September 2010

Environmental Technology Verification Report

ABRAXIS
MICROCYSTIN TEST KITS:
ADDA ELISA TEST KIT
DM ELISA TEST KIT
STRIP TEST KIT

Prepared by
Battelle

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EPA **U.S. Environmental Protection Agency**

ETV ETV ETV

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ETV Advanced Monitoring Systems Center

ABRAXIS MICROCYSTIN TEST KITS: ADDA ELISA TEST KIT DM ELISA TEST KIT STRIP TEST KIT

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Notice

The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for publication. Any opinions expressed in this report are those of the author(s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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

ADQ	audit of data quality
AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
CCV	continuing calibration verification
CR	cross reactivity
CV	coefficient of variation
DI	deionized
DQI	data quality indicator
DQO	data quality objective
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ETV	Environmental Technology Verification
HPLC	high pressure liquid chromatography
HRP	Horseradish Peroxidase
LC-MS-MS	liquid chromatography tandem mass spectrometry
LFM	laboratory fortified matrix
LOQ	limit of quantification
MDL	method detection limit
mg/L	milligram per liter
mL	milliliter
NDEQ	Nebraska Department of Environmental Quality
nm	nanometer
NRC	National Research Council
NRMRL	National Risk Management Research Laboratory
OD	optical density
ppb	parts per billion
%D	percent difference
PEA	performance evaluation audit
PT	performance test
QA	quality assurance
QAO	quality assurance officer
QC	quality control
QMP	quality management plan
%R	percent recovery
r^2	coefficient of determination
RB	reagent blank
RPD	relative percent difference
RSD	relative standard deviation
RW	recreational water
S	standard deviation
SOP	standard operating procedure
SPE	solid phase extraction
TMB	tetramethylbenzidine

TQAP	Test/Quality Assurance Plan
TSA	technical systems audit
µg/L	microgram per liter
WHO	World Health Organization
WSL	Water Sciences Laboratory (University of Nebraska)

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 the ETV program. The AMS Center evaluated the performance of three technologies offered by Abraxis: Microcystin (ADDA) ELISA Test Kit, the Microcystin (DM) ELISA Test Kit, and Microcystin Strip Test Kits.

Chapter 2
Technology Description

This verification report provides results for the verification testing of three Abraxis Test Kits. Following are descriptions of the Microcystin (ADDA) ELISA Test Kit, the Microcystin (DM) ELISA Test Kit, and Microcystin Strip Test Kit (hereafter these technologies will be referred to as the ADDA, DM, and Strip Test, respectively), based on information provided by the vendor. The information provided below was not verified in this test.

2.1 Microcystin ADDA ELISA Test Kit

The ADDA Test Kit is an enzyme-linked immunosorbent assay (ELISA) for the congener independent determination of microcystins and nodularins in water samples. The assay utilizes polyclonal antibodies that have been raised against the ADDA moiety of the molecule, allowing for the detection of microcystins and nodularin variants (over 80 variants are currently known) in drinking, surface, and groundwater at levels below World Health Organization (WHO) guidelines.

The test is an indirect competitive ELISA and is based on the recognition of microcystins, nodularins and their variants by a polyclonal sheep antibody. When present in a sample, microcystins and nodularins compete with a microcystins-protein analog that is immobilized on wells of a microtiter plate for the binding sites of antibodies in solution. After a washing step, a second labeled antibody is added and incubated, antibody- Horseradish Peroxidase (HRP). After a washing step and addition of a substrate/chromogen solution, a color signal is generated. The intensity of the color is inversely proportional to the concentration of the microcystins/nodularins present in the sample. The color reaction is stopped after a specified time and the color is analyzed using a plate photometer to obtain the optical density (OD) at a wavelength of 450 nanometer (nm).

The ADDA Test Kit is not able to distinguish between different microcystin variants. Results from the ADDA test kit are calibrated with respect to a single variant, microcystin-LR. However, other microcystin variants are known (based on information provided by Abraxis) to react to different extents with the antibodies used for detection which is referred to as the cross reactivity (CR) of the variant. For this verification test, cross reactivity values were used to quantify results for different variants based on the LR calibration.

The ADDA ELISA Test Kit (Figure 1) contains:

- Microtiter plate coated with an analog of microcystins conjugated to a protein;
- Standards (6) and positive control (1): 0, 0.15, 0.40, 1.0, 2.0, 5.0 parts per billion (ppb); Positive control has a concentration of 0.75 ppb;
- Antibody solution (monoclonal anti-Microcystins);
- Anti-Sheep-HRP Conjugate;
- Wash Solution 5X Concentrate;
- Color Solution, tetramethylbenzidine (TMB);

- Stop Solution;
- Diluent/zero solution, 25 mL.

The Microcystins ADDA microtiter plate ELISA Test Kit is shown in Figure 1 and measures 9 × 6 × 3 inches (22.9 × 15.2 × 7.6 centimeters) and weighs 4.8 pounds (2.2 kilograms). The cost is $440 for a 96-test kit. Other materials and equipment not provided with the kits are pipettes, pipette tips, a plate photometer capable of reading at 450 nm and distilled or deionized (DI) water. These materials can be purchased separately from the vendor.

Figure 1. Microtiter Plate ELISA Test Kit

2.2 Microcystin DM ELISA Test Kit

The Microcystins (DM) Test Kit is an ELISA for the determination of microcystins and nodularins in water samples. The assay utilizes monoclonal antibodies that have been raised against the ADDA moiety of the molecule, allowing for the detection of numerous microcystins and nodularin variants in drinking, surface, and groundwater at levels below WHO guidelines.

The test is a direct competitive ELISA method and is based on the recognition of microcystins, nodularins and their variants by a monoclonal antibody. Microcystins and nodularins, when present in a sample and a microcystins-HRP analog, compete for the binding sites of anti-microcystins antibodies in solution. The microcystins antibodies are then bound by a second antibody (goat anti-mouse) immobilized on the plate. After a washing step and addition of a substrate/chromogen solution, a color signal is generated. The intensity of the color is inversely proportional to the concentration of the microcystins/nodularins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using a plate photometer at a wavelength of 450 nm.

The DM ELISA Test Kit differs from the ADDA ELISA Test Kit in the coating of the microtiter plate. The Microcystins DM microtiter plate ELISA Test Kit is packaged the same as the ADDA kit (see Figure 1) and contains:

- Microtiter plate coated with a second antibody (goat anti- mouse);
- Standards (6) and positive control (1): 0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb; the positive control samples had a concentration of 0.75 ppb;
- Antibody solution (monoclonal anti-Microcystins);
- Microcystins-HRP Conjugate;
- Wash Solution 5X Concentrate;
- Color Solution (TMB);
- Stop Solution;
- Diluent/zero solution, 25 mL.

The Microcystins DM microtiter plate ELISA Test Kit has a cost of $400 for a 96-test kit. Like the ADDA kit, other materials and equipment not provided are pipettes, pipette tips, a plate photometer capable of reading at 450 nm and distilled or deionized water. These materials can be purchased separately from the vendor.

2.3 Microcystin Strip Test

The Abraxis Microcystin Strip Test is a rapid immunochromatographic test, designed for the use in the qualitative screening of microcystins and nodularins in recreational waters. A rapid cell lysis step (QuikLyse™) performed prior to testing is required to measure total microcystins (dissolved or free, plus cell bound). The Strip Test provides preliminary qualitative test results. If necessary, positive samples can be confirmed by one of the ELISA test kits described above, high pressure liquid chromatography (HPLC) or other conventional methods. The test is designed for field use, requiring no instrumentation or other equipment, no power sources, and no refrigerated storage.

The QuickLyse™ procedure includes shaking the sample in a lysing vial continuing dried lysis reagent. After an eight minute period, a reagent paper is added to the lysis vial. After another eight minute period, the sample is transferred to the Strip Test conical flip-top reagent vial. The reagent vial is shaken vigorously, then the membrane strip is inserted into the vial to obtain a reading.

The test is based on the recognition of microcystins, nodularins and their variants by specific antibodies. The toxin conjugate competes for antibody binding sites with microcystins/nodularins that may be present in the water sample. The test device consists of a conical flip-top vial with specific antibodies for microcystins and nodularins labeled with a gold colloid and a membrane strip to which a conjugate of the toxin is attached. A control line, produced by a non-microcystin antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of microcystins in the water sample, and therefore, it should be present in all reactions. In the absence of toxin in the water sample, the colloidal gold labeled antibody complex moves with the water sample by capillary action to contact the immobilized microcystins conjugate. An antibody-antigen reaction forms one visible line in the test line region**.**

If microcystins are present in the water sample, they compete with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled complex. If a sufficient amount of toxin is present, it will fill all of the available binding sites, thus

preventing attachment of the labeled antibody to the toxin conjugate, therefore preventing the development of a second line in the test line region. If a second line is not visible in the test line region, or if the test line is lighter than the negative control line, microcystins are present at the levels of concern (> 10 ppb). Semi-quantitative results in the range of 0 to 10 ppb can be obtained by comparing the test line intensity to those produced by solutions of known microcystins concentrations (control solutions). During this verification, the results were reported 0 to 10 ppb, or greater than 10 ppb.

The Microcystin Strip Test (Figure 2) contains:

- Microcystin membrane strips (test strips) in a desiccated container
- Sample collection vessels
- Lysis vials
- Graduated disposable pipettes (marked at 1 mL increments)
- Forceps
- Reagent papers
- Conical test vials
- Disposable transfer pipettes
- User's guide and interpretation guide

The Microcystin Strip Test is shown in Figure 2 and measures 9 × 6 × 3 inches (22.9 × 15.2 × 7.6 centimeters) and weighs 2.2 pounds (1 kilogram). The Strip Test (containing 20 test strips) measures 16 × 6 × 3 inches (40.6 × 15.2 × 7.6 centimeters), packaged in two boxes and weighs 8.8 pounds (4 kilograms) total. The cost is $150 for a five-test kit and $480 for a 20-test kit.

Figure 2. Microcystin Strip Test

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]. Evaluating microcystin test kits was identified by the AMS Center stakeholders as a priority area in 2005. With stakeholder input to the design, reference method selection, and submission of recreational waters to be evaluated, the test assessed the performance of microcystin test kits relative to key verification parameters including accuracy, precision, and method detection limit (MDL). This verification test took place from July 26 through August 12, 2010. The reference analysis was performed the week of August 16, 2010.

3.2 Experimental Design

The objective of this verification test was to evaluate the performance of the microcystin test kits against a known concentration of each microcystin variant in ASTM International Type II DI water, as well as microcystin variants in unknown proportions from recreational water (RW) samples. Battelle conducted this verification test with recreational samples provided from the Nebraska Department of Environmental Quality (NDEQ), 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 based on direct injection liquid chromatography tandem mass spectrometry (LC-MS/MS)[3] for the determination of microcystins. To attain lower levels of detection, a sample preparation method was developed by the WSL to extract the microcystins from the water samples and concentrate the samples using solid phase extraction (SPE)[4]. The ELISA kits provided a quantitative or semi-quantitative determination of microcystins. The ADDA (quantitative), DM (quantitative) and Strip (semi-quantitative) test kits were evaluated for:

- Accuracy - comparison of test kit results (samples prepared in DI water) 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 water matrices and chlorophyll-*a* on the results of the test kits
- Operational and Sustainability factors – general operation, data acquisition, setup, consumables, etc.

Each microcystin test kit was operated according to the vendor's instructions by a vendor-trained Battelle technician. The samples were tested according to the kit instructions, and in compliance with the microcystin 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 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 ^{o}C ± 3 ^{o}C until use. The reference samples that were prepared from the test solutions were stored in amber glass bottles at < -10^{o}C until analysis approximately 2 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 specific to individual microcystins, PT samples for each of the three different variants 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 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 and one negative control were analyzed with each ELISA plate. For the Strip Test, a control line is provided on the strip.

3.3.2 PT Samples

PT samples were used to verify the accuracy, precision, linearity, MDL, 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 ppb. These concentration levels were used for microcystin-LR and, because of the 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. For the semi-quantitative Strip Test Kit,

a 15 ppb PT sample was also used to test the semi-quantitative capability of indicating a concentration higher than 10 ppb. EPA Guidelines[5] were followed to estimate the MDL of the quantitative test kits. In doing so, a solution with a concentration five times the vendor's reported detection limit (DL) was used. A minimum of seven replicate analyses of this solution were made individually for each variant to obtain precision data with which to determine the MDL.

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	0.75	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 ppb	3	15
	LA	0.50, 1.0, 2.0, 4.0, 7.0 ppb	3	15
	RR	0.50, 1.0, 2.0, 4.0, 7.0 ppb	3	15
	LR	5 times the vendor stated MDL	7	7
	LA	5 times the vendor stated MDL	7	7
	RR	5 times the vendor stated MDL	7	7
PT Samples - RW Matrix Interference Samples: RW sample and tenfold dilution	LR	4.0 ppb or 2.0 ppb*	3	6
	LA	4.0 ppb or 2.0 ppb*	3	6
	RR	4.0 ppb or 2.0 ppb*	3	6
PT Samples - Chlorophyll-*a* Matrix Interference Samples: Chlorophyll-*a* sample and tenfold dilution	LR	4.0 ppb or 2.0 ppb*	3	6
	LA	4.0 ppb or 2.0 ppb*	3	6
	RR	4.0 ppb or 2.0 ppb*	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 vendor recommended procedure	Unknown	3 samples at unknown concentrations	3	9

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

Additional performance testing was conducted to verify the impact of possible matrix interferences. Two types of possible matrix interferences, RW water and chlorophyll-*a*, were tested. Testing was performed using a RW sample with a low level of native microcystin concentration (based on information from NDEQ). This RW sample was serially 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 (for the Strip Test) or 2.0 ppb (for the ADDA and DM test) 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 interference, 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 a 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 was 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.

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.

The Strip Test kit contains a specific lysing procedure to analyze for microcystin. For this test kit, three of the RW samples were split before the freeze-thaw process to compare the results using the two lysing procedures. The Strip Test Kit was used to analyze the three RW samples with and without the freeze-thaw lysing.

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the AMS Center QMP[2] and the TQAP[1] 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) standards 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; 17 were extracted prior to analysis and five were analyzed by direct injection. One sample duplicate was processed with the 17 extracted samples to assess the DQI. No sample duplicate was included for samples analyzed via direct injection.

The calibration of the LC-MS/MS method was verified by the analysis of a CCV at a minimum of 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.

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

C_s is the measured concentration of the CCV and *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 to 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 LFM spike performed every 20 samples and was assessed by calculating the spike percent recovery (*%Rs*) as 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 %R was required to be within 30% of the spiked amount. The two LFM sample results were within this range for all three of the variants.

The relative percent difference (*RPD*) of the duplicate sample analysis was calculated from the following equation.

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

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. Reference method CCV RPD results are provided in Table 2. Reference method precision of laboratory samples was not determined because the duplicate extraction was performed on the reagent blank sample.

