the tenfold diluted RW samples (p=0.006).

Table 26 shows that the 2.0 ppb spike into DI water generated an average result of 0.31 ppb compared with an average result of 0.77 ppb the spike into 1.0 mg/L chlorophyll-*a* samples. The other statistically significant difference with the RW matrix was between the RR spikes into undiluted and diluted RW (p=0.006). These two samples were not significantly different from

the PT sample spike in DI water, but they were different from each other with average concentrations of 0.77 ppb for the diluted RW and 0.35 ppb for the undiluted RW.
The 2.0 ppb LR spike into DI water was significantly different from the 2.0 ppb LR spike into both 10 mg/L (p=0.002) and 1 mg/L (p=0.003) chlorophyll-*a*. Table 27 shows that the 2.0 ppb spike into DI water generated an average result of 2.4 ppb compared with an average result of 0.45 and 0.53 ppb for the spike into 1.0 mg/L and 10 mg/L chlorophyll-*a*, respectively. For LA, the 1.0 mg/L and 10 mg/L chlorophyll-*a* average results were different by 0.01 ppb and when compared to the DI water results, the p-value for the 1.0 mg/L chlorophyll-*a* solution was 0.05 and therefore considered to be significantly different from the DI water spike. The 10 mg/L chlorophyll-*a* results were also very close to being significantly different at the 95% confidence interval (p= 0.066). The 2.0 ppb spike into DI water generated an average result of 0.53 ppb compared with an average result of 0.14 ppb for the spikes into 1.0 mg/L and 10 mg/L chlorophyll.

There are p-values from 18 tests reported in Table 28 and five of them are smaller than 0.05. At a significance level of 5%, one would expect one test out of every 20 to have a p-value below 0.05 by random chance, even if the null hypothesis were true in each case. A formal multiple comparisons adjustment is not needed here because a performance standard is not being evaluated as this is more of an exploratory test to determine if there is any difference caused by the matrix. However, a conservative Bonferroni correction, for example, would set the p-value associated with a significant result at 0.05 divided by 18, corresponding to a p-value of 0.0028 for the individual tests.

Given that the molecular basis on which the test kits operate is well-characterized and understood from the literature[8], Table 27 provided unexpected results. Two variants (LR and LA) demonstrated an interference effect but the third variant (RR) did not. This could have been caused by a number of factors, such as chlorophyll-*a* source and stability and as mentioned in Section 3.3.2, the fact the chlorophyll-*a* was not in solution when analyzed. However, due to the limited number of replicates that were analyzed, additional testing would be required to provide a better understanding as to whether there is matrix interference due to chlorophyll-*a*, or another variable not investigated in this verification testing.

Table 26. RW Matrix Interferent Sample Results for the Beacon Plate Kit

Variant	Sample Description	Mean Kit Results: LR Equivalents (ppb)	Average Result (ppb)	SD	CR Corrected Conc. By Variant (ppb)
Unknown	Unspiked RW Matrix (RW 9)	0.32	0.33	0.02	
		0.36			
		0.31			
LR	2.0 ppb LR in DI	2.6	2.4	0.20	2.4
		2.3			
		2.3			
	2.0 ppb LR in 10x dilution of RW Matrix	2.1	2.2	0.20	2.1
		2.2			2.2
		2.5			2.5
	2.0 ppb LR in RW Matrix	2.2	2.2	0.10	2.2
		2.4			2.4
		2.1			2.1
LA	2.0 ppb LA in DI	0.28	0.31	0.05	15
		0.22			
		0.33			
		0.32			
		0.34			
		0.36			
	2.0 ppb LA in 10x dilution of RW Matrix	0.83	0.77	0.05	41
		0.73			36
		0.74			37
	2.0 ppb LA in RW Matrix	0.36	0.35	0.02	18
		0.37			18
		0.34			17
RR	2.0 ppb RR in DI	2.7	2.6	0.10	3.6
		2.5			
		2.6			
	2.0 ppb RR in 10x dilution of RW Matrix	4.3	3.7	0.60	5.9
		3.8			5.3
		3.1			4.2
	2.0 ppb RR in RW Matrix	2.7	3.0	0.50	3.7
		3.6			4.9
		2.7			3.8

Table 27. Chlorophyll-*a* Interferent Sample Results for the Beacon Plate Kit

Variant	Sample Description	Mean Kit Results: LR Equivalents (ppb)	Average Result (ppb)	SD	CR Corrected Conc. By Variant (ppb)
LR	2.0 ppb LR in DI	2.6	2.4	0.20	2.4
		2.3			
		2.3			
	2.0 ppb LR in 1.0 mg/L Chlorophyll-*a* DI	0.52	0.45	0.06	0.52
		0.53			0.53
		0.37			0.37
		0.42			0.42
		0.43			0.43
		0.45			0.45
	2.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	0.53	0.53	0.01	0.53
		0.53			0.53
		0.52			0.52
LA	2.0 ppb LA in DI	0.28	0.31	0.05	15
		0.22			
		0.33			
		0.32			
		0.34			
		0.36			
	2.0 ppb LA in 1.0 mg/L Chlorophyll-*a* DI	0.14	0.13	0.01	7.2
		0.14			6.8
		0.13			6.7
		0.14			6.9
		0.12			6.2
	2.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	0.15	0.14	0.01	7.7
		0.15			7.4
		0.13			6.4
RR	2.0 ppb RR in DI	2.7	2.6	0.10	3.6
		2.5			
		2.6			
	2.0 ppb RR in 1.0 mg/L Chlorophyll-*a* DI	3.3	3.1	0.20	4.6
		3.2			4.4
		2.9			4.0
	2.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	2.8	3.1	0.30	3.8
		3.3			4.5
		3.1			4.3

