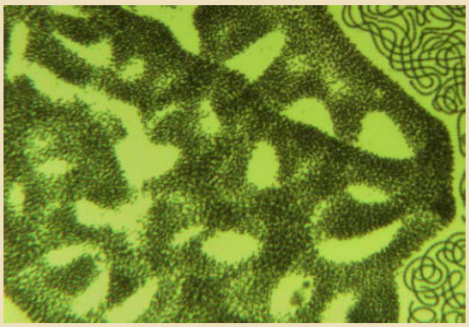
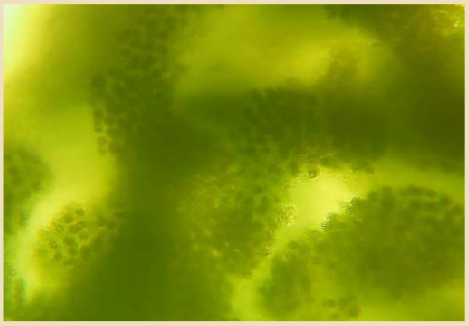
Florida Department of Environmental Protection

Bureau of Laboratories

Division of Environmental Assessment and Restoration



January 2009 Microcystin Round Robin Study



Contents

January 2009 Microcystin Round Robin Study
Prepared by the
Bureau of Laboratories
Florida Department of Environmental Protection

Note:
This report and its associated data analyses are a product of the Florida Department of Environmental Protection's Bureau of Laboratories and may not represent the views of the other participating round robin laboratories. It is important to note that this study was conducted as a research project. **It is NOT a performance test** and should not be used as such to compare one laboratory to another.

Background

During the summer of 2005, the State of Florida saw an increase in the number and severity of cyanobacteria (a.k.a. blue-green algae) blooms in both fresh and marine waters. A number of Florida state agencies and local municipalities were involved with sampling, analyzing, and reporting results for these blooms. It was not uncommon to see different entities reporting widely disparate results for the same bloom. The sources of variability in the results were primarily attributed to differences in sampling and analysis methods.

Staff with the Florida Department of Environmental Protection (FDEP) and the Florida Department of Health (FDoH) created the Cyanobacteria Bloom Sampling and Analysis Standardization Workgroup in an effort to standardize the way cyanobacterial blooms were sampled, analyzed, and reported. In addition to FDEP and FDoH, the workgroup had representatives from numerous state agencies (Florida Fish and Wildlife Conservation Commission/Florida Wildlife Research Institute, South Florida Water Management District, St. John's Water Management District, Florida Department of Agriculture and Consumer Services), Universities (Florida International University, University of Miami), and private laboratories (Greenwater Laboratories, Inc., Mote Marine Laboratory). The workgroup was able to come to consensus on a standard operation procedure for sampling cyanobacteria blooms prior to the 2006 bloom season. (http://www.dep.state.fl.us/labs/biology/hab/docs/cyanobacteria_sop.pdf)

The next step was to assess the variability associated with the laboratory analyses for cyanobacterial toxins. Since the majority of the large 2005 blooms were caused by *Microcystis aeruginosa*, a species of cyanobacteria that is capable of producing potent hepatotoxins known as microcystins, the workgroup decided to focus on the analysis of microcystins. A Plan of Study (POS) was developed for an inter-laboratory variability, or round robin, study in which numerous laboratories would be supplied with identical aliquots of *M. aeruginosa* collected from several different waterbodies; however the 2006 season did not produce significant blooms and the study was delayed.

The spring and early summer of 2007 had few toxin-producing *M. aeruginosa* blooms; therefore the decision was made to initiate the round robin study using two toxin producing *M. aeruginosa* cultures from the FDEP Central Laboratory and a microcystin-LR standard. In July 2007, the FDEP's Bureau of Laboratories conducted the microcystin round robin with twelve laboratories throughout the United States that had volunteered to perform the analyses at no cost. The details of this 2007 round robin and the results, including raw data, can be found at: http://www.dep.state.fl.us/labs/biology/hab/docs/microcystin_rr.pdf

In general, the results of the 2007 round robin showed wide variability in the results both within and between laboratories for a single water sample. The between laboratory variability could not be well estimated as it was confounded by the differences in preparation method and dilution among the laboratories. One of the laboratories found that their dilution standards were low and purchased new certified standards. In discussions after the results were distributed, there was interest in a second round robin where the preparation method and the dilution standard would be controlled. The FDEP Central Laboratory agreed to prepare, split, and ship samples for this second round robin and the laboratories agreed to perform the analyses at no cost. The FDEP laboratory would do the sample preparation so when the participating labs received their samples, they would only need to analyze it.