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 (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% Difference	See Table 3	
Method accuracy	Laboratory Fortified Matrix (LFM) Spikes (Once every 20 samples)	70% - 130% Recovery	LRM 1 93% LR 79% LA 97% RR	LRM 2 103% LR 105% LA 88% RR

Table 3. Summary of Reference Method CCV Percent Recoveries

CCV Conc. (ppb)	Variant % Recovery			Variant 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

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 (ADQ). Audit procedures 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 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 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 water 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 stock solutions used to prepare the calibration standards by the reference laboratory were prepared by dissolving neat standards (not solutions) obtained from EMD Biosciences (microcystin-LR), Sigma Aldrich (microcystin-LA), and ENZO Life Sciences (microcystin-RR). The results from the analyses are presented in Table 4.

Table 4. PEA Results: Analytical Comparison of Microcystin Standards

Standard Source	# of Replicates	Analysis Date	MC-LR (%Recovery)	MC-LA (%Recovery)	MC-RR (%Recovery)
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 recoveries of the NRC and Abraxis standards revealed that the reference laboratory method, using the standards from alternate sources, were outside the acceptance range of ±30%. It was then discussed with the stakeholders, and accepted by the vendors and the EPA Project Officer,

that the reference laboratory use the two available NRC standards (LR and RR) as well as LA from Abraxis for preparing the reference method calibration solutions. This 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 necessary and 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 also developed and used by the reference laboratory for samples expected to be below 5.0 ppb. The MDL of this method was determined from extraction and analysis of eight solutions of LR, LA, and RR at 0.375 ppb. The reference method MDLs for LR, LA, and RR were determined to be 0.095 ppb, 0.141 ppb, and 0.127 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[1] and the AMS Center QMP[2]. As part of the TSA, test procedures were compared to those specified in the TQAP[1], 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. The observations from the TSA were addressed and documented as necessary. The conclusion of the TSA was that verification testing was performed according to the TQAP. TSA records are permanently stored with the QAO.

4.2.3 Data Quality Audit

Two ADQs 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 after the TSA and all observations were addressed prior to the submission of this final report.

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 were 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 each vendor (specific to each test kit), the microcystin-LR equivalents were converted to microcystin concentration by variant as follows:

$$C_{\text{by variant}} = \frac{C_{\text{LR equiv}}}{CR} \quad (4)$$

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:

$$\%D = \frac{C_T - C_R}{C_R} \times 100 \quad (5)$$

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 predicted 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.

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

5.4 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 are given to compare between the 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 2.0 or 4.0 ppb spike concentration). Each paired t-test was performed using the replicate data from each type of sample. The null hypothesis is that the difference between the two sets of data is zero. Therefore, the resulting probability (p)-value gives the likelihood that the null hypothesis would be true. Therefore, at the 95% confidence interval, p-values less than 0.05 will indicate there is a small likelihood of the null hypothesis being true and therefore a significant difference between the two sets of data. Since the number of replicates were predetermined by the test kit instructions and TQAP, power and sample size calculations were not conducted for this assessment. It is important to note that strong inference based on the results cannot be established due to the low power of this study.

Chapter 6
Test Results for the Abraxis ADDA ELISA Test Kit

The following sections provide the results of the quantitative and qualitative evaluations of this verification test for the Abraxis ADDA ELISA Test Kit.

6.1 ADDA Test Kit Summary

As discussed in Chapter 2, the ADDA Test 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 ADDA Test Kit, the CR of microcystin LA is 125% and the CR of microcystin RR is 91%. 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 ADDA Test Kit requires that each standard and sample be analyzed in duplicate and then the raw data output from the plate reader software reports a mean concentration of the duplicate analyses. Therefore, a sample indicated in Table 1 to have three replicates corresponded to six wells being filled as part of the ADDA Test Kit. Each ADDA Test Kit plate contains six calibration solutions. Following the analysis method, the plate reader measures 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 four-parameter curve to quantify the samples. According to Abraxis, if 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 the limit of quantification (<LOQ) or not detectable (ND), respectively. The data from a plate of samples was considered acceptable when the positive control was recovered within 25% of 0.75 ppb. 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 Samples

As described in Section 3.3.1, the QC samples analyzed with the Abraxis ADDA Test Kit included RB samples and the positive and negative 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 non-detect or below the LOQ for the ADDA Test Kit, as presented in Table 6. Two RB samples were analyzed by the reference method and were determined to be below the LOQ for all three variants.

Table 6. RB Sample Results for the Abraxis ADDA Test Kit

Reagent Blank	Plate	Mean Concentration (ppb)
RB 1	1	ND
RB 2	1	ND
RB 3	1	< 0.15 LOQ
RB 4	3	ND
RB 5	3	ND
RB 6	3	ND

The positive controls for the ADDA Test Kit are presented in Table 7. The vendor stated acceptable range for recovery of the positive control is between 75% and 125%. In addition, the coefficient of variation (CV) of the duplicate analyses is reported as a gauge for accurate quantification of microcystins. The variation between the two data points is considered acceptable when the %CV is less than 25%. At least one positive control was analyzed at the end of each plate, and in some instances when space allowed, additional positive controls were analyzed. All ADDA Test Kit plates used for testing produced a positive control result within the acceptable range. During verification testing of the ADDA Test Kit, all plates were within the CV and %R acceptance criteria.

Table 7. Positive Control Sample Results for the Abraxis ADDA Test Kit

Positive Control	Plate	Mean Concentration (ppb)	CV (%)	Percent Recovery (%)
1	1	0.653	8.2	87%
2	2	0.826	4.2	110%
2a	2	0.808	6.2	108%
3	3	0.566	18	75%
4	4	0.625	25	83%
5	5	0.717	2.0	96%
5a	5	0.656	1.7	87%
5b	5	0.769	1.9	103%
6	6	0.903	16	120%

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 have at least three results, but some samples include four or more replicate results because in instances when the %CV were less than 25%, the individual samples were reanalyzed in duplicate (per the vendor instructions). If the resulting %CV was acceptable for both repeat samples, they were both included in the result tables, thus resulting in additional data points. In addition, the 0.50 ppb solutions included all seven replicates from the MDL determination data in addition to the triplicate analyses of the 0.50 ppb PT samples.

6.3.1 Accuracy

Tables 8, 9, and 10 also present the accuracy results for the ADDA Test Kit, expressed as %D when calculated with the theoretical spike concentration and the reference method concentration. As shown in Equation 5 (Section 5.1), the reference method value was used for calculation of accuracy.

Table 8. Abraxis ADDA Test 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 for Theoretical Concentration (% Difference)	Accuracy by Variant for Reference Concentration (% Difference)	Reference Concentration (ppb)
0.10 LR	0.096	0.096	-4%	-4%	0.10
	< LOQ	< LOQ	NA	NA	
	0.087	0.087	-13%	-13%	
	0.110	0.110	10%	10%	
	0.102	0.102	2%	2%	
Avg ± SD	0.083 ± 0.035	0.083 ± 0.035	-1% ± 10%	-1% ± 10%	
0.50 LR	0.588	0.588	18%	40%	0.42
	0.584	0.584	17%	39%	
	0.590	0.590	18%	40%	
	0.553	0.553	11%	32%	
	0.477	0.477	-5%	14%	
	0.538	0.538	8%	28%	
	0.550	0.550	10%	31%	
	0.441	0.441	-12%	5%	
	0.381	0.381	-24%	-9%	
	0.434	0.434	-13%	3%	
Avg ± SD	0.514 ± 0.075	0.514 ± 0.075	3% ± 15%	22% ± 18%	
1.0 LR	1.31	1.31	31%	58%	0.83
	1.00	1.00	0%	21%	
	0.991	0.991	-1%	19%	
Avg ± SD	1.10± 0.181	1.10 ± 0.181	10% ± 18%	33% ± 22%	
2.0 LR	1.87	1.87	-6%	-1%	1.9
	2.63	2.63	32%	39%	
	1.10	1.10	-45%	-42%	
	1.05	1.05	-48%	-45%	
Avg ± SD	1.66 ± 0.749	1.66 ± 0.749	-17% ± 37%	-12% ± 39%	
4.0 LR	3.47	3.47	-13%	-6%	3.7
	3.82	3.82	-5%	3%	
Avg ± SD	3.64 ± 0.245	3.64 ± 0.245	-9% ± 6%	-1% ± 7%	

Table 9. Abraxis ADDA Test 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 for Theoretical Concentration (% Difference)	Accuracy by Variant for Reference Concentration (% Difference)	Reference Concentration (ppb)
0.50 LA	0.667	0.534	7%	33%	0.40
	0.914	0.731	46%	83%	
	0.718	0.574	15%	44%	
	0.969	0.775	55%	94%	
	1.05	0.837	67%	109%	
	0.927	0.742	48%	85%	
	0.842	0.674	35%	68%	
	0.905	0.724	45%	81%	
	0.740	0.592	18%	48%	
	0.750	0.600	20%	50%	
	0.634	0.507	1%	27%	
Avg ± SD	0.828 ± 0.134	0.663 ± 0.107	33% ± 21%	66% ± 27%	
1.0 LA	2.04	1.63	63%	133%	0.70
	1.78	1.43	42%	104%	
	1.55	1.24	24%	77%	
Avg ± SD	1.79 ± 0.246	1.43 ± 0.197	43% ± 20%	104% ± 28%	
2.0 LA	3.60	2.88	44%	69%	1.7
	4.32	3.46	73%	103%	
	4.53	3.62	81%	113%	
Avg ± SD	4.15 ± 0.488	3.32 ± 0.390	66% ± 20%	95% ± 23%	
4.0 LA	7.70	6.16	54%	105%	3.0
	7.99	6.39	60%	113%	
Avg ± SD	7.84 ± 0.206	6.27 ± 0.165	57% ± 4%	109% ± 6%	

Table 10. Abraxis ADDA Test 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 for Theoretical Concentration (% Difference)	Accuracy by Variant for Reference Concentration (% Difference)	Reference Concentration (ppb)
0.50 RR	0.490	0.538	8%	42%	0.38
	0.544	0.598	20%	57%	
	0.552	0.607	21%	60%	
	0.568	0.624	25%	64%	
	0.567	0.623	25%	64%	
	0.518	0.569	14%	50%	
	0.514	0.565	13%	49%	
	0.549	0.603	21%	59%	
	0.563	0.619	24%	63%	
	0.552	0.607	21%	60%	
Avg ± SD	0.542 ± 0.026	0.595 ± 0.029	19% ± 6%	57% ± 8%	
1.0 RR	1.10	1.20	20%	123%	0.54
	0.973	1.07	7%	98%	
	0.972	1.07	7%	98%	
Avg ± SD	1.01 ± 0.071	1.11 ± 0.078	11% ± 8%	106% ± 14%	
2.0 RR	1.80	1.97	-1%	23%	1.6
	2.46	2.70	35%	69%	
	2.20	2.42	21%	51%	
Avg ± SD	2.15 ± 0.335	2.37 ± 0.368	18% ± 18%	48% ± 23%	
4.0 RR	4.36	4.79	20%	50%	3.2
	4.07	4.48	12%	40%	
	3.24	3.56	-11%	11%	
	3.55	3.90	-2%	22%	
Avg ± SD	3.81 ± 0.504	4.18 ± 0.554	5% ± 14%	31% ± 17%	

For the LR spiked samples, the reference method results ranged from 0% to 17% less than the target spike concentration. For LR, the percent difference ranged from -45% to 58%, with overall average percent difference values ranging from -12% to 33%. One 0.10 ppb sample was determined as being less than the LOQ so no %D was calculated, but the other three %Ds were less than 14% from the reference method result. Only two replicate results are given for the 4.0 ppb samples as the samples were repeated four times after the result was reported as "out of range". The sample was not diluted because lower concentrations had already been analyzed. For the 0.50 ppb samples, the %D ranged from -9% to 40%, but the absolute difference from the reference concentration was no more than 0.170 ppb. For the 1.0 ppb samples, the %D ranged from 19% to 58%, corresponding to a maximum absolute difference from the reference concentration of 0.481 ppb. Similarly, for the 2.0 ppb samples, the %D ranged from -45% to 39% and the maximum absolute difference from the reference concentration was 0.860 ppb. For the 4.0 ppb samples, the two reported results had a %D of less than 10%. For LR, the %D when

compared to the theoretical spike concentration range from -48% to 32% with the overall average %D values ranging from -17% to 10%.

For the LA spiked samples, the reference method results ranged from 15% to 33% less than the target spike concentration. For LA, the percent difference ranged from 27% to 276%. For the 0.50 ppb samples, the %D ranged from 27% to 109%, corresponding to an absolute maximum difference from the reference concentration of 0.437 ppb. For the 1.0 ppb samples, the %D ranged from 77% to 133%, corresponding to a maximum absolute difference from the reference concentration of 0.930 ppb. For the 2.0 ppb samples, the %D ranged from 69% to 113% and the maximum absolute difference from the reference concentration was 1.92 ppb. For the 4.0 ppb samples, the two reported results had %Ds of 262% and 276%, corresponding to a maximum absolute difference from the reference concentration of 3.39 ppb. For LA, the %D when compared to the theoretical spike concentration range from 1% to 81% with the overall average %D values ranging from 33% to 66%.

For the RR spiked samples, the reference method results ranged from 20% to 46% less than the target spike concentration. For RR, the percent difference ranged from 11% to 123%. For the 0.50 ppb samples, the %D ranged from 42% to 64%, corresponding to an absolute maximum difference from the reference concentration of 0.244 ppb. For the 1.0 ppb samples, the %D ranged from 98% to 123% and the maximum absolute difference from the reference concentration was 0.663 ppb. For the 2.0 ppb samples, the %D ranged from 23% to 69% corresponding to a maximum absolute difference from the reference concentration of 1.10 ppb. For the 4.0 ppb samples, the %D ranged from 11% to 50%, corresponding to a maximum absolute difference from the reference concentration of 1.59 ppb. For RR, the %D when compared to the theoretical spike concentration range from -11% to 35% with the overall average %D values ranging from 5% to 19%.

6.3.2 Precision

Precision results for the ADDA Test 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 interference and recreational water samples. The RSDs ranged from 5% to 45% for the LR variant; however, seven of the nine sample sets had RSDs lower than 16%. For LA, the RSDs ranged from 3% to 25% and from 4% to 16% for the RR variant. The precision for the RW samples ranged from 3% to 47%, but all but two RW samples sets had RSDs less than 12%. The overall average of all RSDs was 13%, with a minimum of 3% and a maximum of 47%.