Table 28. Statistical Comparisons between Interference Samples

Description of Comparison	p-value (D-different, ND-not different)		
	LR	LA	RR
2.0 ppb in DI compared with 2.0 ppb in 10x dilution of RW	0.470 (ND)	0.006 (D)	0.088 (ND)
2.0 ppb in DI compared with 2.0 ppb in undiluted RW	0.289 (ND)	0.194 (ND)	0.399 (ND)
2.0 ppb in undiluted RW compared with 10x dilution of RW	0.912 (ND)	0.006 (D)	0.244 (ND)
2.0 ppb in DI compared with 2.0 ppb in 1.0 mg/L Chlorophyll-*a* DI	0.002 (D)	0.045 (D)	0.060 (ND)
2.0 ppb in DI compared with 2.0 ppb in 10 mg/L Chlorophyll-*a* DI	0.003 (D)	0.066 (ND)	0.194 (ND)
2.0 ppb in 1.0 mg/L Chlorophyll-*a* DI compared with 2.0 ppb in 10 mg/L Chlorophyll-*a* DI	0.384 (ND)	0.494 (ND)	0.772 (ND)

Shading indicates a statistically significant difference

7.4 RW Sample Results

Table 29 presents the RW results for the plate kit and the reference analysis. The concentrations were determined by the reference method for only three of the approximately 80 variants that are naturally occurring in recreational waters. The total microcystins measured by the plate kit may have other variants present that would not have been detected by the reference method. Therefore, no quantitative comparison was made between the plate kit and the reference method results. The reference data have been converted into LR-equivalents according to the plate kit cross reactivity for the variants. In general, the samples that were determined to have higher total concentrations by the plate kit had higher total concentrations as determined by the reference method. All of the plate kit total microcystin results were greater than the reference method results, which were consistent with the likelihood that all of the microcystins were not being measured by the reference method, which only measured three variants. However, the results of the plate kit were usually within 25% of the reference method, indicating that the LR, LA, and RR variants make up a considerable proportion of the microcystins that are measurable by the plate kit.

Table 29. Recreational Water Sample Results for the Beacon Plate Kit

Sample Description	Test Kit Results: Kit Results: LR Equivalents (ppb)	Dilution Factor	Corrected Conc. (ppb)	Average Conc. (ppb)	Standard Deviation (ppb)	Reference Results (LR-Equivalents ppb): LR	LA	RR	Total
RW 1 (20x dilution)	1.6	20	32	32	1.0	9.6	0.04	14	23
	1.5	20	30						
	1.6	20	32						
	1.6	20	33						
RW 2 (20x dilution)	0.67	20	13	13	0.30	7.2	0	4.6	12
	0.67	20	13						
	0.65	20	13						
RW 3 (20x dilution)	0.67	20	13	13	0.30	7.6	0	1.9	9.6
	0.68	20	14						
	0.65	20	13						
RW 4	0.38	1	0.38	0.35	0.03	0	0.07	0	0.07
	0.35	1	0.35						
	0.33	1	0.33						
RW 4 (4x dilution)	0.23	4	0.92	1.1	0.20	0	0.07	0	0.07
	0.27	4	1.1						
	0.33	4	1.3						
RW 5	1.0	4	4.2	4.3	0.10	3.1	0	0.29	3.3
	1.1	4	4.4						
	1.0	4	4.2						
RW 6 (2x dilution)	1.1	2	2.3	2.4	0.20	1.8	0	0.19	1.9
	1.2	2	2.4						
	1.3	2	2.6						
RW 7	<LOQ	1	<LOQ	<LOQ	NA	0	0	0	0
	<LOQ	1	<LOQ						
	<LOQ	1	<LOQ						
RW 8	0.69	1	0.69	0.67	0.02	0.27	0	0.09	0.36
	0.65	1	0.65						
	0.67	1	0.67						
RW 9 (RW Matrix)	0.32	1	0.32	0.33	0.02	0.18	0	0	0.18
	0.36	1	0.36						
	0.31	1	0.31						

7.5 Operational Factors

During testing activities, the technical operators were instructed to fill out an Ease of Use Questionnaire. This section summarizes these observations as well as other operational considerations about the technology.

7.5.1 Ease of Use

The test kit operator reported that the plate and tube kits were easy to use. The brochure is clear and easy to follow. Sample preparation is minimal, mostly involving diluting the samples that are

above the quantification range. The procedure includes two incubation periods that are 30 minutes each. Previous knowledge or training on the use of micro-pipettes and or multi-channel pipettes with 96-well plates is recommended for consistent readings. The Battelle operator that was trained by the vendor had experience with ELISA kits and pipetting. A spectrophotometer plate reader is necessary for obtaining the spectrophotometric readings that are then analyzed using any commercial ELISA evaluation program (for example, 4-parameters, Logit/Log or alternatively point to point).

7.5.2 Cost and Consumables

According to the vendor, once the analysis is complete, the remaining solutions and tube/plate contents may be flushed down the drain with no hazardous waste being generated for disposal. Since waste disposal requirements vary from state-to-state, the reader is encouraged to consult with the appropriate state government agency for proper waste disposal requirements.

At the time of the verification test, the list price for the plate kit that will analyze 84 samples was $275. According to the vendor, the kits have a 6-month shelf life as received and should be stored at 4 – 8 ^{o}C. Other consumables not included in the kit are pipettes, pipette tips, and distilled or DI water.

Chapter 8
Performance Summary for the Beacon Tube and Plate Test Kits

8.1 Performance Summary for the Beacon Tube Test Kit

The verification of the Beacon tube test kit is summarized by the parameters described in Table 30.