By December 2008, the National Research Council Canada (NRC) had developed certified standards for microcystin-LR and -RR but it was not for commercial sale in the United States (Appendix A). The Abraxis company, a

microcystin Enzyme-Linked Immunosorbent Assay (ELISA) kit manufacturer in the United States, arranged to purchase a single lot quantity of a dilution standard for resale to the round robin participants. This "common standard" was to be used for dilution rather than the one supplied with the ELISA kit. ELISA kits were also purchased from the Envirologix company and some labs used the Envirologix kit standard rather than or in addition to the "common standard" that everyone purchased. In addition, some of the laboratories were able to purchase ELISA kits with the same serial number from the Abraxis or Envirologix companies.

In January 2009, the second round robin was conducted. This report presents the results and a discussion of this event.

Methods

The FDEP Central Biology Laboratory maintains *Microcystis aeruginosa* cultures of various strains or sources. Two cultures, Munson and JaxPond, were isolated from samples collected from North Florida water bodies during toxin-producing *M. aeruginosa* blooms. The Munson culture was originally isolated on October 20, 2006 from samples collected near the Lake Munson public access boat ramp in Southern Leon County, Florida. These environmental samples contained both *M. aeruginosa* colonies and *Anabaena sp*. filaments. Using a dissecting scope the *M. aeruginosa* colonies were separated from the *Anabaena sp.* filaments and inoculated in BG-11 algal growth media. The JaxPond culture was isolated in a similar way from a *M. aeruginosa* bloom in a small pond in Jacksonville, Florida, on March 19, 2008. A sample was collected and analyzed for microcystin toxin and nodularin. Microcystin-LR (20 µg/L Q), microcystin-LA (3.1 µg/L IQ), microcystin-RR (19 µg/L Q), and microcystin-YR (1.7 µg/L IQ) were all detected in the small pond environmental sample by liquid chromatography-mass spectrometry (LC/MS/MS). A third culture, Utex, was purchased from the University of Texas and received November 21, 2006. It was originally isolated in September 1954 from Little Rideau Lake, Ontario, Canada. The Utex culture was inoculated in BG-11 media. All FDEP *Microcystis aeruginosa* cultures are maintained as described in Appendices B and C.

The week before the round robin, all three cultures were tested for microcystin-LR and -RR toxin production by the FDEP Central Chemistry Laboratory using LC/MS/MS. The Munson culture was producing both microcystin-LR and microcystin-RR. The Utex culture was producing only microcystin-LR while the JaxPond culture was not producing toxin in culture. It was decided to use each of the three cultures to make bulk samples to split for the round robin. The JaxPond culture served as a negative control.

After the 2007 round robin, the study participants thought that a split of a prepared microcystin-LR standard sample would provide useful information. In addition, they thought that the FDEP Central Chemistry Laboratory should prepare such a sample for this round robin making it the fourth sample (Std). This standard was spiked with a non-microcystin producing algae, *Dunaliella tertiolecta*, to provide a "green look" to the samples and prevent labs from being able to distinguish between the lower levels of microcystin in the standard and the higher levels of microcystin in the cultures.

Each laboratory was sent 13 blind samples, three replicates each of JaxPond, Utex, and Std, and four replicates of Munson. Cell-lysing preparation of bulk samples for each culture was performed by FDEP one week prior to initiation of the round robin. This was done by autoclaving and then filtering approximately 2 L of culture through a 0.45 µm glass fiber filter under vacuum. The 2 L volume was then diluted to either 8 L (3 replicates) or 10 L (4 replicates) using deionized water. This was done to increase the volume to the amount of sample required and reduce the concentrated amount of toxin found in the culture. The Std sample, created by the FDEP Chemistry Section, was prepared to be 8 L and was not diluted further except for the addition of a small volume of *Dunaliella tertiolecta*. The 8 or 10 L volume was homogenized in a 20 L glass carboy on a stir plate for 15 minutes. Sample vials were filled 1/3 full by siphon tube while the sample was stirred. Once each vial was 1/3 full, the vials were each filled 2/3 full and then finally completely filled. This procedure was performed to minimize variability between sample replicates. The bottle numbering was different for each laboratory so they could not compare results (Table 2, 3, 4).

The samples were then packed in wet ice and shipped via overnight courier to the participating laboratories. The laboratories were asked to hold the samples in the dark at 4ºC for no more than one week before analyzing them according to their routine procedures. They were asked to report their results to FDEP within one month using a standardized reporting form.