Table 11. Abraxis ADDA Test Kit Precision Results

Variant	Sample Concentration in DI	Precision (%RSD)
LR	0.10 ppb	10%
	0.50 ppb	15%
	1.0 ppb	16%
	2.0 ppb	45%
	4.0 ppb	7%
	2.0 ppb LR in 1 mg/L Chlorophyll-*a* DI	11%
	2.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	5%
	2.0 ppb LR in 10x dilution of RW Matrix	7%
	2.0 ppb LR in RW Matrix	31%
LA	0.50 ppb	16%
	1.0 ppb	14%
	2.0 ppb	12%
	4.0 ppb	3%
	2.0 ppb LA in 1 mg/L Chlorophyll-*a* DI	18%
	2.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	6%
	2.0 ppb LA in 10x dilution of RW Matrix	19%
	2.0 ppb LA in RW Matrix	25%
RR	0.50 ppb	5%
	1.0 ppb	7%
	2.0 ppb	16%
	4.0 ppb	13%
	2.0 ppb RR in 1 mg/L Chlorophyll-*a* DI	14%
	2.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	15%
	2.0 ppb RR in 10x dilution of RW Matrix	4%
	2.0 ppb RR in RW Matrix	8%
Unknown	RW 1	10%
	RW 2	5%
	RW 3 (10x dilution)	47%
	RW 3 (20x dilution)	8%
	RW 4 (4x dilution)	12%
	RW 5 (4x dilution)	12%
	RW 6 (2x dilution)	22%
	RW 7	NA
	RW 8	5%
	RW 9	3%

NA - Result was less than the LOQ so no calculation of RSD

6.3.3 Linearity

The linearity of the ADDA Test Kit measurements was assessed by performing a linear regression of the ADDA test kit results against the reference method results for the five PT samples ranging from 0.10 to 4.0 ppb of microcystin LR in DI water and four PT samples ranging from 0.50 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 0.906 for LR, 0.990 for LA, and 0.961 for RR.

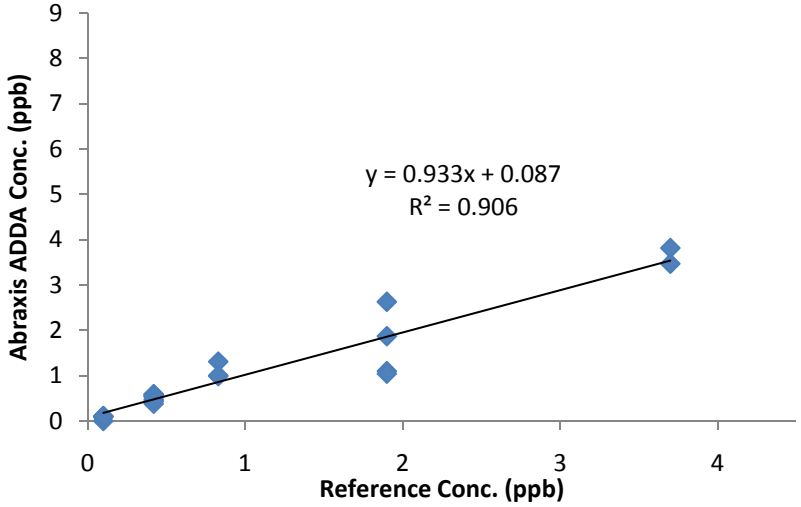

Figure 3. Linearity for the Abraxis ADDA Test Kit for LR

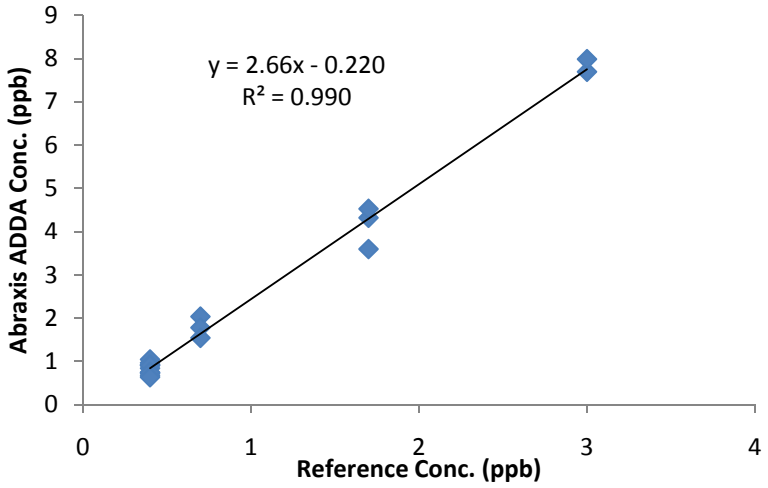

Figure 4. Linearity for the Abraxis ADDA Test Kit for LA

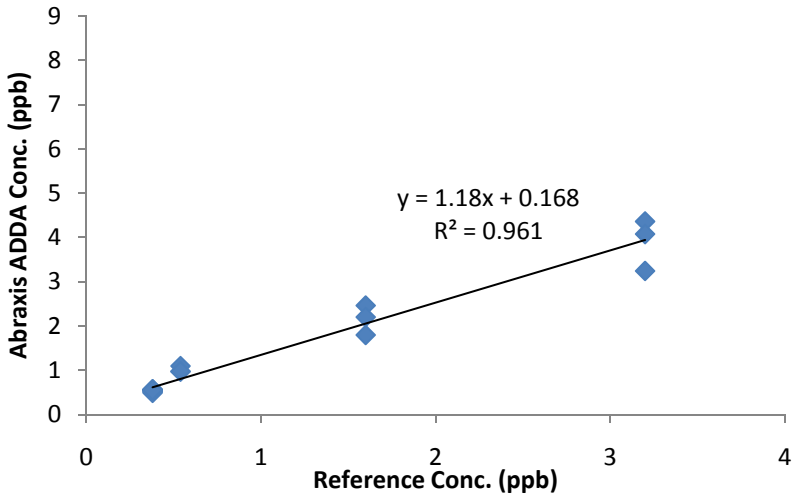

Figure 5. Linearity for the Abraxis ADDA Test Kit for RR

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 0.10 ppb). Table 12 lists the replicate results, the %CV of the duplicate ADDA Test 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 0.14, 0.22, and 0.05 ppb for LR, LA, and RR, respectively.

Table 12. Detection Limit Results for the Abraxis ADDA Test Kit

Variant	LR		LA		RR	
Sample Concentration (ppb)	**Mean Conc. (ppb)**	**%CV**	**Mean Conc. (ppb LR Equivalents)**	**%CV**	**Mean Conc. (ppb LR Equivalents)**	**%CV**
0.50	0.59	8.9	0.91	5.5	0.49	1.3
0.50	0.58	2.5	0.72	1.2	0.54	0.50
0.50	0.59	2.3	0.97	3.6	0.55	3.3
0.50	0.55	11	1.0	4.5	0.57	17
0.50	0.48	15	0.67	24	0.57	8.9
0.50	0.54	3.6	0.93	5.6	0.52	0.80
0.50	0.55	9.8	0.84	3.6	0.51	12
0.50	0.44	18	0.91	2.2	0.55	6.3
0.50	0.38	7.0	0.74	0.60	0.56	8.9
0.50	0.43	1.6	0.75	12	0.55	4.7
0.50	NA	NA	0.63	2.1	NA	NA
Standard Deviation	0.08		0.13		0.03	
t value	1.8		1.8		1.8	
n	10		11		10	
MDL	**0.14**		**0.22**		**0.05**	

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. All RPDs were less than 13% and all but four were less than 6%.

Table 13. Inter-kit lot Comparison of Kit Calibration Standards for the ADDA Test Kit

	OD Values		
Standard	**Set A**	**Set B**	**RPD**
Std 0 ppb	1.48	1.34	11%
	1.47	1.29	13%
Std 0.15 ppb	1.14	1.10	3%
	1.16	1.05	11%
Std 0.40 ppb	0.836	0.786	6%
	0.841	0.790	6%
Std 1.0 ppb	0.561	0.544	3%
	0.560	0.551	2%
Std 2.0 ppb	0.434	0.413	5%
	0.458	0.456	0%
Std 5.0 ppb	0.335	0.337	1%
	0.340	0.299	13%

6.3.6 Matrix Effect

Matrix interference effects were assessed by using a t-test to compare the ADDA Test 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 chlorophyll-*a* at 10 mg/L and 1 mg/L. Tables 14 and 15 provide the ADDA Test 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 the difference between the two sets of data is zero. The resulting probability (p)-value gives the likelihood that the null hypothesis would be true. Therefore, at the 95% confidence interval, p-values less than 0.05 will indicate there is a small likelihood of the null hypothesis being true and therefore a significant difference between the two sets of data (at low power).

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, three out of 16 comparisons resulted in statistically significant differences (two comparisons could not be performed because there were only two replicate results for the LR spiked undiluted RW samples). The 2.0 ppb LA spike into DI water was significantly different from the 2.0 ppb LA spike into both 1 mg/L (p=0.005) and 10 mg/L (p=0.006) chlorophyll-*a*. Table 15 shows that the 2.0 ppb spike into DI

water generated an average result of 4.15 ppb compared with an average result of 0.552 and 0.580 ppb for the spike into 1.0 mg/L and 10 mg/L chlorophyll-*a*, respectively. The other statistically significant difference was between the RR spikes into undiluted and diluted RW ($p=0.01$). 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 1.35 ppb for the diluted RW and 2.56 ppb for the undiluted RW. There were three other pairs of data that were close to being statistically significant differences with p-values of less than 0.1. The LR spike into DI was nearly different from the chlorophyll-*a* spikes and the RR spike into DI was nearly different from the RW spikes.

Given that the molecular basis on which the test kits operate is well-characterized and understood from the literature[8], Table 15 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. 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 14. RW Matrix Interference Sample Results for the Abraxis ADDA Test 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)	0.639 0.600 0.621	0.620	0.020	
LR	2.0 ppb LR in DI	1.87 2.63 1.10 1.05	1.66	0.749	1.87 2.63 1.10 1.05
	2.0 ppb LR in tenfold dilution of RW Matrix	1.70 1.90 1.66	1.76	0.130	1.70 1.90 1.66
	2.0 ppb LR in RW Matrix	4.05 2.60	3.32	1.03	4.05 2.60
LA	2.0 ppb LA in DI	3.60 4.32 4.53	4.15	0.488	2.88 3.46 3.62
	2.0 ppb LA in tenfold dilution of RW Matrix	2.66 3.86 3.03	3.18	0.617	2.12 3.09 2.42
	2.0 ppb LA in RW Matrix	3.01 2.17 1.89	2.36	0.583	2.41 1.74 1.52
RR	2.0 ppb RR in DI	1.80 2.46 2.20	2.15	0.335	1.97 2.70 2.42
	2.0 ppb RR in tenfold dilution of RW Matrix	1.37 1.39 1.29	1.35	0.054	1.50 1.52 1.41
	2.0 ppb RR in RW Matrix	2.78 2.38 2.50	2.56	0.208	3.06 2.61 2.75

Table 15. Chlorophyll-*a* Interferent Sample Results for the Abraxis ADDA Test 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	1.87	1.66	0.749	1.87
		2.63			2.63
		1.10			1.10
		1.05			1.05
	2.0 ppb LR in 1 mg/L Chlorophyll-*a* DI	0.391	0.436	0.048	0.391
		0.432			0.432
		0.486			0.486
	2.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	0.488	0.504	0.024	0.488
		0.532			0.532
		0.493			0.493
LA	2.0 ppb LA in DI	3.60	4.15	0.488	2.88
		4.32			3.46
		4.53			3.62
	2.0 ppb LA in 1 mg/L Chlorophyll-*a* DI	0.533	0.552	0.102	0.426
		0.669			0.535
		0.581			0.465
		0.425			0.340
	2.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	0.576	0.580	0.035	0.461
		0.617			0.494
		0.548			0.438
RR	2.0 ppb RR in DI	1.80	2.15	0.335	1.97
		2.46			2.70
		2.20			2.42
	2.0 ppb RR in 1 mg/L Chlorophyll-*a* DI	1.98	1.64	0.230	2.17
		1.44			1.58
		1.61			1.77
		1.57			1.73
	2.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	2.24	1.96	0.285	2.46
		1.77			1.94
		1.57			1.73
		2.20			2.42
		2.01			2.21

Table 16. Statistical Comparisons between Interference Samples for the Abraxis ADDA Test Kit

	p-value (D-different, ND-not different)		
Description of Comparison	**LR**	**LA**	**RR**
2.0 ppb in DI compared with 2.0 ppb in tenfold dilution of RW	0.788 (ND)	0.084 (ND)	0.054 (ND)
2.0 ppb in DI compared with 2.0 ppb in undiluted RW	NA	0.102 (ND)	0.327 (ND)
2.0 ppb in undiluted RW compared with tenfold dilution of RW	NA	0.311 (ND)	0.010 (D)
2.0 ppb in DI compared with 2.0 ppb in 1 mg/L Chlorophyll-*a* DI	0.089 (ND)	0.005 (D)	0.307 (ND)
2.0 ppb in DI compared with 2.0 ppb in 10 mg/L Chlorophyll-*a* DI	0.087 (ND)	0.006 (D)	0.510 (ND)
2.0 ppb in 1 mg/L Chlorophyll-*a* DI compared with 2.0 ppb in 10 mg/L Chlorophyll-*a* DI	0.156 (ND)	0.677 (ND)	0.242 (ND)

Shading indicates a statistically significant difference
NA- The p-value could not be determined with only 2 replicate results of the undiluted RW LR spiked samples

6.4 RW Sample Results

Table 17 presents the RW results for the ADDA Test 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 ADDA Test Kit may have other variants present that would not have been detected by the reference method. Therefore, no quantitative comparison was made between the ADDA Test Kit and the reference method results. The reference data were converted into LR-equivalents according to the ADDA Test Kit cross reactivity for the variants. In general, the samples that were determined to have higher total concentrations by the ADDA Test Kit had higher total concentrations as determined by the reference method. All of the ADDA Test Kit total microcystin results were greater than the reference method results that only quantified three variants. However, the results of the ADDA Test Kit were usually within a factor of two of the reference method. The LR, LA, and RR variants likely make up a significant proportion of the microcystins that are measurable by the ADDA Test Kit.