Table 30. Beacon Tube Test Kit Performance Summary

Verification Parameters	LR	LA	RR
Accuracy (ppb, range of %D)			
0.10	< LOQ		
0.50	-76%	450% to 3,400% - LR equivalent values were closer to the spiked values suggesting that the 2% CR for LA may differ from those provided by Beacon.	36% to 63%
1.0	5% to 21%		120% to 190%
2.0	16% to 21%		45% to 110%
4.0	-1% to 21 %		8% to 17%
7.0			20% to 28%
Precision (range of %RSD)	3% to 10%	0% to 18%	3% to 22%
Precision (RW samples)	2% to 99%, all except 2 RSDs were < 14%		
Linearity (y=)	1.2x - 0.14 r^2 = 0.98	3.1x + 13 r^2 = 0.90	0.063x + 0.27 r^2 = 0.90
Method Detection Limit (ppb)	0.18	0.34	0.52

Inter-kit lot reproducibility. Calibration standards from two different lots were measured and the RPD of the resulting optical densities were less than 14%.

Matrix Interference. Matrix interference effects were assessed by using a t-test to compare results from samples made by spiking undiluted and diluted interference matrices with the PT sample results at 2.0 ppb spiked concentration. Across both the chlorophyll-*a* and RW results, two of 18 comparisons resulted in statistically significant differences: 1) the diluted RW, 2.0 ppb LR spikes and 2.0 ppb DI water with a p-value of 0.016; and 2) the other statistically significant difference was between the LA DI spikes and LA spikes into diluted RW (p=0.041). None of the comparisons of the chlorophyll-*a* samples had significant differences.

Recreational Water (RW). Because the reference method did not measure all possible microcystin variants, no quantitative comparison was made between the tube kit and the reference method results. The reference data were converted into LR-equivalents according to the tube kit cross reactivity for the variants. In general, the samples were in agreement when comparing the tube kit to the reference method. In particular, results from RW 1, RW 3, and RW 6 were with 1 ppb of the reference method result. This indicates that the LR, LA, and RR variants make up a significant proportion of the microcystins that are measurable by the tube kit.

Operational Factors. The test kit operator reported that the tube kit was easy to use. Solution or sample preparation is minimal, involving diluting the wash solution or the samples that are above the quantification range. The procedure includes two incubation periods that are 20 minutes each. A spectrophotometer tube reader is necessary for obtaining the spectrophotometric readings that are then analyzed using any commercial ELISA evaluation program (for example, four parameters, Logit/Log or alternatively point to point).

The listed price for the tube kit at the time of the verification test was $200 for a 40 tube kit that will analyze 24 samples. The kit has a 6-month shelf life as received and should be stored at 4 – 8 ^{o}C. Other consumables not included in the kit are pipettes, pipette tips, and distilled or DI water.

8.2 Performance Summary for the Beacon Plate Test Kit

The verification of the Beacon Plate Test Kit is summarized by the parameters described in Table 31.

Table 31. Beacon Plate Test Kit Performance Summary

Verification Parameters	LR	LA	RR
Accuracy (ppb, range of %D)			
0.10	34% to 81%		
0.50	16% to 72%	270% to 2900% The LR equivalent values were closer to the spiked values suggesting that the 2% CR for LA may differ from those provided by Beacon.	49% to 170%
1.0	26% to 47%		170% to 190%
2.0	21% to 38%		59% to 100%
4.0			
7.0			
Precision (range of %RSD)	1% to 15%	3% to 16%	4% to 18%
Precision (RW samples)	All RSD results < 9%, except one at 59%		
Linearity (y=)	1.2x + 0.052 r^2 = 0.99	2.9x + 9.8 r^2 = 0.76	1.6x + 0.29 r^2 = 0.91
Method Detection Limit (ppb)	0.15	0.043	0.20

Inter-kit lot reproducibility. Calibration standards from two different lots were measured and the RPD of the resulting optical densities were all less than 14%.

Matrix Interference. Matrix interference effects were assessed by using a t-test to compare the plate kit results generated from samples made by spiking undiluted and diluted interference matrices with the PT sample results at the same concentration. For chlorophyll-*a* and RW matrices, five comparisons resulted in statistically significant differences: 1) 4 ppb LA spike into DI water was significantly different from the 4 ppb LA spike into the tenfold diluted RW samples (p=0.006); 2) the RW matrix was between the RR spikes into undiluted and diluted RW (p=0.006); 3) 4 ppb LR spike into DI water was significantly different from the 4 ppb LR spike into the 10 mg/L (p=0.002); 4) the 1 mg/L (p=0.003) chlorophyll-*a*; and 5) for LA, the 1 mg/L and 10 mg/L chlorophyll-*a* solutions average results were different by 0.007 ppb and when compared to the DI water results. The 1.0 mg/L chlorophyll-*a* solution results were statistically different (p = 0.045) and the 10 mg/L chlorophyll-*a* results were very close to being significant at the 95% confidence interval (p= 0.066).

Recreational Water (RW). Because the reference method did not measure all possible microcystin variants, no quantitative comparison was made between the plate kit and the reference method results. The reference data were converted into LR-equivalents according to the plate kit cross reactivity for the variants. In general, the samples that were determined to have higher total concentrations by the plate kit had higher total concentrations as determined by the reference method. All of the plate kit total microcystin results were greater than the reference method results, this was consistent with the likelihood that all of the microcystins were not being measured by the reference method, which only measured three variants. However, the results of the plate kit were usually within 25% of the reference method, indicating that the LR, LA, and RR variants make up a significant proportion of the microcystins that are measurable by the plate kit.