Results

ELISA Kit Results Overview

Thirteen laboratories performed microcystin analyses using ELISA kits from two manufacturers, Abraxis and Envirologix. Twelve of the labs used a common standard for (7)-desmethyl microcystin-LR, microcystin-LR, microcystin-RR, and nodularin-R purchased from National Research Council, Canada, Institute for Marine Biosciences. Lab 11 and Lab 5 used standards supplied by the kit manufacturers. All labs used polyclonal kits with the exception of Lab 4 which used a monoclonal Abraxis kit and Lab 11 which used a monoclonal Envirologix kit in addition to a polyclonal Abraxis kit. There is not enough information determine variability between monoclonal and polyclonal kits as only two labs used monoclonal kits. The ELISA results are presented in Table 2. Each result along with the serial number for the kit is plotted in Figures 1, 5, and 9 for each sample. Average total microcystin values for each laboratory and sample combination are presented in Figures 2, 6, and 10.

High Performance Liquid Chromatography/Mass Spectroscopy (LCMS) Results Overview

Seven laboratories performed microcystin analyses using single or tandem quadrupole liquid chromatography mass spectroscopy (LCMS). Four laboratories (1,4,16,18) used LC/MS and three used LC/MS/MS (2,3,7). These results are presented in Table 3. Each result is plotted in Figures 1, 5, and 9 for each sample. Average total microcystin values for each laboratory are presented in Figures 3, 7, and 11. While the labs were instructed to perform no further preparations for the samples as they had already been autoclaved and filtered, the extraction methods differed among the participating labs. Labs 1, 3, 4 and 7 did not perform any additional preparations. Lab 2 sonicated the samples. Lab 16 performed solid phase extraction, and Lab 18 prepared the samples for analysis by performing lyophilization and sonication in 50% methanol.

Not all laboratories measured the same group of toxins (Table 3). While all laboratories reported microcystin-LR and RR, some also reported microcystin-YR, (7)-desmethyl microcystin-LR, microcystin LA, microcystin desmethyl-RR and nodularin. Microcystin LA, microcystin-YR, and nodularin were undetected in all samples. The total microcystins value reported for these samples is a sum of the individual toxin groups (microcystin-LR, microcystin-RR, desmethyl-LR, etc.) so the difference in the groups analyzed has an effect on the final reported result. Comparisons can also be made between the labs total microcystin-LR+microcystin-RR values to reduce variability due to the extra microcystin congeners analyzed by some of the labs.

Protein Phosphatase Inhibition Assay (PPIA) Results Overview

Three laboratories performed microcystin analyses using the protein phosphatase inhibition assay (PPIA). Lab 5 performed no additional preparation. Lab 15 received two sets of samples and performed no additional preparations on one set. On the second set of samples, nitrogen gas blow down was used to dry the sample, and it was reconstituted with 50% methanol in 1% acetic acid. Lab 18 performed lyophilization and sonication in 10mL of 50% methanol. The PPIA results are presented in Table 4. Each result is plotted in Figures 1, 5, and 9 for each sample. Average total microcystin values for each laboratory and sample combination are presented in Figures 4, 8, and 12.

Blank Sample

No congeners or degradation products of microcystin were detected for the JaxPond samples by any laboratory. The non-detects for JaxPond are not reported in the tables.

Microcystin-LR Standard (Std)

ELISA Kit Results

Values reported for the Microcystin-LR Standard samples ranged from 2.0 to 18.9 µg/L. The overall mean and coefficient of variation (% CV) for all ELISA kit results was 4.7 µg/L and 53% respectively. Lab 14 reported one result of 18.9 µg/L which is an extreme outlier (>3.0 times the interquartile range). The next highest result was 7.9 µg/L . The overall mean and % CV for all ELISA kit results excluding the outlier reported by Lab 14 was 4.4 µg/L and 31% respectively.

Values reported for the Microcystin-LR Standard samples using the NRC ELISA Standard ranged from 2.0 to 6.5 µg/L. Excluding the Lab 14 outlier, the overall mean and % CV for all ELISA kit results was 4.3 µg/L and 30% respectively.

Abraxis values (excluding the Lab 14 outlier) reported for the Microcystin-LR Standard samples ranged from 2.1 to 6.1 µg/L. The mean and % CV for the Abraxis ELISA kit results was 4.4 µg/L and 28% respectively.

Envirologix values reported for the Microcystin-LR Standard samples ranged from 2.0 to 7.9 µg/L. The mean and % CV for the Envirologix ELISA kit results was 4.5 µg/L and 35% respectively.