Table 17. Recreational Water Sample Results for the Abraxis ADDA Test Kit

Sample Description	Test Kit Results					Reference Results (ppb)			
	Kit Results: LR Equivalents (ppb)	Dilution Factor	Corrected Conc. (ppb)	Average Conc. (ppb)	Standard Deviation (ppb)	LR	LA	RR	Total
RW 1 (20x dilution)	2.132	20	43	40	4.1	9.6	2.3	17	29
	1.843	20	37						
RW 2 (10x dilution)	2.235	10	22	22	1.1	7.2	2.8	5.7	16
	2.298	10	23						
	2.087	10	21						
RW 3 (10x dilution)	1.742	10	17	24	6.7	7.6	< 0.1	2.4	10
	3.497	10	35						
RW 3 (20x dilution)	1.019	20	20						
	1.202	20	24						
	1.101	20	22						
RW 4 (4x dilution)	2.258	4	9.0	9.9	1.2	< 0.1	4.4	< 0.1	4.4
	2.667	4	11						
RW 5 (4x dilution)	1.574	4	6.3	5.9	0.69	3.1	0.19	0.36	3.6
	1.587	4	6.3						
	1.280	4	5.1						
RW 6 (2x dilution)	1.388	2	2.8	2.7	0.57	1.8	< 0.1	0.23	2.0
	1.405	2	2.8						
	1.710	2	3.4						
	1.207	2	2.4						
	0.931	2	1.9						
RW 7	< LOQ	1	< LOQ	<LOQ	NA	< 0.1	< 0.1	< 0.1	< 0.1
	< LOQ	1	< LOQ						
	< LOQ	1	< LOQ						
RW 8	0.885	1	0.89	0.85	0.04	0.27	< 0.1	0.11	0.38
	0.807	1	0.81						
	0.857	1	0.86						
RW 9 (RW Matrix)	0.639	1	0.64	0.62	0.02	0.18	< 0.1	< 0.1	0.18
	0.600	1	0.60						
	0.621	1	0.62						

NA - Standard Deviation was not calculated because sample results were less than the LOQ

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 ADDA Test Kit was easy to use. The brochure and flow charts with illustrations were clear and easy to follow. Solution and sample preparation were minimal, involving dilution of the samples that were initially above the quantification range. The procedure includes three incubation periods that total 2.5 hours. 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 Abraxis had more than 10 years of analytical laboratory experience, but was not experienced with ELISA analysis. A spectrophotometer plate reader is necessary for obtaining the spectrophotometric readings that are then analyzed using any commercial ELISA evaluation program (four-parameters are recommended by the vendor). Once the analysis was complete, the remaining solutions were disposed in the trash in accordance with local regulations.

6.5.2 Cost and Consumables

The listed price for the ADDA Test Kit at the time of the verification test was $440. The kit has a 12-month shelf life as received and should be stored at 4 to 8 ^{o}C. Of the 96-wells on one plate, 16 wells are needed for calibration and control samples. The remaining 80 wells are for sample analyses that are performed in duplicate. Other consumables required for the test, but not included in the kit are pipettes, pipette tips, and distilled or DI water. These can be obtained from the vendor.

Chapter 7
Test Results for the Abraxis DM ELISA Test Kit

The following sections provide the results of the quantitative and qualitative evaluations of this verification test for the Abraxis DM ELISA Test Kit.

7.1 DM Test Kit Summary

As discussed in Chapter 2, the DM Test 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 DM Test Kit, the CR of microcystin LA is 48% and the CR of microcystin RR is 53%. 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 DM Test Kit requires that each standard and sample be analyzed in duplicate, and then the raw data output from the plate reader software reports a mean concentration of the duplicate analyses. Therefore, a sample indicated in Table 1 to have three replicates corresponded to six wells being filled as part of the DM Test Kit. Each DM Test Kit plate contains six calibration solutions. 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 four-parameter curve to quantify the rest of the samples. According to the Abraxis kit instructions, if a sample was out of range and it was determined to be either above or below the calibration range, then it would either be diluted or reported as <LOQ or ND, respectively. The data from a plate of samples was considered acceptable when the positive control was recovered within 25% of 0.75 ppb. 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.

7.2 Test Kit QC Sample

As described in Section 3.3.1, the QC samples analyzed with the Abraxis DM Test Kit included RB samples and the positive and negative 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 below the limit of quantification for the DM Test Kit and are presented in Table 18. Two RB samples were analyzed by the reference method and determined to be below the limit of quantification. It was concluded that microcystin contamination resulting from sample handling did not occur.

Table 18. RB Sample Results for the Abraxis DM Test Kit

Reagent Blank	Plate	Mean Concentration (ppb)
RB 1	1	< 0.15 LOQ
RB 2	1	< 0.15 LOQ
RB 3	1	< 0.15 LOQ
RB 4	3	< 0.15 LOQ
RB 5	3	< 0.15 LOQ
RB 6	3	< 0.15 LOQ

The positive controls for the DM Test Kit are presented in Table 19. The vendor kit instructions stated acceptable range for recovery of the positive control was between 75% and 125%. In addition, the CV of the duplicate analyses was reported as a gauge for accurate quantification of microcystins. The variation between the two data points was considered acceptable when the %CV was less than 25%. A positive control was analyzed at the end of each plate, and in some instances, there were additional positive controls analyzed. This was done to fill in the final column of wells on the 96-well plates. All DM Test Kit plates used for testing produced a positive control result within the acceptable range. During verification testing of the DM Test Kit, all plates were within the %CV and %R acceptance criteria.

Table 19. Positive Control Sample Results for the Abraxis DM Test Kit

Positive Control	Plate	Mean Concentration (ppb)	CV (%)	Percent Recovery (%)
1	1	0.836	9.0	111%
2a	2	0.746	9.1	99%
2b	2	0.740	4.7	99%
3	3	0.603	0.10	80%
4	4	0.756	2.7	101%
5a	5	0.616	6.4	82%
5b	5	0.505	1.3	67%

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 have at least three results, but some samples include four or more replicate results because in instances when the %CV were less than 25%, the individual samples were reanalyzed in duplicate(vendor test kit instructions). If the resulting %CV was acceptable for both repeat samples, they were both included in the result tables, thus resulting in additional data points. In addition, the 0.50 ppb solutions included all seven replicates from the MDL determination data in addition to the triplicate analyses of the 0.50 ppb PT samples.

7.3.1 Accuracy

Tables 20, 21, and 22 also present the accuracy results for the DM Test Kit, expressed as %D. As calculated by Equation 5 (Section 5.1), the reference method value was used for calculation of accuracy. For LR, the reference method ranged from 0% to 17% less than the target spike concentration. For LA the reference values ranged from 15% to 33% lower than the target spike concentration and for RR, they were from 20% to 46% lower 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. Abraxis DM Test 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 for Theoretical Concentration (% Difference)	Accuracy by Variant for Reference Concentration (% Difference)	Reference Concentration (ppb)
0.10 LR	0.176	0.176	76%	76%	0.10
	0.157	0.157	57%	57%	
	0.150	0.150	50%	50%	
	0.183	0.183	83%	83%	
	0.194	0.194	94%	94%	
Avg ± SD	0.172 ± 0.018	0.172 ± 0.018	72% ± 18%	72% ± 18%	
0.50 LR	0.701	0.701	40%	67%	0.42
	0.729	0.729	46%	74%	
	0.683	0.683	37%	63%	
	0.705	0.705	41%	68%	
	0.734	0.734	47%	75%	
	0.619	0.619	24%	47%	
	0.680	0.680	36%	62%	
	0.777	0.777	55%	85%	
	0.816	0.816	63%	94%	
	0.776	0.776	55%	85%	
Avg ± SD	0.722 ± 0.057	0.722 ± 0.057	44% ± 11%	72% ± 14%	
1.0 LR	1.42	1.42	42%	71%	0.83
	1.36	1.36	36%	63%	
	1.33	1.33	33%	61%	
Avg ± SD	1.37 ± 0.046	1.37 ± 0.046	37% ± 5%	65% ± 6%	
2.0 LR	2.44	2.44	22%	28%	1.9
	2.72	2.72	36%	43%	
	2.76	2.76	38%	45%	
Avg ± SD	2.64± 0.174	2.64± 0.174	35% ± 9%	39% ± 9%	
4.0 LR	4.61	4.61	15%	24%	3.7
	4.61	4.61	15%	24%	
	4.77	4.77	19%	29%	
Avg ± SD	4.66 ± 0.097	4.66 ± 0.097	17% ± 2%	26% ± 3%	

Table 21. Abraxis DM Test 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 for Theoretical Concentration (% Difference)	Accuracy by Variant for Reference Concentration (% Difference)	Reference Concentration (ppb)
0.50 LA	0.559	1.17	133%	191%	0.40
	0.575	1.20	140%	199%	
	0.631	1.32	163%	229%	
	0.506	1.05	111%	164%	
	0.536	1.12	123%	179%	
	0.474	0.988	98%	147%	
	0.516	1.08	115%	169%	
	0.534	1.11	123%	178%	
	0.523	1.10	118%	172%	
	0.536	1.12	123%	179%	
Avg ± SD	0.539 ± 0.043	1.12 ± 0.089	125% ± 18%	181% ± 22%	
1.0 LA	0.998	2.08	108%	197%	0.70
	0.975	2.03	103%	190%	
	1.05	2.18	118%	211%	
Avg ± SD	1.01± 0.036	2.10 ± 0.074	110% ± 7%	199% ± 11%	
2.0 LA	2.06	4.30	115%	153%	1.7
	2.29	4.78	139%	181%	
	2.12	4.42	121%	160%	
Avg ± SD	2.16 ± 0.120	4.50 ± 0.249	125% ± 12%	165% ± 15%	
4.0 LA	3.72	7.74	93%	158%	3.0
	3.82	7.97	99%	165%	
	3.75	7.81	95%	160%	
Avg ± SD	3.76 ± 0.055	7.84 ± 0.115	96% ± 3%	161% ± 4%	

Table 22. Abraxis DM Test 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 for Theoretical Concentration (% Difference)	Accuracy by Variant for Reference Concentration (% Difference)	Reference Concentration (ppb)
0.50 RR	0.501	0.945	89%	149%	0.38
	0.482	0.909	82%	139%	
	0.471	0.889	78%	134%	
	0.506	0.955	91%	151%	
	0.411	0.775	55%	104%	
	0.417	0.787	57%	107%	
	0.399	0.753	51%	98%	
	0.364	0.687	37%	81%	
	0.532	1.00	101%	164%	
	0.468	0.883	77%	132%	
Avg ± SD	0.455 ± 0.054	0.859 ± 0.103	72% ± 21%	126% ± 27%	
1.0 RR	1.01	1.90	90%	252%	0.54
	1.03	1.94	94%	260%	
	1.03	1.93	93%	258%	
Avg ± SD	1.02 ± 0.012	1.93 ± 0.022	93% ± 2%	257% ± 4%	
2.0 RR	2.07	3.90	95%	144%	1.6
	1.95	3.69	84%	130%	
	1.95	3.68	84%	130%	
Avg ± SD	1.99 ± 0.066	3.75 ± 0.125	88% ± 6%	135% ± 8%	
4.0 RR	3.84	7.24	81%	126%	3.2
	3.72	7.03	76%	120%	
	3.67	6.92	73%	116%	
Avg ± SD	3.74 ± 0.087	7.06 ± 0.164	77% ± 4%	121% ± 5%	

For the LR spiked samples, the reference method results ranged from 0% to 17% less than the target spike concentration. For LR, the %D ranged from 24% to 94%. For the 0.10 ppb samples, the %D ranged from 50% to 94%, but the absolute difference from the reference concentration was never more than 0.094 ppb. For the 0.50 ppb samples, the %D ranged from 47% to 94%, corresponding to a maximum absolute difference from the reference concentration of 0.396 ppb. For the 1.0 ppb samples, the %D ranged from 61% to 71% and the maximum absolute difference from the reference concentration was 0.592 ppb. For the 2.0 ppb samples, the %D ranged from 28% to 45% and the maximum absolute difference from the reference concentration was 0.856 ppb. For the 4.0 ppb samples, the three reported results had %Ds of less than 29%. For LR, the %D when compared to the theoretical spike concentration ranged from -24% to 94% with the overall average %D values ranging from 26% to 72%.

For the LA spiked samples, the reference method results ranged from 15% to 33% less than the target spike concentration, and 20 to 46% lower than the target spike concentration for the RR spiked samples. For LA and RR, the %D ranged from 147% to 229% and 81% to 260%, respectively. These %Ds are calculated based on the concentration being corrected for the CR of the LA and RR variant. However, for both variants, the data suggest that the uncorrected results in LR equivalents would provide concentrations that were much more similar to the reference method concentrations.

7.3.2 Precision

Precision results for the Abraxis DM Test 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 2% to 11% for the LR variant, from 1% to 9% for the LA variant, and from 1% to12 % for the RR variant. The RSD results for the RW samples ranged from 2% to 9%. The overall average of all RSDs was 7%, with a minimum of 1% and a maximum of 35%.

Table 23. Abraxis DM Test Kit Precision Results

Variant	Sample Concentration in DI	Precision (%RSD)
LR	0.10 ppb	11%
	0.50 ppb	8%
	1.0 ppb	3%
	2.0 ppb	7%
	4.0 ppb	2%
	2.0 ppb LR in 1 mg/L Chlorophyll-*a* DI	10%
	2.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	3%
	2.0 ppb LR in 10x dilution of RW Matrix	5%
	2.0 ppb LR in RW Matrix	6%
LA	0.50 ppb	8%
	1.0 ppb	4%
	2.0 ppb	6%
	4.0 ppb	1%
	2.0 ppb LA in 1 mg/L Chlorophyll-*a* DI	3%
	2.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	9%
	2.0 ppb LA in 10x dilution of RW Matrix	3%
	2.0 ppb LA in RW Matrix	4%
RR	0.50 ppb	12%
	1.0 ppb	1%
	2.0 ppb	3%
	4.0 ppb	2%
	2.0 ppb RR in 1 mg/L Chlorophyll-*a* DI	3%
	2.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	4%
	2.0 ppb RR in 10x dilution of RW Matrix	2%
	2.0 ppb RR in RW Matrix	2%
Unknown	RW 1 (10x dilution)	7%
	RW 1 (20x dilution)	3%
	RW 2 (10x dilution)	6%
	RW 2 (20x dilution)	5%
	RW 3 (10x dilution)	3%
	RW 3 (20x dilution)	4%
	RW 4	8%
	RW 4 (4x dilution)	5%
	RW 5	2%
	RW 5 (4x dilution)	4%
	RW 6	9%
	RW 6 (2x dilution)	2%
	RW 7	NA
	RW 8	6%
	RW 9	5%

NA - Result was less than the LOQ so no calculation of RSD

7.3.3 Linearity

The linearity of the DM Test Kit measurements was assessed by performing a linear regression of the DM Test Kit results against the reference method results for the five PT samples ranging from 0.10 to 4.0 ppb of microcystin LR in DI water and four PT samples ranging from 0.50 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. All linear regressions compared to the reference method results had $r^2 > 0.98$.