Operational Factors. The test kit operator reported that the plate kit was easy to use. Solution or sample preparation is minimal, mostly involving diluting the wash solution or the samples that are above the quantification range. The procedure includes two incubation periods that are 30 minutes each. Previous knowledge or training on the use of micro-pipettes and or multi-channel pipettes with 96-well plates is recommended for consistent readings. A spectrophotometer plate reader is necessary for obtaining the spectrophotometric readings that are then analyzed using any commercial ELISA evaluation program (for example, four parameters, Logit/Log or alternatively point to point).

At the time of the verification test, the list price for the plate kit that will analyze 84 samples was $275. According to the vendor, the kits have a 6-month shelf life as received and should be stored at 4 – 8 ^{o}C. Other consumables not included in the kit are pipettes, pipette tips, and distilled or DI water.

Chapter 9
References

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5. *"Guidelines Establishing Test Procedures for the Analysis of Pollutants."*, USEPA, Editor. 2000, U.S. Code of Federal Regulations.
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7. 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.

APPENDIX A
Reference Laboratory Method Detection Limit Memo

July 14, 2010

To: Anne Gregg and Ryan James, Battelle Laboratories

From: Daniel Snow and David Cassada, UNL Water Sciences Laboratory

Re: Summary of Microcystin SPE method validation – July 13-14, 2010

Microcystins LA, LR and RR were spiked into water and extracted using solid phase extraction (SPE) to evaluate method accuracy and precision, and method detection limits. The method described in Cong et al. 2006 was modified to allow for extraction of a larger sample by using higher capacity polymeric (Waters Oasis, HLB) SPE cartridges. Briefly, 400-milliliter (mL) of purified reagent water was fortified with 1500 µL of a diluted mixed stock (0.1 ng/µL) obtained from Battelle to produce 0.375 µg/L of each analyte. Nodularin (1600 µL of a 0.1 ng/µL solution) was also added to produce a concentration of 0.40 µg/L . Eight 50 mL portions of this fortified water were weighed into 125 mL amber glass bottles and each portion separately spiked with 100 µL of the enkephalin-Leu internal standard (IS) solution (0.1 ng/µL) to give a concentration of 2.0 µg/L. A single method blank was prepared by spiking with IS and surrogate only.

After capping and shaking each solution to equilibrate, samples were drawn under vacuum through pre-conditioned 200 mg Oasis HLB SPE cartridges at a rate of approximately 10 mL/min. When the sample had completely passed through the cartridge, it was allowed to air-dry under vacuum, removed from the extraction apparatus and prepared for elution. Ten (10) milliliters of high purity methanol (Fisher Optima Grade) were used to elute analyte, IS and surrogate compounds from the cartridges. The methanol was evaporated under nitrogen to approximately 0.4 mL and the extracts transferred to low volume inserts for analysis on the LCQ ion trap tandem LC/MS system. Calibration solutions (5, 10, 30, 60 and 75 ng/mL) were prepared in water from the same mixed stock as the spiking solutions. A table summarizing the results of the validation is copied below (Table A-1.).

A second 10-mL aliquot of methanol was passed through 4 of SPE cartridges and collected separately to check for completeness of analyte elution. These second aliquots were blown down to the same 0.4 mL volume as the MDLs eluants and analyzed. The resulting absolute areas of the analyte, surrogate, and internal standard peaks obtained were approximately 1% of the areas obtained in the first portion. This suggests that lower elution volumes can result in decreased analyte recovery.

References

Cong, L.; Huang, B.; Chen, Q.; Lu, B.; Zhang, J.; Ren, Y. (2006) Determination of trace amount of microcystins in water samples using liquid chromatography coupled with triple quadrupole mass spectrometry. *Anal. Chim. Acta*, **569** (1-2), 157-168.

Table A-1. Average, standard deviation, method detection limits (MDL = S x tN-1) and recoveries of microcystins obtained from extraction and analysis of 8 fortified reagent water (0.375 µg/L) samples.

50 mL sample Aliquot	Amount obtained (ng)				Concentration (µg/L)			
	Nodularin	**MC-RR**	**MC-LR**	**MC-LA**	**Nodularin**	**MC-RR**	**MC-LR**	**MC-LA**
MDL 1	23.994	19.679	18.084	19.913	0.480	0.394	0.362	0.398
MDL 2	24.647	19.661	21.985	21.752	0.493	0.393	0.440	0.435
MDL 3	22.716	17.660	20.524	18.404	0.454	0.353	0.410	0.368
MDL 4	23.157	19.715	21.022	20.304	0.463	0.394	0.420	0.406
MDL 5	26.361	19.731	20.462	21.182	0.527	0.395	0.409	0.424
MDL 6	19.618	18.214	18.322	18.393	0.392	0.364	0.366	0.368
MDL 7	20.254	14.533	20.046	21.490	0.405	0.291	0.401	0.430
MDL 8	19.889	15.247	17.518	14.614	0.398	0.305	0.350	0.292
AVG	22.580	18.055	19.745	19.507	0.452	0.361	0.395	0.390
STD DEV	2.460	2.113	1.586	2.360	0.049	0.042	0.032	0.047
MDL	**7.371**	**6.333**	**4.753**	**7.072**	**0.147**	**0.127**	**0.095**	**0.141**
%REC	**112.9**	**96.3**	**105.3**	**104.0**	**112.9**	**96.3**	**105.3**	**104.0**
Expected value	20.0	18.75	18.75	18.75	0.4	0.375	0.375	0.375