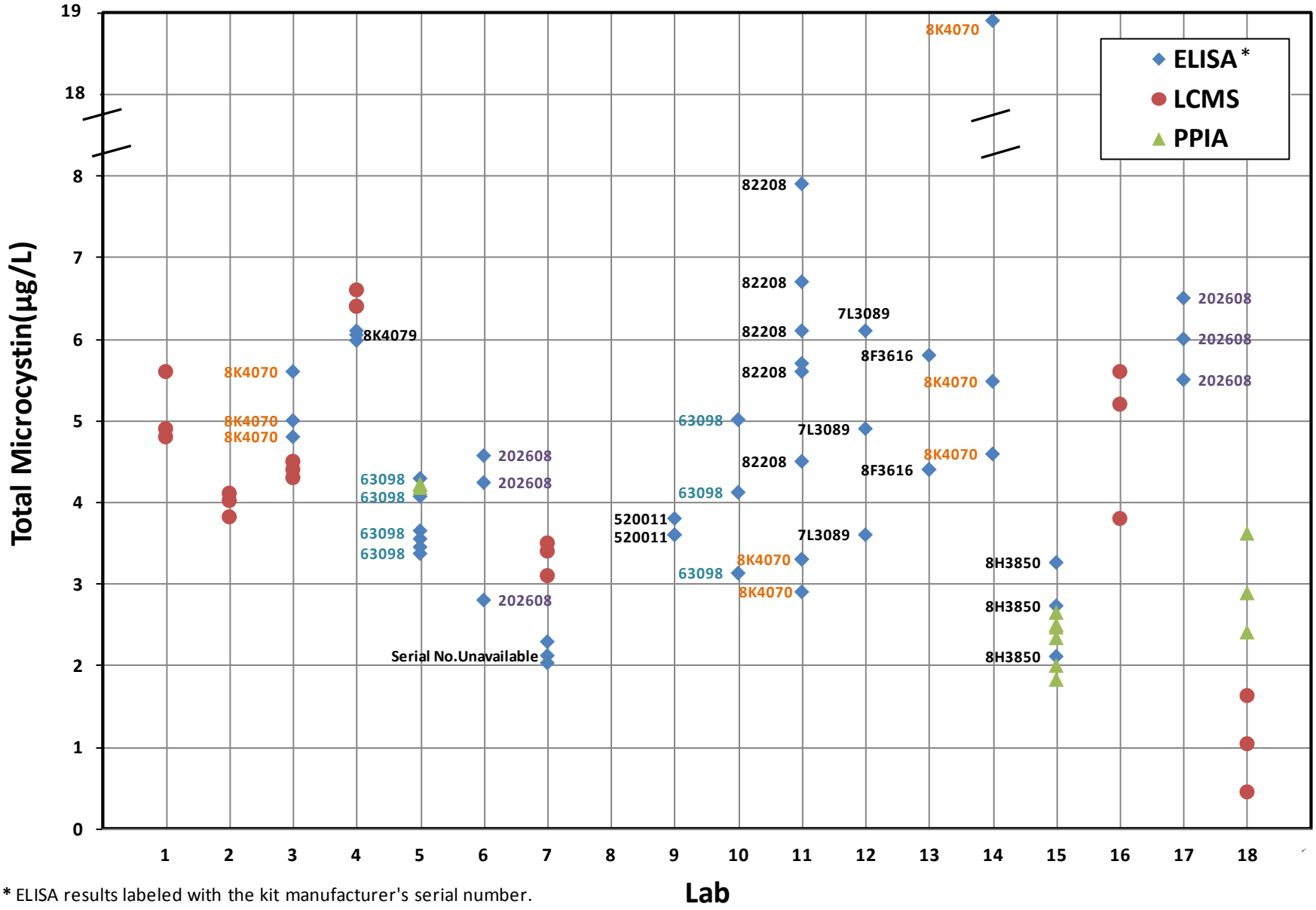
High Performance Liquid Chromatography/Mass Spectroscopy Results

Total microcystin values for all congeners reported for the Microcystin-LR Standard sample ranged from 0.5 to 6.6 µg/L. The overall mean and % CV was 4.2 µg/L and 40%, respectively. The within laboratory % CV for the replicates ranged from 2 to 56%. Microcystin-RR, microcystin-YR, microcystin desmethyl-LR, microcystin-LA, microcystin desmethyl-RR and nodularin were undetected in the standard sample.