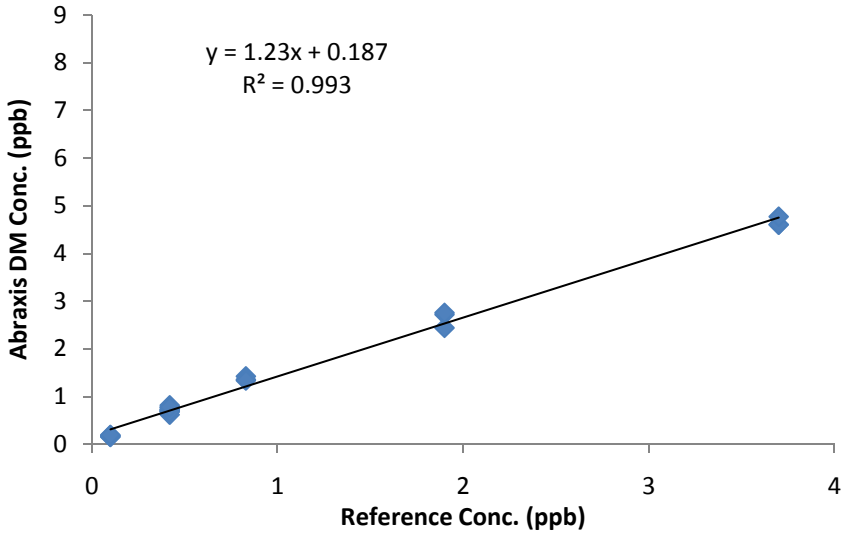

Figure 6. Linearity for the Abraxis DM Test Kit for LR

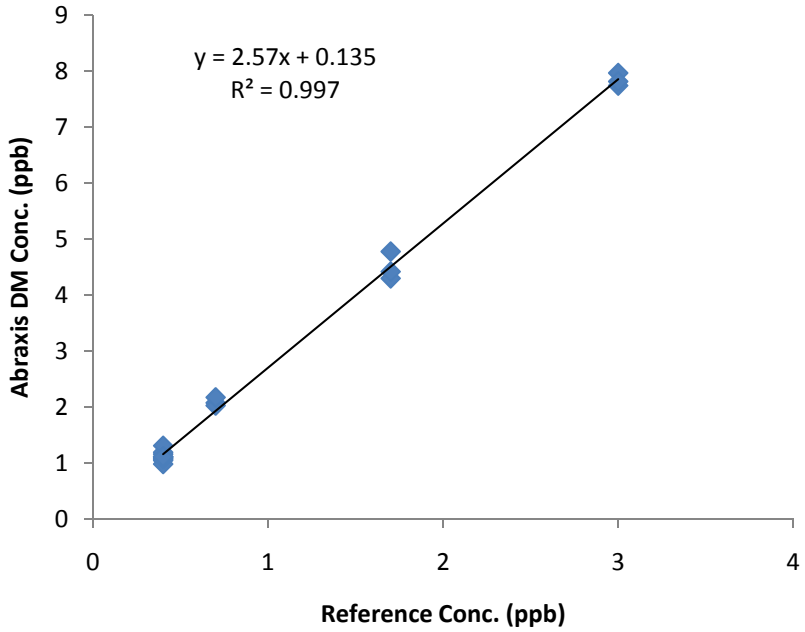

Figure 7. Linearity for the Abraxis DM Test Kit for LA

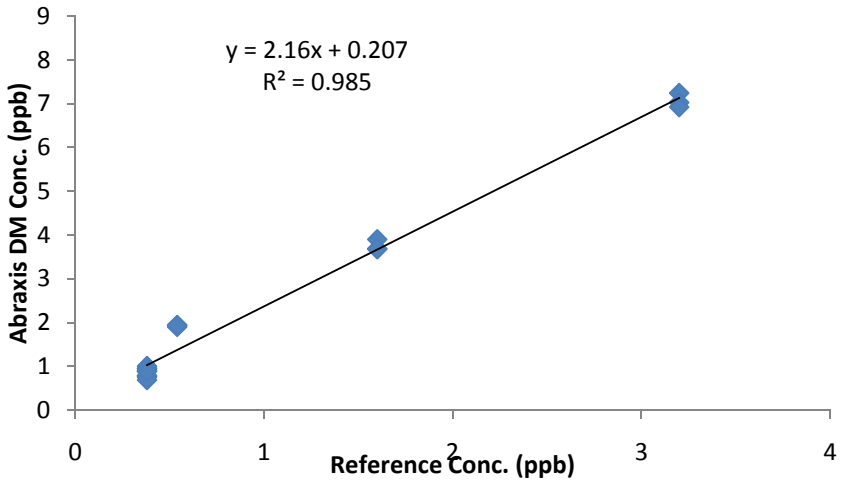

Figure 8. Linearity for the Abraxis DM Test Kit for RR

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 DM Test 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 0.11, 0.08, and 0.10 ppb for LR, LA, and RR, respectively.

Table 24. Detection Limit Results for the Abraxis DM Test Kit

Variant	LR		LA		RR	
Sample Concentration (ppb)	Mean Conc. (ppb)	%CV	Mean Conc. (ppb LR Equivalents)	%CV	Mean Conc. (ppb LR Equivalents)	%CV
0.50	0.70	2.0	0.56	2.2	0.50	13
0.50	0.73	2.9	0.58	11	0.48	4.9
0.50	0.68	4.7	0.63	4.4	0.47	5.5
0.50	0.71	17	0.51	7.2	0.51	3.9
0.50	0.73	11	0.54	6.7	0.41	4.0
0.50	0.62	3.5	0.47	3.4	0.42	13
0.50	0.68	2.8	0.52	2.5	0.40	6.2
0.50	0.78	1.4	0.53	2.8	0.36	2.6
0.50	0.82	5.1	0.52	13	0.53	2.3
0.50	0.78	7.6	0.54	4.2	0.47	2.8
Standard Deviation	0.06		0.04		0.05	
t (n=10)	1.8		1.8		1.8	
MDL	**0.11**		**0.08**		**0.10**	

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 data are presented in Table 25. 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. The RPDs were less than 10% and all but five were less than 5%.

Table 25. Inter-kit lot Comparison of Kit Calibration Standards for the DM Test Kit

Standard (ppb)	OD Values		RPD
	Set A	Set B	
0	1.14	1.12	2%
	1.12	1.16	4%
0.15	0.975	0.953	2%
	0.996	0.957	4%
0.40	0.762	0.772	1%
	0.719	0.711	1%
1.0	0.489	0.442	10%
	0.451	0.439	3%
2.0	0.294	0.276	6%
	0.288	0.265	8%
5.0	0.144	0.154	7%
	0.146	0.158	8%

7.3.6 Matrix Effects

Matrix interference effects were assessed by using a t-test to compare the DM Test Kit results generated from samples made by 2.0 ppb spiking of 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 chlorophyll-*a* at 1 mg/L and 10 mg/L. Tables 26 and 27 provide the DM Test 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 the difference between the two sets of data is zero. The resulting probability (p)-value gives the likelihood that the null hypothesis would be true. Therefore, at the 95% confidence interval, p-values less than 0.05 indicate there is a small likelihood of the null hypothesis being true and therefore a significant difference between the two sets of data (at low power).

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. Both the RW matrix results for LA and the 10x RW sample were significantly different from the DI water results and the diluted and undiluted RW were also significantly different from one another. Table 26 shows the 2.0 ppb LA spike into DI water generated an average result of 2.16 ppb compared with the average result of 1.71 and 2.02 ppb for the diluted and undiluted RW samples, respectively. The chlorophyll-*a* results for LR, LA, and RR were all statistically different when compared to the DI results except the 1 mg/L chlorophyll-*a* solutions spiked with RR ($p = 0.169$). Table 27 shows the 2.0 ppb LR spike into DI water generated an average result of 2.64 ppb compared with an average result of 0.470 and 0.480 ppb for the spike into 1.0 mg/L and 10 mg/L chlorophyll-*a*, respectively. For LA, the 2.0 ppb spike into DI water generated an average result of 2.16 ppb compared with an average result of 0.340 and 0.320 ppb for the spike into 1.0 mg/L and 10 mg/L chlorophyll-*a*, respectively.

There was no difference determined when comparing the two levels of chlorophyll-*a* solution results for all three variants. All of the undiluted and diluted RW samples were significantly different from one another for all three variants. The spiked undiluted RW samples each exhibited a higher microcystin concentration than did the diluted RW sample even after the samples were corrected for any background microcystin present.

Table 26. RW Matrix Interferent Sample Results for the Abraxis DM Test 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)	0.44	0.470	0.025	
		0.48			
		0.47			
LR	2.0 ppb LR in DI	2.43	2.64	0.174	2.43
		2.72			2.72
		2.75			2.75
	2.0 ppb LR in tenfold dilution of RW Matrix	2.34	2.44	0.111	2.34
		2.40			2.40
		2.56			2.56
	2.0 ppb LR in RW Matrix	3.48	3.26	0.197	3.48
		3.10			3.10
		3.20			3.20
LA	2.0 ppb LA in DI	2.06	2.16	0.120	4.30
		2.29			4.78
		2.12			4.42
	2.0 ppb LA in tenfold dilution of RW Matrix	1.69	1.71	0.045	3.53
		1.75			3.66
		1.67			3.48
	2.0 ppb LA in RW Matrix	1.93	2.02	0.090	4.04
		2.11			4.41
		2.00			4.17
RR	2.0 ppb RR in DI	2.06	1.99	0.066	3.90
		1.95			3.69
		1.94			3.68
	2.0 ppb RR in tenfold dilution of RW Matrix	1.81	1.80	0.040	3.42
		1.83			3.47
		1.75			3.32
	2.0 ppb RR in RW Matrix	2.13	2.16	0.049	4.04
		2.21			4.19
		2.12		0.025	4.02

Table 27. Chlorophyll-*a* Interferent Sample Results for the Abraxis DM Test 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	2.44	2.64	0.174	2.44
		2.72			2.72
		2.76			2.76
	2.0 ppb LR in 1 mg/L Chlorophyll-*a* DI	0.482	0.470	0.047	0.482
		0.503			0.503
		0.413			0.413
	2.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	0.492	0.480	0.016	0.492
		0.477			0.477
		0.460			0.460
LA	2.0 ppb LA in DI	2.06	2.16	0.120	4.30
		2.29			4.78
		2.12			4.42
	2.0 ppb LA in 1 mg/L Chlorophyll-*a* DI	0.350	0.340	0.011	0.729
		0.343			0.715
		0.328			0.683
	2.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	0.293	0.320	0.030	0.610
		0.323			0.673
		0.352			0.733
RR	2.0 ppb RR in DI	2.07	1.99	0.066	3.90
		1.95			3.69
		1.95			3.68
	2.0 ppb RR in 1 mg/L Chlorophyll-*a* DI	1.82	1.86	0.054	3.43
		1.92			3.62
		1.84			3.46
	2.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	2.14	2.06	0.077	4.04
		1.99			3.76
		2.04			3.84

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. 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 28. Statistical Comparisons between Interference Samples for the Abraxis DM Test Kit

Description of Comparison	p-value (D-different, ND-not different)		
	LR	LA	RR
2.0 ppb in DI compared with 2.0 ppb in tenfold dilution of RW	0.087 (ND)	0.011 (D)	0.042 (D)
2.0 ppb in DI compared with 2.0 ppb in undiluted RW	0.097 (ND)	0.017 (D)	0.090 (ND)
2.0 ppb in undiluted RW compared with tenfold dilution of RW	0.034 (D)	0.012 (D)	0.002 (D)
2.0 ppb in DI compared with 2.0 ppb in 1 mg/L Chlorophyll-*a* DI	0.003 (D)	0.001 (D)	0.169 (ND)
2.0 ppb in DI compared with 2.0 ppb in 10 mg/L Chlorophyll-*a* DI	0.003 (D)	0.001 (D)	0.044 (D)
2.0 ppb in 1 mg/L Chlorophyll-*a* DI compared with 2.0 ppb in 10 mg/L Chlorophyll-*a* DI	0.672 (ND)	0.529 (ND)	0.111 (ND)

Shading indicates a statistically significant difference

7.4 RW Sample Results

Table 29 presents the RW results for the DM Test 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 DM Test Kit may have other variants present that would not have been detected by the reference method. Therefore, no quantitative comparison was made between the ADDA Test Kit and the reference method results. The reference data were converted into LR-equivalents according to the DM Test Kit cross reactivity for the variants. In general, the samples that were determined to have higher total concentrations by the DM Test Kit had higher total concentrations as determined by the reference method. All of the DM Test Kit total microcystin results were greater than the reference method results, which only quantified three variants. However, the results of the DM Test Kit were usually within a factor of two of the reference method. The LR, LA, and RR variants likely make up a significant proportion of the microcystins that are measurable by the DM Test Kit.

Table 29. Recreational Water Sample Results for the Abraxis DM Test Kit

Sample Description	Test Kit Results: Results: LR Equivalents (ppb)	Dilution Factor	CR Corrected Conc. (ppb)	Average Conc. (ppb)	Standard Deviation (ppb)	Reference Results (LR Equivalents ppb): LR	LA	RR	Total
RW 1 (10x dilution)	2.417	10	24	23	1.0	9.6	0.87	10	20
	2.194	10	22						
RW 1 (20x dilution)	1.168	20	23						
	1.157	20	23						
	1.226	20	25						
RW 2 (10x dilution)	1.410	10	14	14	0.72	7.2	1.1	3.3	12
	1.298	10	13						
RW 2 (20x dilution)	0.721	20	14						
	0.668	20	13						
	0.735	20	15						
RW 3 (10x dilution)	1.427	10	14	14	0.49	7.6	< 0.1	1.4	9.0
	1.362	10	14						
	1.337	10	13						
RW 3 (20x dilution)	0.737	20	15						
	0.698	20	14						
	0.690	20	14						
RW 4	3.914	1	3.9	4.1	0.26	< 0.1	1.7	< 0.1	1.7
	4.039	1	4.0						
	4.543	1	4.5						
RW 4 (4x dilution)	1.079	4	4.3						
	0.974	4	3.9						
	1.003	4	4.0						
RW 5	4.568	1	4.6	4.6	0.16	3.1	0.07	0.21	3.3
	4.608	1	4.6						
	4.410	1	4.4						
RW 5 (4x dilution)	1.128	4	4.5						
	1.221	4	4.9						
	1.154	4	4.6						
RW 6	1.682	1	1.7	1.8	0.11	1.8	< 0.1	0.14	1.9
	1.881	1	1.9						
	2.009	1	2.0						
RW 6 (2x dilution)	0.904	2	1.8						
	0.934	2	1.9						
	0.924	2	1.8						
RW 7	<LOQ	1	<LOQ	<LOQ	NA	< 0.1	< 0.1	< 0.1	< 0.1
	<LOQ	1	<LOQ						
	ND	1	ND						
RW 8	0.633	1	0.63	0.67	0.04	0.27	< 0.1	0.06	0.33
	0.715	1	0.72						
	0.667	1	0.67						
RW 9 (RW Matrix)	0.441	1	0.44	0.47	0.03	0.18	< 0.1	< 0.1	0.18
	0.489	1	0.49						
	0.475	1	0.48						

NA - Standard Deviation was not calculated because sample results were less than the LOQ

7.5 Operational Factors

During testing activities, the technical operators were instructed to fill out an Ease of Use Questionnaire that is an appendix in the TQAP[1] for this verification test. 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 DM test kit was easy to use. The brochure and flow charts with illustrations were clear and easy to follow. 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 total 2 hours. The solutions in the kit produce a color change in the wells, confirming that those wells contain the solution. This feature is extremely helpful as technicians can become confused about which wells have had the solution added and which ones have not when analyzing 96-well plates. Previous knowledge or training on the use of micro-pipettes and/or multi-channel pipettes is recommended for consistent readings. The Battelle operator that was trained by Abraxis had more than 10 years of analytical laboratory experience, but was not experienced with ELISA analysis. A spectrophotometer plate reader is necessary for obtaining the readings that are then analyzed using any commercial ELISA evaluation program (four-parameter is recommended by the vendor). Once the analysis was complete, the remaining solutions and well contents were disposed in the trash in accordance with local regulations.