APPENDIX B
Beacon Test Kit Raw Data

Table B-1. Beacon Tube Kit Raw Data

Sample Description	Variant	OD Value	Conc. (ppb)
Reagent Blank	RB	1.245	Range?
Reagent Blank	RB	1.253	Range?
Reagent Blank	RB	1.131	0.061
Reagent Blank	RB	1.172	Range?
Reagent Blank	RB	1.162	Range?
Reagent Blank	RB	1.163	Range?
Positive Control 1	LR	0.503	0.908
Positive Control 10	LR	0.589	1.059
Positive control 11	LR	0.526	1.102
Positive control 12	LR	0.54	0.984
Positive Control 3	LR	0.568	0.941
Positive Control 4	LR	0.546	0.982
Positive Control 5	LR	0.524	0.983
Positive Control 6	LR	0.474	1.046
Positive Control 7	LR	0.483	0.909
Positive Control 8	LR	0.509	0.821
Positive Control 9	LR	0.319	1.118
Negative Diff Lot	LR	1.053	0.076
Std 0.3ppb Diff Lot	LR	0.735	0.438
Std 0.8 ppb Diff Lot	LR	0.658	0.608
Std 2.0 ppb Diff Lot	LR	0.37	2.451
Std 5.0 ppb Diff Lot	LR	0.261	5.82
0.1 LR	LR	1.122	0.051
0.1 LR	LR	1.125	0.05
0.1 LR	LR	1.066	0.081
0.5 LA	LA	0.964	0.23
0.5 LA	LA	0.916	0.278
0.5 LA	LA	0.952	0.241
0.5 LR	LR	1.069	0.066
0.5 LR	LR	1.018	0.1
0.5 LR	LR	1.037	0.087
0.5 RR	RR	0.76	0.376
0.5 RR	RR	0.753	0.389
0.5 RR	RR	0.72	0.452
1.0 LA	LA	0.915	0.279
1.0 LA	LA	0.845	0.361
1.0 LA	LA	0.858	0.345

Table B-1. Beacon Tube Kit Raw Data Continued			
Sample Description	**Variant**	**OD Value**	**Conc. (ppb)**
1.0 LR	LR	0.48	1.006
1.0 LR	LR	0.512	0.873
1.0 LR	LR	0.49	0.962
1.0 RR	RR	0.511	1.154
1.0 RR	RR	0.57	0.882
1.0 RR	RR	0.515	1.133
1.5 LA	LA	0.832	0.266
1.5 LA	LA	0.674	0.556
1.5 LA	LA	0.834	0.263
1.5 LA	LA	0.879	0.207
1.5 LA	LA	0.637	0.655
1.5 LA	LA	0.852	0.239
1.5 LA	LA	0.824	0.276
1.5 LR	LR	0.455	1.522
1.5 LR	LR	0.446	1.594
1.5 LR	LR	0.429	1.743
1.5 LR	LR	0.458	1.499
1.5 LR	LR	0.451	1.553
1.5 LR	LR	0.435	1.688
1.5 LR	LR	0.456	1.514
1.5 RR	RR	0.435	1.256
1.5 RR	RR	0.443	1.208
1.5 RR	RR	0.419	1.365
1.5 RR	RR	0.407	1.457
1.5 RR	RR	0.462	1.104
1.5 RR	RR	0.386	1.891
1.5 RR	RR	0.414	1.615
2.0 LA	LA	0.814	0.402
2.0 LA	LA	0.816	0.4
2.0 LA	LA	0.837	0.371
2.0 LR	LR	0.341	2.178
2.0 LR	LR	0.34	2.194
2.0 LR	LR	0.334	2.292
2.0 RR	RR	0.403	1.715
2.0 RR	RR	0.405	1.696
2.0 RR	RR	0.348	2.432
4.0 LA	LA	0.772	0.464
4.0 LA	LA	0.778	0.455
4.0 LA	LA	0.759	0.485
4.0 LR	LR	0.267	4.308
4.0 LR	LR	0.264	4.474
4.0 LR	LR	0.323	3.672

Table B-1. Beacon Tube Kit Raw Data Continued			
Sample Description	**Variant**	**OD Value**	**Conc. (ppb)**
4.0 RR	RR	0.333	2.733
4.0 RR	RR	0.343	2.525
4.0 RR	RR	0.337	2.646
7.0 LA	RR	0.688	0.522
7.0 LA	LA	0.733	0.53
7.0 LA	LA	0.713	0.566
7.0 RR	RR	0.296	3.913
7.0 RR	RR	0.316	3.972
7.0 RR	RR	0.312	4.167
4ppb Chloro 10x LA	LA	0.696	0.416
4ppb Chloro 10x LA	LA	0.677	0.444
4ppb Chloro 10x LA	LA	0.658	0.474
4ppb Chloro 10x RR	RR	0.279	3.346
4ppb Chloro 10x RR	RR	0.283	3.194
4ppb Chloro 10x RR	RR	0.292	2.897
4ppb Chloro LA	LA	0.673	0.498
4ppb Chloro LA	LA	0.673	0.498
4ppb Chloro LA	LA	0.673	0.498
4ppb Chloro LR	LR	0.301	3.361
4ppb Chloro LR	LR	0.294	3.673
4ppb Chloro LR	LR	0.324	3.632
4ppb Chloro LR 10x	LR	0.289	3.934
4ppb Chloro LR 10x	LR	0.311	4.219
4ppb Chloro LR 10x	LR	0.324	3.632
4ppb Chloro RR	RR	0.297	2.754
4ppb Chloro RR	RR	0.28	3.307
4ppb Chloro RR	RR	0.285	3.122
4ppb Matrix 10x LA	LA	0.57	0.661
4ppb Matrix 10x LA	LA	0.454	1.019
4ppb Matrix 10x LA	LA	0.622	0.555
4ppb Matrix 10x LR	LR	0.326	3.556
4ppb Matrix 10x LR	LR	0.268	3.854
4ppb Matrix 10x LR	LR	0.283	3.194
4ppb Matrix 10x RR	RR	0.251	4.34
4ppb Matrix 10x RR	RR	0.319	3.838
4ppb Matrix 10x RR	RR	0.341	3.071
4ppb Matrix LA	LA	0.526	0.771
4ppb Matrix LA	LA	0.432	1.119
4ppb Matrix LA	LA	0.454	1.019
4ppb Matrix LR	LR	0.272	3.652
4ppb Matrix LR	LR	0.282	3.23
4ppb Matrix LR	LR	0.287	3.054