Protein Phosphatase Inhibition Assay Results

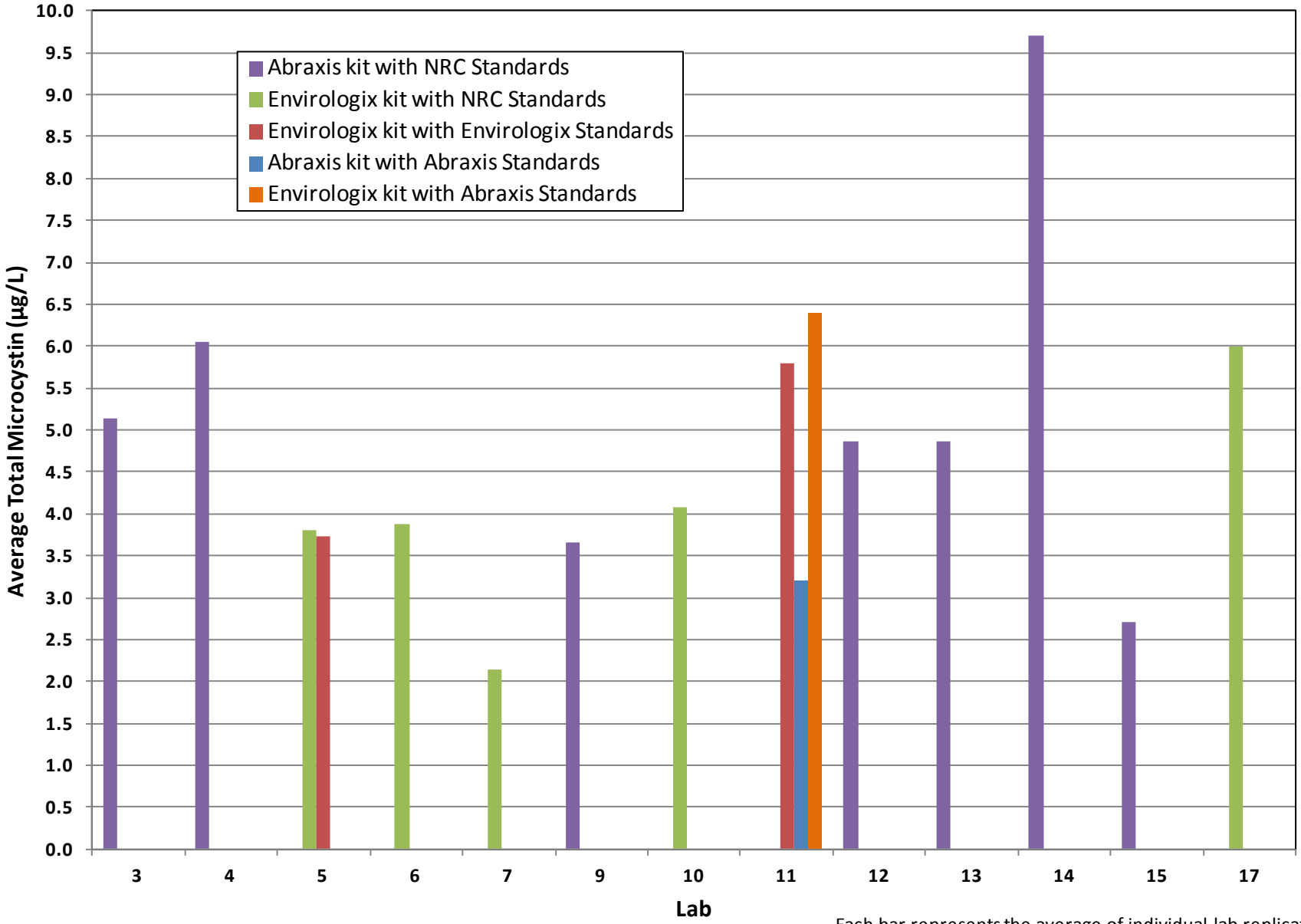
Total microcystin values reported for the Microcystin-LR Standard samples ranged from 1.8 to 4.2 µg/L. (Maximum and minimum values both attained from the no additional preparations method). The overall mean and % CV was 2.9 µg/L and 30%, respectively. The within laboratory % CV for the replicates ranged from 1 to 21%. Preparation methods comparisons can be made with Lab 15 for the nitrogen gas blow down/reconstitution and no additional prep method. The % CV for the nitrogen gas blow down/reconstitution was 4% and for no additional preparation method was 13%. Lab 5 also performed no additional preparations so comparisons between Lab 5 and Lab 15 can be made. The overall mean and % CV for the standard sample-no additional preparations was 3.1 µg/L and 38%.