7.5.2 Cost and Consumables

The listed price for the DM test kit at the time of the verification test was $400. The kit has a 12-month shelf life as received and should be stored at 4 to 8 ^{o}C. Of the 96-wells on one plate, 16 wells are needed for calibrators and controls. The remaining 80 wells are for sample analyses that are performed in duplicate. Other consumables required for the test, but not included in the kit are pipettes, pipette tips, and distilled or DI water. These can be obtained from the vendor.

Chapter 8
Test Results for the Abraxis Strip Test Kit

The following subsections provide the results of the Abraxis Strip Test Kit.

8.1 Abraxis Strip Test Summary

As described in Section 2.3, the Strip Test is a qualitative test for microcystins and nodularins. The Strip Test semi-quantitative results in the range of 0 to 10 ppb can be obtained by comparing the test line intensity to those that have been produced by solutions of known microcystin concentrations and are shown in the Strip Test brochure. In summary, the test strip is exposed to a water sample; as the water moves up the test strip through capillary action, the water does not inhibit an antibody antigen interaction. This uninhibited antibody antigen interaction results in the appearance of a control line in the test line region of the test strip. The control line should be present on the test strip when wetted, and by design, is not influenced by the presence or absence of microcystins in the water sample. Therefore, it should be present whenever testing is being conducted. In the absence of microcystins or nodularins in water being tested, a second line that is the same color, but can vary in darkness in comparison to the control line, appears in the test line region. However, if microcystins or nodularins are present, the second line appears lighter in color than the control line or does not appear at all, leaving only the control line. The Strip Test brochure contains an interpretation figure that depicts the relative darkness of the second line to the control line over the microcystin detection range. This figure was used to interpret the observations of the test strips. During this ETV test, when the control line and the test line were observed to be the same color (denoted as dark line,line, or faint line), the microcystin concentration was interpreted as 0 to 10 ppb and when the test line was lighter in color than the control line or absent (denoted as no line) the concentration was interpreted to be greater than 10 ppb. The Strip Test includes a lysing procedure for the determination of total microcystins called, QuikLyse^{TM}. The Strip Test and QuikLyse^{TM} reagents are designed to be used in combination. According to Abraxis, use of the Strip Test without the QuikLyse^{TM} reagents will adversely affect the performance of the test, producing inaccurate results.

8.2 Test Kit QC Sample

The QC samples analyzed with the Abraxis Strip Test Kit included RB samples. In addition, each test strip included the control line as described above. Ten percent of all samples analyzed were RB samples. The results of all RB sample results were 0 to 10 ppb and are presented in Table 30. The RB sample was analyzed by the reference method and was determined to be below the LOQ. The control line on each test strip for the RB samples and all test samples appeared as expected and therefore, all results were accepted and none of the samples were re-analyzed.

Table 30. RB Sample Results for the Abraxis Strip Test Kit

Sample Description	Batch	Control line? (Y/N)	Observation	Interpretation
RB 1	1	y	Line	0 – 10 ppb
RB 2	1	y	Line	0 – 10 ppb
RB 3	1	y	Line	0 – 10 ppb
RB 4	4	y	Dark Line	0 – 10 ppb
RB 5	4	y	Dark Line	0 – 10 ppb
RB 6	4	y	Dark Line	0 – 10 ppb
RB 7	7	y	Dark Line	0 – 10 ppb
RB 8	7	y	Dark Line	0 – 10 ppb
RB 9	7	y	Dark Line	0 – 10 ppb

8.3 PT Samples

8.3.1 DI Water Samples

Tables 31, 32, and 33 present the results for the PT samples (concentrations between 0.10 and 15 ppb) for the three variants of microcystin used during this verification test. All of the samples were analyzed in triplicate and each of them produced a control line on the Test Strip to indicate that the Test Strip was functioning properly. As the concentrations of the various microcystin variants increased, the line generated in the test area region was observed to change as expected. The lowest concentration samples generated dark lines, the mid-level concentrations generated lighter lines, and the highest concentrations were either very faint lines or generated no line at all. The line colors were consistent within the three replicates at each concentration and were consistent with the results from the reference method with one exception. The 7.0 ppb RR PT sample did not generate a line, which indicated a concentration of greater than 10 ppb. The results of the Strip Test kit identified solutions with > 10 ppb microcystin concentration.

Table 31. Abraxis Strip Test Kit Microcystin-LR DI Water Sample Results

Sample Description	Control line? (Y/N)	Observation	Interpretation	Reference Concentration (ppb)
0.10 ppb LR	y	Dark Line	0 – 10 ppb	0.10
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
0.50 ppb LR	y	Line	0 – 10 ppb	0.42
	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
1.0 ppb LR	y	Line	0 – 10 ppb	0.83
	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
2.0 ppb LR	y	Line	0 – 10 ppb	1.9
	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
4.0 ppb LR	y	Faint line	0 – 10 ppb	3.7
	y	Faint line	0 – 10 ppb	
	y	Faint line	0 – 10 ppb	
15 ppb LR	y	No line	> 10 ppb	15
	y	No line	> 10 ppb	
	y	No line	> 10 ppb	

Table 32. Abraxis Strip Test Kit Microcystin-LA DI Water Sample Results

Sample Description	Control line? (Y/N)	Observation	Interpretation	Reference Concentration (ppb)
0.50 ppb LA	y	Dark Line	0 – 10 ppb	0.40
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
1.0 ppb LA	y	Dark Line	0 – 10 ppb	0.70
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
2.0 ppb LA	y	Line	0 – 10 ppb	1.7
	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
4.0 ppb LA	y	Faint line	0 – 10 ppb	3.0
	y	Faint line	0 – 10 ppb	
	y	Faint line	0 – 10 ppb	
7.0 ppb LA	y	Very Faint line	0 – 10 ppb	4.7
	y	Very Faint line	0 – 10 ppb	
	y	Very Faint line	0 – 10 ppb	
15 ppb LA	y	No line	> 10 ppb	12
	y	No line	> 10 ppb	
	y	No line	> 10 ppb	

Table 33. Abraxis Strip Test Kit Microcystin-RR DI Water Sample Results

Sample Description	Control line? (Y/N)	Observation	Interpretation	Reference Concentration (ppb)
0.50 ppb RR	y	Dark Line	0 – 10 ppb	0.38
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
1.0 ppb RR	y	Line	0 – 10 ppb	0.54
	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
2.0 ppb RR	y	Line	0 – 10 ppb	1.6
	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
4.0 ppb RR	y	Faint line	0 – 10 ppb	3.2
	y	Faint line	0 – 10 ppb	
	y	Faint line	0 – 10 ppb	
7.0 ppb RR	y	No line	> 10 ppb	4.5
	y	No line	> 10 ppb	
	y	No line	> 10 ppb	
15 ppb RR	y	No line	> 10 ppb	15
	y	No line	> 10 ppb	
	y	No line	> 10 ppb	

8.3.2 Matrix Interference Samples

Matrix interference Table 34 presents the RW matrix interference sample results for the Strip Test kit and Table 35 presents the chlorophyll-*a* interference sample results. The triplicate analyses of the samples all agreed and the interpretation of the results is consistent with the spiked amount of microcystins in the samples. Consequently, there was no indication that the different matrices affected the test kit performance.

Table 34. RW Matrix Interferent Sample Results for the Strip Test Kit

Variant	Sample Description	Control line? (Y/N)	Observation	Interpretation
LR	4.0 ppb LR in DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb LR in 10x dilution of RW Matrix	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb LR in RW Matrix	y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
LA	4.0 ppb LA in DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb LA in 10x dilution of RW Matrix	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb LA in RW Matrix	y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
RR	4.0 ppb RR in DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb RR in 10x dilution of RW Matrix	y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
	4.0 ppb RR in RW Matrix	y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb

Table 35. Matrix Interferent Sample Results for the Abraxis Strip Test Kit

Variant	Sample Description	Control line? (Y/N)	Observation	Interpretation
LR	4.0 ppb LR in DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb LR in 1 mg/L Chlorophyll-*a* DI	y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
	4.0 ppb LR in 10 mg/L Chlorophyll-*a* DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
LA	4.0 ppb LA in DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb LA in 1 mg/L Chlorophyll-*a* DI	y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
	4.0 ppb LA in 10 mg/L Chlorophyll-*a* DI	y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
		y	Very Faint line	0 – 10 ppb
RR	4.0 ppb RR in DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb RR in 1 mg/L Chlorophyll-*a* DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
	4.0 ppb RR in 10 mg/L Chlorophyll-*a* DI	y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb
		y	Faint line	0 – 10 ppb

8.4 RW Samples

Table 36 presents the RW results for the Strip Test Kit and the reference method results. The microcystin concentrations in the samples that were determined by the reference method represent three of the approximately 80 variants that are naturally occurring in RWs. The total measured microcystin result may have other variants present that cannot be detected by the reference method. To assess the lysing procedure, aliquots of RW 6, RW 7, and RW 8 were analyzed before and after the sample went through the freeze–thaw lysing procedure. The observations and interpretations of these RW samples were not different with respect to lysing procedures. With the exception of RW 5, the triplicate measurements of the samples generated lines that were very similar in intensity. In two replicates, RW 5 generated faint lines indicating a 0 to 10 ppb concentration and one replicate that had no line, indicating a concentration of greater than 10 ppb. Again, except for one exception for RW 5, the sample results were consistent with the reference laboratory results of the RW samples. That is, with the one RW 5 exception, when the reference concentrations were greater than 10 ppb, there was no line generated; when the reference concentration was between 2.0 and 4.0 ppb, the lines were medium dark or faint, and when the reference concentrations were lower than 2.0, the lines generated were dark.

Table 36. Recreational Water Sample Results for the Abraxis Strip Test Kit

Sample Description	Control line? (Y/N)	Observation	Interpretation	Reference Concentration (ppb)
RW 1	y	No line	> 10 ppb	30
	y	No line	> 10 ppb	
	y	No line	> 10 ppb	
RW 2	y	No line	> 10 ppb	16
	y	No line	> 10 ppb	
	y	No line	> 10 ppb	
RW 3	y	No line	> 10 ppb	10
	y	No line	> 10 ppb	
	y	No line	> 10 ppb	
RW 4	y	Very Faint line	0 – 10 ppb	3.5
	y	Very Faint line	0 – 10 ppb	
	y	Very Faint line	0 – 10 ppb	
RW 5	y	Very Faint line	0 – 10 ppb	3.6
	y	Very Faint line	0 – 10 ppb	
	y	No line	> 10 ppb	
RW 6	y	Line	0 – 10 ppb	2.0
	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
RW 6 Not lysed by freeze-thaw method	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
	y	Line	0 – 10 ppb	
RW 7	y	Dark Line	0 – 10 ppb	< LOQ
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
RW 7 Not lysed by freeze-thaw method	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
RW 8	y	Dark Line	0 – 10 ppb	0.39
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
RW 8 Not lysed by freeze-thaw method	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	
RW 9 (RW Matrix)	y	Dark Line	0 – 10 ppb	0.18
	y	Dark Line	0 – 10 ppb	
	y	Dark Line	0 – 10 ppb	

8.5 Operational Factors

During testing activities, the technical operators were instructed to fill out an Ease of Use Questionnaire that is an appendix in the TQAP[1] for this verification test. This section summarizes these observations, as well as other considerations regarding the technology.

8.5.1 Ease of Use

The test kit operator reported that the Strip Test Kit was very easy to use and needs no technical skills to operate (although the Battelle operator was a trained technician). The brochure and flow charts with illustrations were clear and easy to follow. There was no solution or sample preparation needed. The entire procedure is approximately 40 minutes long, including the QuikLyse™ procedure and the microcystins analysis. The QuikLyse™ process uses 1 mL of sample through 2 × 8 minute incubation periods. Then the sample is transferred into the microcystins reagent conical tube. The sample is incubated for 10 minutes and then the test strip is added to the conical tube. The test strip is interpreted according to the figure in the brochure after 5 minutes of exposure to the sample.

8.5.2 Cost and Consumables

There were no consumables required for this technology. The test strips were disposed in the regular trash after use, producing no hazardous waste.

The listed price for the Strip Test kit at the time of the verification test was $480 for a 20 strip kit and $150 for a five strip kit. Each kit has a 12-month shelf life as received and should be stored at room temperature.

Chapter 9
Performance Summary for the Abraxis ADDA, DM, and Strip Test

9.1 Performance Summary for the ADDA ELISA Test Kit

The verification of the Abraxis ADDA ELISA Test Kit is summarized by the parameters described in Table 37.

Table 37. Abraxis ADDA Test Kit Performance Summary

Verification Parameters	LR	LA	RR
Accuracy (range of % difference from reference method; low %Ds indicate increased accuracy)			
0.10 ppb	-13% to 2%		
0.50 ppb	-9% to 40%	27% to 109%	42% to 64%
1.0 ppb	19% to 58%	77% to 133%	98% to 123%
2.0 ppb	-45% to 39%	69% to 113%	23% to 69%
4.0 ppb	-6% and 3%	105% and 113%	11% to 50%
Precision (range of %RSD)	5% to 45% (7 of 9 samples < 16%)	3% to 25%	4% to 16%
Precision (RW samples)	3% to 47%, all except 2 RSDs were < 12%		
Linearity (y=)	0.933x + 0.087 r^2=0.906	2.66x - 0.220 r^2=0.990	1.18x + 0.168 r^2=0.961
Method Detection Limit (ppb)	0.137	0.218	0.047

Inter-kit lot reproducibility. Calibration standards from two different lots were measured and the RPD of the resulting optical densities ranged from 0% to 13% with eight of the 12 being less than 6%.

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. For chlorophyll-*a* and RW matrices, only three of the 18 comparisons resulted in statistically significant differences: 1) 2.0 ppb LA spike into DI water was significantly different from the 2.0 ppb LA spike into both 1 mg/L (p=0.005); 2) 10 mg/L (p=0.006) chlorophyll-*a*; and 3) The other statistically significant difference was between the RR spikes into undiluted and diluted RW (p=0.01). Due to the limited number of replicates and low statistical power of this study, additional testing would be required to provide a better understanding as to whether there is a matrix interference due to chlorophyll-*a*.