Table B-1. Beacon Tube Kit Raw Data Continued			
Sample Description	**Variant**	**OD Value**	**Conc. (ppb)**
4ppb Matrix RR	RR	0.29	2.687
4ppb Matrix RR	RR	0.264	3.589
4ppb Matrix RR	RR	0.354	2.742
RW1 (10x dil)	Unknown	0.44	2.372
RW1 (10x dil)	Unknown	0.471	1.981
RW1 (10x dil)	Unknown	0.424	2.613
RW2 (10x dil)	Unknown	0.73	0.49
RW2 (10x dil)	Unknown	0.723	0.503
RW2 (10x dil)	Unknown	0.717	0.515
RW3 (10x dil)	Unknown	0.528	1.45
RW3 (10x dil)	Unknown	0.637	0.7
RW3 (10x dil)	Unknown	0.631	0.717
RW4	Unknown	0.457	0.62
RW4	Unknown	0.436	0.668
RW4	Unknown	0.439	0.66
RW 5 (4x dil)	Unknown	1.049	0.104
RW 5 (4x dil)	Unknown	1.041	0.111
RW 5 (4x dil)	Unknown	1.054	0.1
RW6	Unknown	0.45	1.576
RW6	Unknown	0.45	1.576
RW6	Unknown	0.418	1.873
RW6 (2x dil)	Unknown	0.584	0.864
RW6 (2x dil)	Unknown	0.573	0.903
RW6 (2x dil)	Unknown	0.598	0.816
RW7	Unknown	1.176	0.015
RW7	Unknown	1.198	0.001
RW7	Unknown	1.185	0.009
RW8	Unknown	0.691	0.569
RW8	Unknown	0.696	0.558
RW8	Unknown	0.745	0.462
RW9 Matrix	Unknown	0.910	0.213
RW9 Matrix	Unknown	0.596	1.022
RW9 Matrix	Unknown	0.928	0.193

Table B-2. Beacon Plate Kit Raw Data

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
Reagent Blank	RB	0.033	0.008	23.3
Reagent Blank	RB	0.032	0.007	22.4
Reagent Blank	RB	0.054	0.007	13.2
Reagent Blank	RB	0.025	0.007	29.4
Reagent Blank	RB	0.035	0.018	52
Reagent Blank	RB	0.054	0.007	13.2
Positive Control 1	LR	0.98	0.004	0.5
Positive Control 2a	LR	1.123	0.095	8.5
Positive Control 2b	LR	1.267	0.006	0.5
Positive Control 4	LR	1.069	0.003	0.3
Positive control 5a	LR	1.268	0.128	10.1
Positive control 5b	LR	1.336	0.297	22.2
Positive Control 6	LR	0.965	0.134	13.9
Positive Control 6	LR	1.064	0.113	10.6
Positive Control 6	LR	1.281	0.028	2.2
Std 0 Diff Lot	LR	0.02	0.017	85.1
Std 0.1 Diff Lot	LR	0.164	0.005	2.9
Std 0.3 Diff Lot	LR	0.397	0.008	2.1
Std 0.8 Diff Lot	LR	0.966	0.105	10.9
Std 2.0 Diff Lot	LR	3.16	0.612	19.4
0.1 LR	LR	0.134	0.001	0.8
0.1 LR	LR	0.151	0.018	11.9
0.1 LR	LR	0.181	0.027	14.9
0.5 LA	LA	0.175	0.023	13.3
0.5 LA	LA	0.186	0.019	10.1
0.5 LA	LA	0.184	0.007	4
0.5 LA	LA	0.192	0.007	3.7
0.5 LA	LA	0.172	0.002	1
0.5 LA	LA	0.18	0.014	7.8
0.5 LA	LA	0.188	0.021	11
0.5 LA	LA	0.185	0.003	1.4
0.5 LA	LA	0.205	0.012	5.7
0.5 LA	LA	0.24	0.034	14.1
0.5 LA	LA	0.239	0.004	1.5
0.5 LR	LR	0.597	0.046	7.7
0.5 LR	LR	0.503	0.029	5.7
0.5 LR	LR	0.488	0.015	3.1
0.5 LR	LR	0.602	0.05	8.4
0.5 LR	LR	0.62	0.006	1
0.5 LR	LR	0.721	0.008	1.1
0.5 LR	LR	0.668	0.102	15.3
0.5 LR	LR	0.528	0.037	7
0.5 LR	LR	0.494	0.063	12.8
0.5 LR	LR	0.501	0.012	2.4
0.5 RR	RR	0.42	0.02	4.9
0.5 RR	RR	0.412	0.01	2.5
0.5 RR	RR	0.566	0.015	2.7
0.5 RR	RR	0.709	0.066	9.3
0.5 RR	RR	0.657	0.094	14.3