Figure 1. Microcystin-LR Standard Results - All Methods



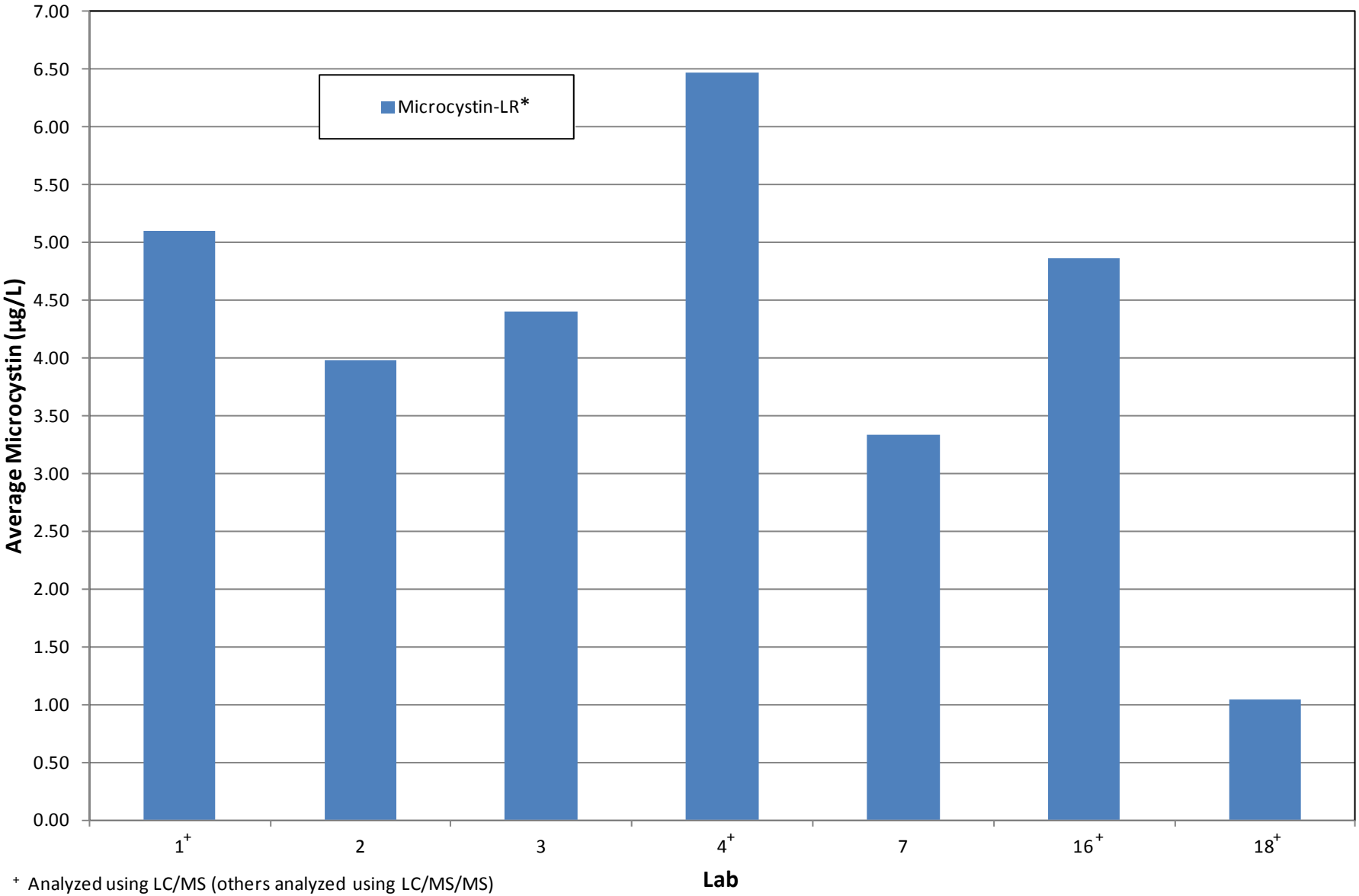
* ELISA results labeled with the kit manufacturer's serial number.

Figure 2. ELISA Microcystin-LR Standard Results



Each bar represents the average of individual lab replicates.