Recreational Water (RW). Because the reference method did not measure all possible microcystin variants, no quantitative comparison was made between the ADDA Test Kit and the reference method results. The reference data were converted into LR-equivalents according to the ADDA Test Kit cross reactivity for the variants. In general, the samples that were determined to have higher total concentrations by the ADDA Test Kit had higher total concentrations as determined by the reference method. All of the ADDA test kit total microcystin results were greater than the reference method results, which was consistent with the likelihood that all of the microcystins were not being measured by the reference method.

Operational Factors. The test kit operator reported that the ADDA Test Kit was easy to use. Solution or sample preparation was minimal, mostly involving diluting the samples that were initially above the quantification range. The procedure included three incubation periods that total 2.5 hours. 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 (four-parameter is recommended by the vendor). Once the analysis was complete, the remaining solutions were disposed of in the trash in accordance with local regulations.

The listed price for the ADDA Test Kit at the time of the verification test was $440. The kit has a 12-month shelf life as received and should be stored at 4 to 8 ^{o}C. Of the 96-wells on one plate, 16 wells are needed for calibration and control samples. The remaining 80 wells are for sample analyses that are performed in duplicate. Other consumables not included in the kit are pipettes, pipette tips, and distilled or DI water can be provided by the vendor.

9.2 Performance Summary for the DM ELISA Test Kit

The verification of the Abraxis DM ELISA Test Kit is summarized by the parameters described in Table 38.

Table 38. Abraxis DM Test Kit Performance Summary

Verification Parameters	LR	LA	RR
Accuracy (range of % difference from reference method; low %Ds indicate increased accuracy)			
0.10 ppb	50% to 94%		
0.50 ppb	47% to 94%	For LA, 147% to 229%, and for RR, 81% to 260%. For both variants, the data suggested that the uncorrected results in LR equivalents would provide concentrations that were more similar to the reference method concentrations.	
1.0 ppb	61% to 71%		
2.0 ppb	28% to 45%		
4.0 ppb	24% to 29%		
Precision (range of %RSD)	2% to 11%	1% to 9%	1% to12 %
Precision (RW samples)	2% to 9%		
Linearity (y=)	1.23x + 0.187 r^2 = 0.993	2.57x + 0.135 r^2 = 0.997	2.16x + 0.207 r^2 = 0.985
Method Detection Limit (ppb)	0.105	0.078	0.099

Inter-kit lot reproducibility. Calibration standards from two different lots were measured and the RPD of the resulting optical densities ranged from 1% to 10% with seven of the 12 being less than 5%.

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. Both the RW matrix results for LA and the 10x RW sample were significantly different from the DI water results and the diluted and undiluted RW were also significantly different from one another. The chlorophyll-*a* results for LR, LA, and RR were all statistically different when compared to the DI results except the 1 mg/L chlorophyll-*a* solutions spiked with RR ($p = 0.169$). There was no difference determined when comparing the two levels of chlorophyll-*a* solution results for all three variants. All of the undiluted and diluted RW samples were significantly different from one another for all three variants. The spiked undiluted RW samples each exhibited a higher microcystin concentration than did the diluted RW sample even after the samples were corrected for any background

microcystin present. Due to the limited number of replicates and low statistical power of this study, additional testing would be required to provide a better understanding as to whether there is a matrix interference due to chlorophyll-*a*.

Recreational Water (RW). Because the reference method did not measure all possible microcystin variants, no quantitative comparison was made between the DM Test Kit and the reference method results. The reference data were converted into LR-equivalents according to the DM Test Kit cross reactivity for the variants. As for the ADDA Test Kit, the samples that were determined to have higher total concentrations by the DM Test Kit had higher total concentrations as determined by the reference method. All of the DM Test Kit total microcystin results were greater than the reference method results, which was consistent with the likelihood that all of the microcystins were not being measured by the reference method.

Operational Factors. The test kit operator reported that the DM test kit was easy to use. Solution or sample preparation was minimal, mostly involving diluting samples that were above the quantification range. The procedure included two incubation periods that total 2 hours. The solutions in the kit produce a color change in the wells, confirming that those wells contain the solution. This feature was extremely helpful as technicians can become confused about what wells have had the solution added and which ones have not when analyzing 96-well plates. 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 (four-parameter is recommended by the vendor). Once the analysis was complete, the remaining solutions were disposed of in the trash in accordance with local regulations.

The listed price for the DM test kit at the time of the verification test was $400. The kit has a 12-month shelf life as received and should be stored at 4 to 8 ^{o}C. Of the 96-wells on one plate, 16 wells were needed for calibrators and controls. The remaining 80 wells are for sample analyses that are performed in duplicate. Other consumables not included in the kit are pipettes, pipette tips, and distilled or DI water can be provided by the vendor.

9.3 Performance Summary for the Strip Test Kit

The verification of the Abraxis Strip Test is summarized in Table 39 and by the parameters described below.

Table 39. Abraxis Strip Test Kit Performance Summary

Sample Description	LR Results		LA Results		RR Results	
Spiked Conc.	(Observation and Interpretation)		(Observation and Interpretation)		(Observation and Interpretation)	
0.10 ppb	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb
	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb
	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb
0.50 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
1.0 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
2.0 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
4.0 ppb	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb
	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb
	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb
7.0 ppb	NA	NA	Very Faint line	0 – 10 ppb	No line	> 10 ppb
	NA	NA	Very Faint line	0 – 10 ppb	No line	> 10 ppb
	NA	NA	Very Faint line	0 – 10 ppb	No line	> 10 ppb
15 ppb	No line	> 10 ppb	No line	> 10 ppb	No line	> 10 ppb
	No line	> 10 ppb	No line	> 10 ppb	No line	> 10 ppb
	No line	> 10 ppb	No line	> 10 ppb	No line	> 10 ppb

NA = not applicable; 7.0 ppb level was not performed for LR samples.

Accuracy. The DI samples (concentrations between 0.10 and 15 ppb) for the three variants of microcystin were used during this verification test. All of the samples were analyzed in triplicate and each of them produced a control line on the Strip Test to indicate that the Strip Test was functioning properly. As the concentrations of the various microcystin variants increased, the line generated in the test area region was observed to change as expected. The lowest concentration samples generated dark lines, the mid-level concentrations generated lighter lines, and the highest concentrations were either very faint lines or generated no line at all. The Strip Test kit correctly detected each of the 15 ppb samples as being greater than 10 ppb.

Precision. The line colors were consistent within the three replicates at each concentration and were consistent with the results from the reference method results with one exception. The 7.0 ppb RR DI water sample did not generate a line, indicating a concentration of greater than 10 ppb. With the exception of RW 5, the triplicate measurements of the samples generated lines that were very similar in intensity. In two replicates, RW 5 generated faint lines indicating a 0 to 10 ppb concentration and one replicate that had no line, indicating a concentration of greater than 10 ppb.

Matrix Interference. The RW matrix interferent and chlorophyll-*a* interferent sample results for the Strip Test Kit all agreed and the interpretation of the results is consistent with the spiked

amount of microcystins in the samples. There was no indication that different matrices affected the test kit performance.

Recreational Water (RW). RW sample results for the Strip Test Kit were also consistent with the reference method results. While the total measured microcystin result may have other variants present that cannot be detected by the reference method, with one exception for RW 5, the sample results were all consistent with the reference laboratory results of the RW samples. That is, when the reference concentrations were greater than 10 ppb, there was no line generated, when the reference concentration was between 2.0 and 4.0 ppb, the lines were normal or faint, and when the reference concentrations were lower than 2.0, the lines generated were dark.

Operational Factors. The test kit operator reported that the Strip Test kit was very easy to use and needs no technical skills to operate. The brochure and flowcharts with illustrations were clear and easy to follow. There was no solution or sample preparation needed. The entire procedure is approximately 40 minutes long, including the QuikLyse™ procedure and the microcystins analysis. The QuikLyse™ process uses 1 mL of sample through 2 × 8 minute incubation periods. Then the sample is transferred into the microcystins reagent conical tube. The sample is incubated for 10 minutes and then the test strip is added to the conical tube. The test strip is interpreted according to the figure in the brochure after 5 minutes of exposure to the sample.

There were no consumables required for this technology. The test strips were disposed in the regular trash after use, producing no hazardous waste.

The listed price for the Strip Test Kit at the time of the verification test was $480 for a 20 strip kit and $150 for a five strip kit. The kit has a 12-month shelf life as received and should be stored at room temperature.

Chapter 10
References

1. *Test/Quality Assurance Plan for Verification of Microcystin Test KitsTest/Quality Assurance Plan for Verification of Microcystin Test Kits.* U.S. Environmental Technology Verification Program, Battelle, July 2010.
2. *Quality Management Plan for the ETV Advanced Monitoring Systems Center, Version 7.* U.S. Environmental Technology Verification Program, Battelle, November 2008.
3. Cong, L.H., B.; Chen, Q.; Lu, B.; Zhang, J.; Ren, Y., *Determination of trace amount of microcystins in water samples using liquid chromatography coupled with triple quadrupole mass spectrometry. Anal. Chim. Acta*, 2006. **569** (1-2): p. 157-168.
4. Cassada, D., *Standard Operating Procedure (SOP) Determination of algaltoxin residues in water extracts by solid phase extraction (SPE), liquid chromatography (LC)-atmospheric pressure electrospray ionization tandem mass spectrometry (MS/MS)*. July, 2010, Water Sciences Laboratory, University of Nebraska.
5. *"Guidelines Establishing Test Procedures for the Analysis of Pollutants."*, USEPA, Editor. 2000, U.S. Code of Federal Regulations.
6. *SOP# SWS-2320.1A: Microcystin Analysis Using the Abraxis ELISA (Enzyme-Linked Immuno-Sorbent Assay) Method.* Nebraska Department of Environmental Quality.
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.
8. Fischer, W.; Garthwaite, I.; Miles, C.; Ross, K.; Aggen, J.; Chamberlin, A.; Towers, N.; Dietrich, D. *Congener-Independent Immunoassay for Microcystins and Nodularins*, *Environ. Sci. Technol.,* **2001**, 35, 4849-4856.

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 uL 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
Abraxis Test Kit Raw Data

Table B-1. Abraxis ADDA ELISA Test Kit Raw Data

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
Reagent Blank	RB	Range?	Range?	Range?
Reagent Blank	RB	Range?	Range?	Range?
Reagent Blank	RB	0.015	0	0.5
Reagent Blank	RB	Range?	Range?	Range?
Reagent Blank	RB	Range?	Range?	Range?
Reagent Blank	RB	Range?	Range?	Range?
Positive Control 1	LR	0.653	0.054	8.2
Positive Control 2	LR	0.826	0.035	4.2
Positive Control 2a	LR	0.808	0.050	6.2
Positive Control 3	LR	0.566	0.101	17.8
Positive control 4	LR	0.625	0.156	24.9
Positive Control 5	LR	0.717	0.014	2
Positive Control 5a	LR	0.656	0.011	1.7
Positive Control 5b	LR	0.769	0.015	1.9
Positive Control 6	LR	0.903	0.147	16.3
Std 0 diff lot	LR	0.072	0.016	21.8
Std 0.15 diff lot	LR	0.195	0.027	13.7
Std 0.4 diff lot	LR	0.467	0.004	0.8
Std 1.0 diff lot	LR	1.086	0.025	2.3
Std 2.0 diff lot	LR	2.034	0.430	21.2
Std 5.0 diff lot	LR	13.829	11.59	83.8
0.1 LR	LR	0.096	0.003	3.5
0.1 LR	LR	0.022	0.005	21.4
0.1 LR	LR	0.087	0.010	11.2
0.1 LR	LR	0.110	0.021	18.8
0.1 LR	LR	0.102	0.011	11
0.5 LA	LA	0.667	0.157	23.5
0.5 LA	LA	0.914	0.050	5.5
0.5 LA	LA	0.718	0.009	1.2
0.5 LA	LA	0.969	0.035	3.6
0.5 LA	LA	1.046	0.047	4.5
0.5 LA	LA	0.927	0.052	5.6
0.5 LA	LA	0.842	0.030	3.6
0.5 LA	LA	0.905	0.020	2.2
0.5 LA	LA	0.740	0.004	0.6
0.5 LA	LA	0.750	0.094	12.5
0.5 LA	LA	0.634	0.014	2.1
0.5 LR	LR	0.588	0.052	8.9
0.5 LR	LR	0.584	0.014	2.5
0.5 LR	LR	0.590	0.013	2.3
0.5 LR	LR	0.553	0.058	10.5
0.5 LR	LR	0.477	0.071	14.8
0.5 LR	LR	0.538	0.019	3.6

Table B-1. Abraxis ADDA ELISA Test Kit Raw Data Continued				
Sample Description	**Variant**	**Mean Conc. (ppb)**	**Standard Deviation (ppb)**	**CV%**
0.5 LR	LR	0.550	0.054	9.8
0.5 LR	LR	0.441	0.083	18.7
0.5 LR	LR	0.381	0.027	7
0.5 LR	LR	0.434	0.007	1.6
0.5 RR	RR	0.490	0.007	1.3
0.5 RR	RR	0.544	0.003	0.5
0.5 RR	RR	0.552	0.018	3.3
0.5 RR	RR	0.568	0.096	17
0.5 RR	RR	0.567	0.051	8.9
0.5 RR	RR	0.518	0.004	0.8
0.5 RR	RR	0.514	0.060	11.7
0.5 RR	RR	0.549	0.035	6.3
0.5 RR	RR	0.563	0.050	8.9
0.5 RR	RR	0.552	0.026	4.7
1.0 LA	LA	2.037	0.173	8.5
1.0 LA	LA	1.781	0.074	4.1
1.0 LA	LA	1.545	0.071	4.6
1.0 LR	LR	1.311	0.265	20.2
1.0 LR	LR	1.003	0.236	23.5
1.0 LR	LR	0.991	0.018	1.8
1.0 RR	RR	1.095	0.104	9.5
1.0 RR	RR	0.973	0.039	4
1.0 RR	RR	0.972	0.015	1.6
2.0 LA	LA	3.598	0	0
2.0 LA (2x dil)	LA	2.160	0.007	0.3
2.0 LA (2x dil)	LA	2.264	0.038	1.7
2.0 LR	LR	1.873	0.465	24.8
2.0 LR	LR	2.632	0.348	13.2
2.0 LR	LR	1.101	0.061	5.6
2.0 LR	LR	1.045	0.182	17.4
2.0 RR	RR	1.796	0.022	1.2
2.0 RR	RR	2.461	0.603	24.5
2.0 RR	RR	2.200	0.232	10.5
4.0 LA (4x dil)	LA	1.924	0.36	18.7
4.0 LA (4x dil)	LA	1.997	0.011	0.5
4.0 LR	LR	3.473	0	0
4.0 LR	LR	3.819	0	0
4.0 RR	RR	4.359	0.234	5.4
4.0 RR	RR	4.074	0.562	13.8
4.0 RR	RR	3.241	0.594	18.3
4.0 RR	RR	3.550	0.125	3.5
2.0 LA Chloro	LA	0.576	0.119	20.7
2.0 LA Chloro	LA	0.617	0.052	8.4
2.0 LA Chloro	LA	0.548	0.106	19.4
2.0 LA Chloro 10X	LA	0.533	0.041	7.7
2.0 LA Chloro 10X	LA	0.669	0.106	15.8
2.0 LA Chloro 10X	LA	0.581	0.019	3.2
2.0 LA Chloro 10X	LA	0.425	0.042	9.9
52671-36-09 2x	LA	3.014	0.247	8.2
52671-36-09 2x	LA	2.173	0.284	13.1