Table B-2. Beacon Plate Kit Raw Data Continued

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
0.5 RR	RR	0.745	0.046	6.1
0.5 RR	RR	0.639	0.018	2.8
0.5 RR	RR	0.622	0.1	16
0.5 RR	RR	0.636	0.072	11.3
0.5 RR	RR	0.565	0.031	5.4
1.0 LA	LA	0.213	0.011	5.3
1.0 LA	LA	0.211	0.034	16.3
1.0 LA	LA	0.291	0.047	16.3
1.0 LA	LA	0.295	0.035	11.7
1.0 LA	LA	0.306	0.066	21.5
1.0 LA	LA	0.269	0.022	8.1
1.0 LR	LR	1.054	0.04	3.8
1.0 LR	LR	1.217	0.237	19.5
1.0 LR	LR	1.046	0.092	8.8
1.0 RR	RR	1.108	0.016	1.4
1.0 RR	RR	1.055	0.011	1.1
1.0 RR	RR	1.151	0.246	21.4
2.0 LA	LA	0.279	0.012	4.5
2.0 LA	LA	0.223	0.047	21.3
2.0 LA	LA	0.328	0.001	0.3
2.0 LA	LA	0.321	0.037	11.7
2.0 LA	LA	0.339	0.001	0.4
2.0 LA	LA	0.364	0.082	22.6
2.0 LR	LR	1.312	0.01	0.7
2.0 LR	LR	1.153	0.206	17.9
2.0 LR	LR	1.163	0.08	6.9
2.0 RR	RR	1.014	0.009	0.9
2.0 RR	RR	1.169	0.068	5.8
2.0 RR	RR	0.926	0.067	7.2
4.0 LA	LA	0.38	0.06	15.8
4.0 LA	LA	0.304	0.002	0.7
4.0 LA	LA	0.339	0.018	5.4
4.0 LA	LA	0.457	0.005	1
4.0 LA	LA	0.442	0.005	1.1
4.0 LA	LA	0.438	0.014	3.2
4.0 LR	LR	125.744	0	0
4.0 LR	LR	Range?	Range?	Range?
4.0 LR	LR	Range?	Range?	Range?
4.0 RR	RR	9.029	0	0
4.0 RR	RR	170.126	0	0
4.0 RR	RR	Range?	Range?	Range?
7.0 LA	LA	0.393	0.074	18.9
7.0 LA	LA	0.424	0.019	4.5
7.0 LA	LA	0.352	0.083	23.5
7.0 LA	LA	0.553	0.007	1.3
7.0 LA	LA	0.52	0.032	6.2
7.0 LA	LA	0.445	0.007	1.5
0.1 LR	LR	0.134	0.001	0.8

Table B-2. Beacon Plate Kit Raw Data

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
0.1 LR	LR	0.151	0.018	11.9
0.1 LR	LR	0.181	0.027	14.9
0.5 LA	LA	0.175	0.023	13.3
0.5 LA	LA	0.186	0.019	10.1
0.5 LA	LA	0.184	0.007	4
0.5 LA	LA	0.192	0.007	3.7
0.5 LA	LA	0.172	0.002	1
0.5 LA	LA	0.18	0.014	7.8
0.5 LA	LA	0.188	0.021	11
0.5 LA	LA	0.185	0.003	1.4
0.5 LA	LA	0.205	0.012	5.7
0.5 LA	LA	0.24	0.034	14.1
0.5 LA	LA	0.239	0.004	1.5
0.5 LR	LR	0.597	0.046	7.7
0.5 LR	LR	0.503	0.029	5.7
0.5 LR	LR	0.488	0.015	3.1
0.5 LR	LR	0.602	0.05	8.4
0.5 LR	LR	0.62	0.006	1
0.5 LR	LR	0.721	0.008	1.1
0.5 LR	LR	0.668	0.102	15.3
0.5 LR	LR	0.528	0.037	7
0.5 LR	LR	0.494	0.063	12.8
0.5 LR	LR	0.501	0.012	2.4
0.5 RR	RR	0.42	0.02	4.9
0.5 RR	RR	0.412	0.01	2.5
0.5 RR	RR	0.566	0.015	2.7
0.5 RR	RR	0.709	0.066	9.3
0.5 RR	RR	0.657	0.094	14.3
0.5 RR	RR	0.745	0.046	6.1
0.5 RR	RR	0.639	0.018	2.8
0.5 RR	RR	0.622	0.1	16
0.5 RR	RR	0.636	0.072	11.3
0.5 RR	RR	0.565	0.031	5.4
1.0 LA	LA	0.213	0.011	5.3
1.0 LA	LA	0.211	0.034	16.3
1.0 LA	LA	0.291	0.047	16.3
1.0 LA	LA	0.295	0.035	11.7
1.0 LA	LA	0.306	0.066	21.5
1.0 LA	LA	0.269	0.022	8.1
1.0 LR	LR	1.054	0.04	3.8
1.0 LR	LR	1.217	0.237	19.5
1.0 LR	LR	1.046	0.092	8.8
1.0 RR	RR	1.108	0.016	1.4
1.0 RR	RR	1.055	0.011	1.1
1.0 RR	RR	1.151	0.246	21.4
2.0 LA	LA	0.279	0.012	4.5