Figure 3. LCMS Microcystin-LR Standard Results

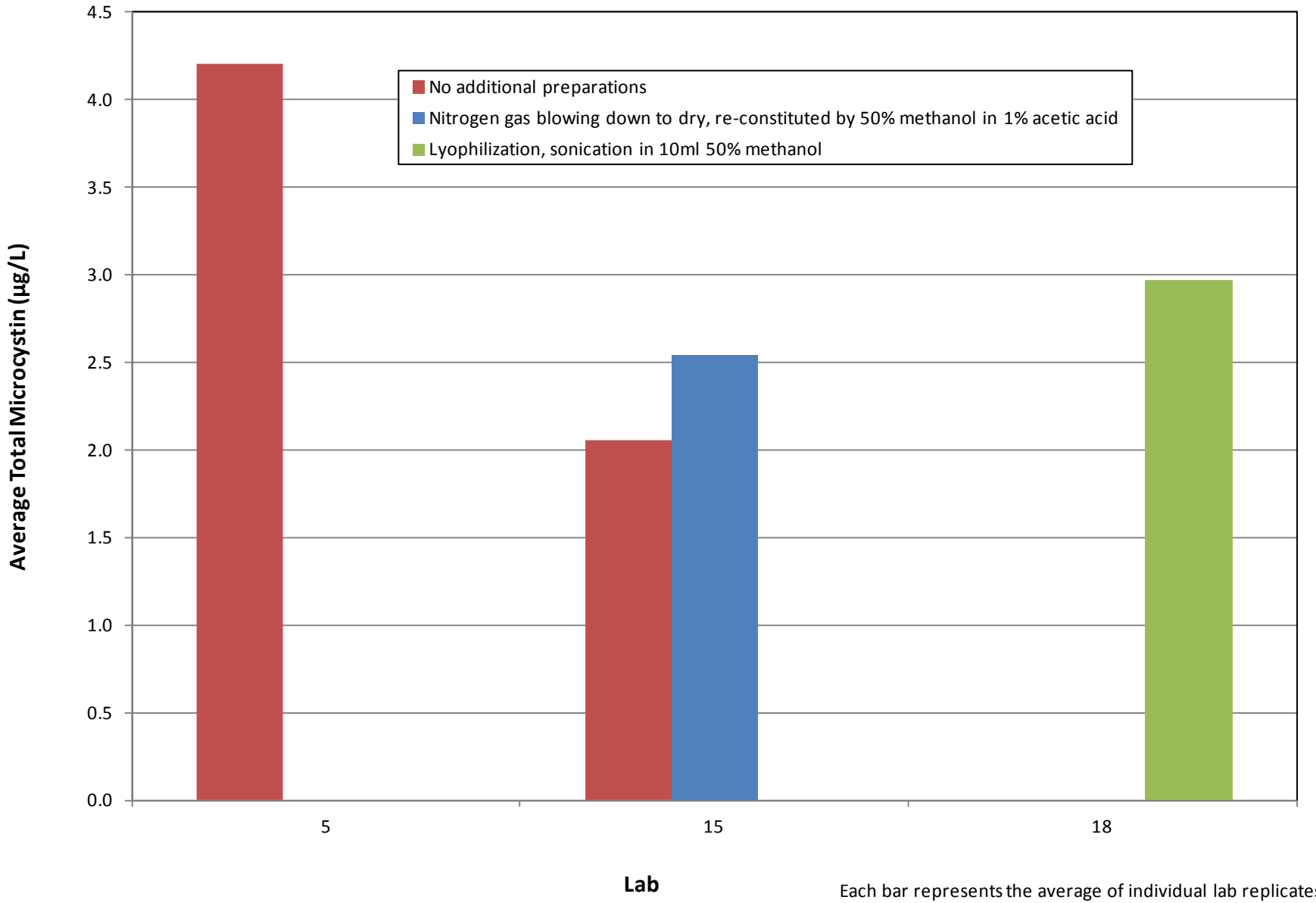


+ Analyzed using LC/MS (others analyzed using LC/MS/MS)

* All other congeners and degradation products were undetected in this sample.

Each bar represents the average of individual lab replicates.

Figure 4. PPIA Microcystin-LR Standard Results



Each bar represents the average of individual lab replicates.

FDEP culture of University of Texas *Microcystis aeruginosa* isolate (Utex)

ELISA Kit Results

Values reported for the University of Texas isolate ranged from 60 to 310 µg/L. The overall mean and % CV for all ELISA kit results was 177 µg/L and 38% respectively.

Values reported for the University of Texas isolate using the NRC ELISA Standard ranged from 92 to 309 µg/L. The overall mean and % CV for all ELISA kit results was 180µg/L and 32% respectively.

Abraxis values reported for the University of Texas isolate ranged from 130 to 310 µg/L. The mean and % CV for the Abraxis ELISA kit results was 207 µg/L and 26% respectively.

Envirologix values reported for the University of Texas isolate ranged from 60 to 309 µg/L. The mean and % CV for the Envirologix ELISA kit results was 147 µg/L and 45% respectively.