Table B-1. Abraxis ADDA ELISA Test Kit Raw Data Continued				
Sample Description	**Variant**	**Mean Conc. (ppb)**	**Standard Deviation (ppb)**	**CV%**
52671-36-09 2x	LA	1.894	0.127	6.7
2.0 LA Matrix 10x	LA	3.861	0.123	3.2
2.0 Matrix 10x LA (2x dil)	LA	3.030	0.103	3.4
52671-36-12 2x	LA	2.655	0.362	13.6
2.0 LR Chloro	LR	0.488	0.015	3.1
2.0 LR Chloro	LR	0.532	0.064	12.1
2.0 LR Chloro	LR	0.493	0.094	19.2
2.0 LR Chloro 10x	LR	0.391	0.048	12.2
2.0 LR Chloro 10x	LR	0.432	0.019	4.4
2.0 LR Chloro 10x	LR	0.486	0.067	13.7
2.0 LR Matrix	LR	4.050	0.500	12.3
2.0 LR Matrix	LR	2.598	0.236	9.1
2.0 LR Matrix 10x	LR	1.703	0.144	8.5
2.0 LR Matrix 10x	LR	1.903	0.082	4.3
2.0 LR Matrix 10x	LR	1.658	0.226	13.6
2.0 RR Chloro	RR	2.238	0.070	3.1
2.0 RR Chloro	RR	1.769	0.187	10.6
2.0 RR Chloro	RR	1.574	0.317	20.1
2.0 RR Chloro	RR	2.012	0.099	4.9
2.0 RR Chloro	RR	2.204	0.413	18.7
2.0 RR Chloro 10x	RR	1.975	0.072	3.7
2.0 RR Chloro 10x	RR	1.437	0.255	17.8
2.0 RR Chloro 10x	RR	1.611	0.106	6.6
2.0 RR Chloro 10x	RR	1.573	0.114	7.2
2.0 RR Matrix	RR	2.784	0.330	11.8
2.0 RR Matrix	RR	2.378	0.115	4.8
2.0 RR Matrix	RR	2.505	0.204	8.2
2.0 RR Matrix 10x	RR	1.368	0.131	9.6
2.0 RR Matrix 10x	RR	1.387	0.211	15.2
2.0 RR Matrix 10x	RR	1.286	0.044	3.4
RW1 (20x dil)	Unknown	2.132	0.141	6.6
RW1 (20x dil)	Unknown	1.843	0.023	1.2
RW2 (10x dil)	Unknown	2.235	0.272	12.2
RW2 (10x dil)	Unknown	2.298	0.159	6.9
RW2 (10x dil)	Unknown	2.087	0.022	1.1
RW3 (10x dil)	Unknown	1.742	0.168	9.7
RW3 (10x dil)	Unknown	3.497	0.521	14.9
RW3 (20x dil)	Unknown	1.019	0.112	10.9
RW3 (20x dil)	Unknown	1.202	0.089	7.4
RW3 (20x dil)	Unknown	1.101	0.032	2.9
RW 4 (4x dil)	Unknown	2.258	0.314	13.9
RW 4 (4x dil)	Unknown	2.667	0.018	0.7
RW 5 (4x dil)	Unknown	1.574	0.267	17
RW 5 (4x dil)	Unknown	1.587	0.113	7.1
RW 5 (4x dil)	Unknown	1.280	0.061	4.7
RW6 (2x dil)	Unknown	1.388	0.050	3.6
RW6 (2x dil)	Unknown	1.405	0.203	14.5
RW6 (2x dil)	Unknown	1.710	0.268	15.7
RW6 (2x dil)	Unknown	1.207	0.01	0.8

Table B-1. Abraxis ADDA ELISA Test Kit Raw Data Continued				
Sample Description	**Variant**	**Mean Conc. (ppb)**	**Standard Deviation (ppb)**	**CV%**
RW6 (2x dil)	Unknown	0.931	0.024	2.6
RW6	Unknown	4.588	0.931	20.3
RW7	Unknown	0.012	0.004	33
RW7	Unknown	0.015	0.008	55.1
RW7	Unknown	0.050	0.015	28.9
RW8	Unknown	0.885	0.147	16.6
RW8	Unknown	0.807	0.021	2.6
RW8	Unknown	0.857	0.026	3.1
RW 9 Matrix	Unknown	0.639	0.038	5.9
RW 9 Matrix	Unknown	0.600	0.087	14.4
RW 9 Matrix	Unknown	0.621	0.119	19.1

Table B-2. Abraxis DM ELISA Test Kit Raw Data

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
Reagent Blank	RB	0.038	0	0
Reagent Blank	RB	0.004	0	0
Reagent Blank	RB	0.044	0	0
Reagent Blank	RB	0.061	0.007	12
Reagent Blank	RB	0.061	0.010	17.3
Reagent Blank	RB	0.060	0.004	7
Positive Control 1	LR	0.836	0.075	9
Positive Control 2a	LR	0.746	0.068	9.1
Positive Control 2b	LR	0.740	0.035	4.7
Positive Control 3	LR	0.603	0.001	0.1
Positive control 4	LR	0.756	0.020	2.7
Positive Control 5a	LR	0.616	0.039	6.4
Positive Control 5b	LR	0.505	0.007	1.3
Std 0 diff lot	LR	0.018	0	0
Std 0.15 diff lot	LR	0.169	0.003	1.6
Std 0.4 diff lot	LR	0.415	0.061	14.7
Std 1.0 diff lot	LR	1.094	0.009	0.8
Std 2.0 diff lot	LR	2.164	0.080	3.7
Std 5.0 diff lot	LR	4.632	0.149	3.2
0.1 LR	LR	0.176	0.017	9.5
0.1 LR	LR	0.157	0.033	20.7
0.1 LR	LR	0.150	0.001	0.5
0.1 LR	LR	0.183	0.003	1.6
0.1 LR	LR	0.194	0.025	13.1
0.5 LA	LA	0.559	0.012	2.2
0.5 LA	LA	0.575	0.063	11
0.5 LA	LA	0.631	0.028	4.4
0.5 LA	LA	0.506	0.037	7.2
0.5 LA	LA	0.536	0.036	6.7
0.5 LA	LA	0.474	0.016	3.4
0.5 LA	LA	0.516	0.013	2.5
0.5 LA	LA	0.534	0.015	2.8
0.5 LA	LA	0.523	0.067	12.8
0.5 LA	LA	0.536	0.022	4.2
0.5 LR	LR	0.701	0.014	2
0.5 LR	LR	0.729	0.021	2.9
0.5 LR	LR	0.683	0.032	4.7
0.5 LR	LR	0.705	0.118	16.7
0.5 LR	LR	0.734	0.083	11.4
0.5 LR	LR	0.619	0.022	3.5
0.5 LR	LR	0.680	0.019	2.8
0.5 LR	LR	0.777	0.011	1.4
0.5 LR	LR	0.816	0.042	5.1
0.5 LR	LR	0.776	0.059	7.6
0.5 RR	RR	0.501	0.064	12.8
0.5 RR	RR	0.482	0.024	4.9
0.5 RR	RR	0.471	0.026	5.5
0.5 RR	RR	0.506	0.020	3.9
0.5 RR	RR	0.411	0.016	4

Table B-2. Abraxis DM ELISA Test Kit Raw Data Continued

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
0.5 RR	RR	0.417	0.052	12.5
0.5 RR	RR	0.399	0.025	6.2
0.5 RR	RR	0.364	0.010	2.6
0.5 RR	RR	0.532	0.012	2.3
0.5 RR	RR	0.468	0.013	2.8
1.0 LA	LA	0.998	0.194	19.4
1.0 LA	LA	0.975	0.102	10.4
1.0 LA	LA	1.045	0.083	8
1.0 LR	LR	1.422	0.003	0.2
1.0 LR	LR	1.356	0.077	5.7
1.0 LR	LR	1.334	0.219	16.4
1.0 RR	RR	1.007	0.122	12.1
1.0 RR	RR	1.029	0.079	7.7
1.0 RR	RR	1.025	0.010	1
2.0 LA	LA	2.064	0.037	1.8
2.0 LA	LA	2.294	0.076	3.3
2.0 LA	LA	2.122	0.140	6.6
2.0 LR	LR	2.439	0.002	0.1
2.0 LR	LR	2.720	0.117	4.3
2.0 LR	LR	2.756	0.293	10.6
2.0 RR	RR	2.066	0.018	0.9
2.0 RR	RR	1.954	0.024	1.2
2.0 RR	RR	1.948	0.028	1.4
4.0 LA	LA	3.715	0.101	2.7
4.0 LA	LA	3.823	0.067	1.7
4.0 LA	LA	3.750	0.036	1
4.0 LR	LR	4.605	0.167	3.6
4.0 LR	LR	4.606	0.080	1.7
4.0 LR	LR	4.773	0.060	1.3
4.0 RR	RR	3.838	0.151	3.9
4.0 RR	RR	3.723	0.090	2.4
4.0 RR	RR	3.668	0.267	7.3
2.0 LA Chloro	LA	0.293	0.002	0.6
2.0 LA Chloro	LA	0.323	0.066	20.4
2.0 LA Chloro	LA	0.352	0.019	5.5
2.0 LA Chloro 10X	LA	0.350	0.050	14.4
2.0 LA Chloro 10X	LA	0.343	0.014	4.1
2.0 LA Chloro 10X	LA	0.328	0.010	3.1
2.0 LA Matrix	LA	1.939	0.079	4.1
2.0 LA Matrix	LA	2.117	0.229	10.8
2.0 LA Matrix	LA	2.003	0.023	1.2
2.0 LA Matrix 10x	LA	1.695	0.083	4.9
2.0 LA Matrix 10x	LA	1.758	0.074	4.2
2.0 LA Matrix 10x	LA	1.672	0.076	4.5
2.0 LR Chloro	LR	0.492	0.013	2.6
2.0 LR Chloro	LR	0.477	0.028	5.8
2.0 LR Chloro	LR	0.460	0.053	11.6
2.0 LR Chloro 10x	LR	0.482	0.010	2
2.0 LR Chloro 10x	LR	0.503	0.009	1.8
2.0 LR Chloro 10x	LR	0.413	0.052	12.5

Table B-2. Abraxis DM ELISA Test Kit Raw Data Continued

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
2.0 LR Matrix	LR	3.484	0.082	2.3
2.0 LR Matrix	LR	3.102	0.033	1.1
2.0 LR Matrix	LR	3.208	0.127	4
2.0 LR Matrix 10x	LR	2.345	0.071	3
2.0 LR Matrix 10x	LR	2.405	0.025	1
2.0 LR Matrix 10x	LR	2.560	0.109	4.3
2.0 RR Chloro	RR	2.143	0.064	3
2.0 RR Chloro	RR	1.993	0.057	2.9
2.0 RR Chloro	RR	2.035	0.091	4.5
2.0 RR Chloro 10x	RR	1.819	0.050	2.7
2.0 RR Chloro 10x	RR	1.920	0.060	3.1
2.0 RR Chloro 10x	RR	1.835	0.084	4.6
2.0 RR Matrix	RR	2.139	0.056	2.6
2.0 RR Matrix	RR	2.219	0.045	2
2.0 RR Matrix	RR	2.129	0.019	0.9
2.0 RR Matrix 10x	RR	1.813	0.004	0.2
2.0 RR Matrix 10x	RR	1.837	0.043	2.3
2.0 RR Matrix 10x	RR	1.759	0.048	2.7
RW1 (10x dil)	Unknown	2.417	0.110	4.6
RW1 (10x dil)	Unknown	2.194	0.126	5.7
RW1 (10x dil)	Unknown	1.168	0.019	1.6
RW1 (20x dil)	Unknown	1.157	0.064	5.5
RW1 (20x dil)	Unknown	1.226	0.027	2.2
RW2 (10x dil)	Unknown	1.410	0.175	12.4
RW2 (10x dil)	Unknown	1.298	0.038	2.9
RW2 (20x dil)	Unknown	0.721	0.055	7.7
RW2 (20x dil)	Unknown	0.668	0.094	14
RW2 (20x dil)	Unknown	0.735	0.068	9.3
RW3 (10x dil)	Unknown	1.427	0.170	11.9
RW3 (10x dil)	Unknown	1.362	0.016	1.2
RW3 (10x dil)	Unknown	1.337	0.098	7.3
RW3 (20x dil)	Unknown	0.737	0.028	3.8
RW3 (20x dil)	Unknown	0.698	0.065	9.3
RW3 (20x dil)	Unknown	0.690	0.046	6.7
RW 4	Unknown	3.914	0.170	4.3
RW 4	Unknown	4.039	0.074	1.8
RW 4	Unknown	4.543	0.201	4.4
RW 4 (4x dil)	Unknown	1.079	0.084	7.8
RW 4 (4x dil)	Unknown	0.974	0.007	0.7
RW 4 (4x dil)	Unknown	1.003	0.077	7.7
RW 5	Unknown	4.568	0.346	7.6
RW 5	Unknown	4.608	0.417	9
RW 5	Unknown	4.410	0.192	4.4
RW 5 (4x dil)	Unknown	1.128	0.036	3.2
RW 5 (4x dil)	Unknown	1.221	0.087	7.1
RW 5 (4x dil)	Unknown	1.154	0.197	17.1
RW6	Unknown	1.682	0.203	12.1
RW6	Unknown	1.881	0.055	2.9
RW6	Unknown	2.009	0.122	6.1
RW 6 (2x dil)	Unknown	0.904	0.015	1.6

Table B-2. Abraxis DM ELISA Test Kit Raw Data Continued

Sample Description	Variant	Mean Conc. (ppb)	Standard Deviation (ppb)	CV%
RW 6 (2x dil)	Unknown	0.934	0.015	1.6
RW 6 (2x dil)	Unknown	0.924	0.018	2
RW7	Unknown	0.054	0	0
RW7	Unknown	0.057	0	0
RW7	Unknown	Range?	Range?	Range?
RW8	Unknown	0.633	0.022	3.4
RW8	Unknown	0.715	0.074	10.4
RW8	Unknown	0.667	0.008	1.2
RW 9 Matrix	Unknown	0.441	0	0.1
RW 9 Matrix	Unknown	0.489	0.013	2.6
RW 9 Matrix	Unknown	0.475	0.006	1.2