Table B-2. Beacon Plate Kit Raw Data Continued

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
2.0 LA	LA	0.223	0.047	21.3
2.0 LA	LA	0.328	0.001	0.3
2.0 LA	LA	0.321	0.037	11.7
2.0 LA	LA	0.339	0.001	0.4
2.0 LA	LA	0.364	0.082	22.6
2.0 LR	LR	1.312	0.01	0.7
2.0 LR	LR	1.153	0.206	17.9
2.0 LR	LR	1.163	0.08	6.9
2.0 RR	RR	1.014	0.009	0.9
2.0 RR	RR	1.169	0.068	5.8
2.0 RR	RR	0.926	0.067	7.2
4.0 LA	LA	0.38	0.06	15.8
4.0 LA	LA	0.304	0.002	0.7
4.0 LA	LA	0.339	0.018	5.4
4.0 LA	LA	0.457	0.005	1
4.0 LA	LA	0.442	0.005	1.1
4.0 LA	LA	0.438	0.014	3.2
7.0 LA	LA	0.393	0.074	18.9
7.0 LA	LA	0.424	0.019	4.5
7.0 LA	LA	0.352	0.083	23.5
7.0 LA	LA	0.553	0.007	1.3
7.0 LA	LA	0.52	0.032	6.2
7.0 LA	LA	0.445	0.007	1.5
7.0 LA	LA	0.393	0.074	18.9
7.0 LA	LA	0.424	0.019	4.5
7.0 LA	LA	0.352	0.083	23.5
7.0 LA	LA	0.553	0.007	1.3
7.0 LA	LA	0.52	0.032	6.2
7.0 LA	LA	0.445	0.007	1.5
2.0 LA Chloro	LA	0.153	0.001	0.5
2.0 LA Chloro	LA	0.147	0.029	19.7
2.0 LA Chloro	LA	0.127	0	0.3
2.0 LA Chloro 10X	LA	0.143	0.012	8.4
2.0 LA Chloro 10X	LA	0.135	0.007	5.1
2.0 LA Chloro 10X	LA	0.134	0.001	0.6
2.0 LA Chloro 10X	LA	0.138	0.012	8.8
2.0 LA Chloro 10X	LA	0.124	0.004	3.6
2.0 LA Matrix	LA	0.357	0.013	3.7
2.0 LA Matrix	LA	0.368	0.008	2.2
2.0 LA Matrix	LA	0.335	0.022	6.6
2.0 LA Matrix 10x	LA	0.827	0.069	8.3
2.0 LA Matrix 10x	LA	0.729	0.079	10.9
2.0 LA Matrix 10x	LA	0.742	0.165	22.3
2.0 LR Chloro	LR	0.53	0.051	9.5
2.0 LR Chloro	LR	0.531	0.015	2.8
2.0 LR Chloro	LR	0.52	0.02	3.9
2.0 LR Chloro 10x	LR	0.523	0.007	1.3
2.0 LR Chloro 10x	LR	0.528	0.063	12

Table B-2. Beacon Plate Kit Raw Data Continued

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
2.0 LR Chloro 10x	LR	0.373	0.022	5.8
2.0 LR Chloro 10x	LR	0.424	0.001	0.2
2.0 LR Chloro 10x	LR	0.428	0.01	2.3
2.0 LR Chloro 10x	LR	0.449	0.01	2.2
2.0 LR Matrix	LR	2.196	0.146	6.7
2.0 LR Matrix	LR	2.355	0.177	7.5
2.0 LR Matrix	LR	2.113	0.037	1.7
2.0 LR Matrix 10x	LR	2.093	0.034	1.6
2.0 LR Matrix 10x	LR	2.156	0.066	3
2.0 LR Matrix 10x	LR	2.481	0.198	8
2.0 RR Chloro	RR	1.957	0.062	3.2
2.0 RR Chloro	RR	2.072	0.246	11.9
2.0 RR Chloro	RR	1.943	0.014	0.7
2.0 RR Chloro 10x	RR	1.913	0.26	13.6
2.0 RR Chloro 10x	RR	1.932	0.08	4.1
2.0 RR Chloro 10x	RR	1.943	0.244	12.6
2.0 RR Matrix	RR	2.267	0.179	7.9
2.0 RR Matrix	RR	2.428	0.14	5.8
2.0 RR Matrix	RR	2.122	0.126	5.9
2.0 RR Matrix 10x	RR	2.018	0.043	2.1
2.0 RR Matrix 10x	RR	2.22	0.055	2.5
2.0 RR Matrix 10x	RR	2.176	0.413	19
RW1 (20x dil)	Unknown	1.59	0.243	15.3
RW1 (20x dil)	Unknown	1.489	0.139	9.3
RW1 (20x dil)	Unknown	1.594	0.338	21.2
RW1 (20x dil)	Unknown	1.639	0.192	11.7
RW2 (20x dil)	Unknown	0.669	0.066	9.8
RW2 (20x dil)	Unknown	0.67	0.004	0.6
RW2 (20x dil)	Unknown	0.647	0.021	3.3
RW3 (20x dil)	Unknown	0.674	0.012	1.8
RW3 (20x dil)	Unknown	0.681	0.028	4.2
RW3 (20x dil)	Unknown	0.653	0.043	6.6
RW 4	Unknown	0.376	0.019	5
RW 4	Unknown	0.35	0.011	3.1
RW 4	Unknown	0.327	0.017	5.2
RW 4 (4x dil)	Unknown	0.231	0.026	11.4
RW 4 (4x dil)	Unknown	0.269	0.002	0.6
RW 4 (4x dil)	Unknown	0.329	0.058	17.5
RW 5 (4x dil)	Unknown	1.042	0.031	3
RW 5 (4x dil)	Unknown	1.106	0.046	4.1
RW 5 (4x dil)	Unknown	1.043	0.095	9.1
RW6 (2x dil)	Unknown	1.144	0.078	6.8
RW6 (2x dil)	Unknown	1.179	0.011	1
RW6 (2x dil)	Unknown	1.304	0.145	11.1
RW7	Unknown	0.053	0.001	2.4
RW7	Unknown	0.059	0.005	8.1
RW7	Unknown	0.06	0.008	14
RW8	Unknown	0.686	0	0

Table B-2. Beacon Plate Kit Raw Data Continued

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
RW8	Unknown	0.646	0.069	10.7
RW8	Unknown	0.666	0.133	20
RW 9 Matrix	Unknown	0.323	0	0.1
RW 9 Matrix	Unknown	0.358	0.002	0.4
RW 9 Matrix	Unknown	0.31	0.025	8.2