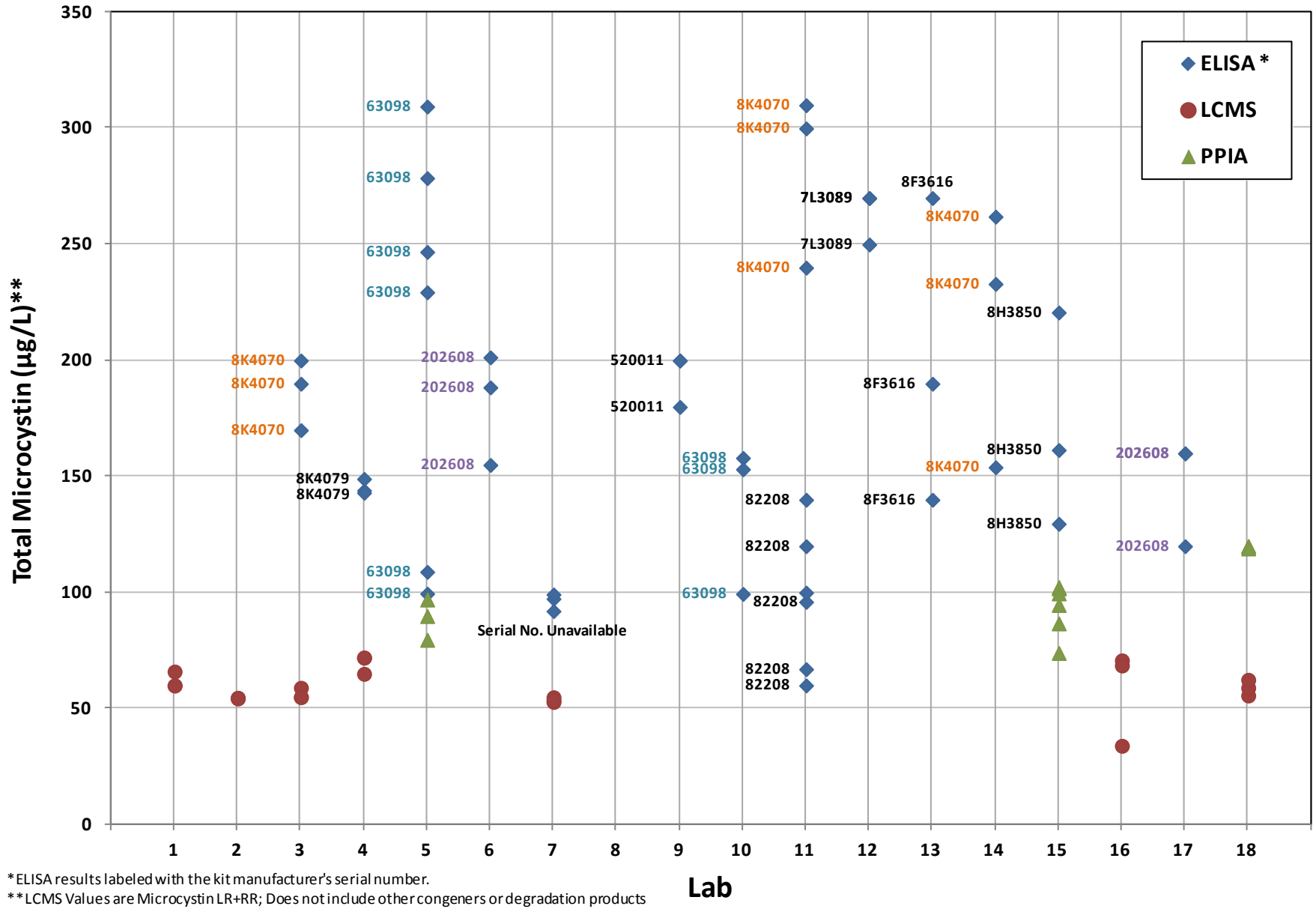
High Performance Liquid Chromatography/Mass Spectroscopy Results

Total microcystin values for all congeners reported for the University of Texas isolate samples ranged from 34 to 97µg/L. The overall mean and % CV was 70 µg/L and 21% respectively. The within laboratory % CV for the replicates ranged from less than 1% to 36%. Total microcystin-LR and -RR values reported ranged from 34 to 72 µg/L. The overall mean for microcystin-LR and -RR and % CV was 59 µg/L and 9% respectively. The within laboratory % CV ranged from less than 1% to 36%. Microcystin-LA, microcystin-YR, microcystin-RR, microcystin desmethyl-RR and nodularin were undetected in the University of Texas isolate samples. The higher variability associated with the total microcystins values when compared with the values for total microcystin-LR and -RR can be attributed to the microcystin desmethyl-LR analyzed for and detected by four labs (Lab 2, Lab 4, Lab 7 and Lab 18). The total microcystin value was higher in these four labs when compared with their own microcystin-LR and -RR values and with the labs that did not analyze for microcystin desmethyl-LR.

Protein Phosphatase Inhibition Assay Results

Total microcystin values reported for the University of Texas isolate samples ranged from 74 to 120 µg/L. The overall mean % CV and was 99 µg/L and 15%, respectively. The within laboratory % CV for the replicates ranged from less than 1 to 12%. Preparation methods comparisons can be made with Lab 15 for the nitrogen gas blow down/reconstitution and no additional prep method. The % CV for the nitrogen gas blow down/reconstitution was 12% and for no additional preparation method was 1%. Lab 5 also performed no additional preparations so comparisons between Lab 5 and Lab 15 can be made. The overall mean and % CV for the University of Texas isolate-no additional preparations was 95 µg/L and 9%.

Figure 5. FDEP Culture of University of Texas *Microcystis aeruginosa* Isolate Results - All Methods



*ELISA results labeled with the kit manufacturer's serial number.
**LCMS Values are Microcystin LR+RR; Does not include other congeners or degradation products

Figure 6. ELISA FDEP Culture of University of Texas *Microcystis aeruginosa* Isolate Results

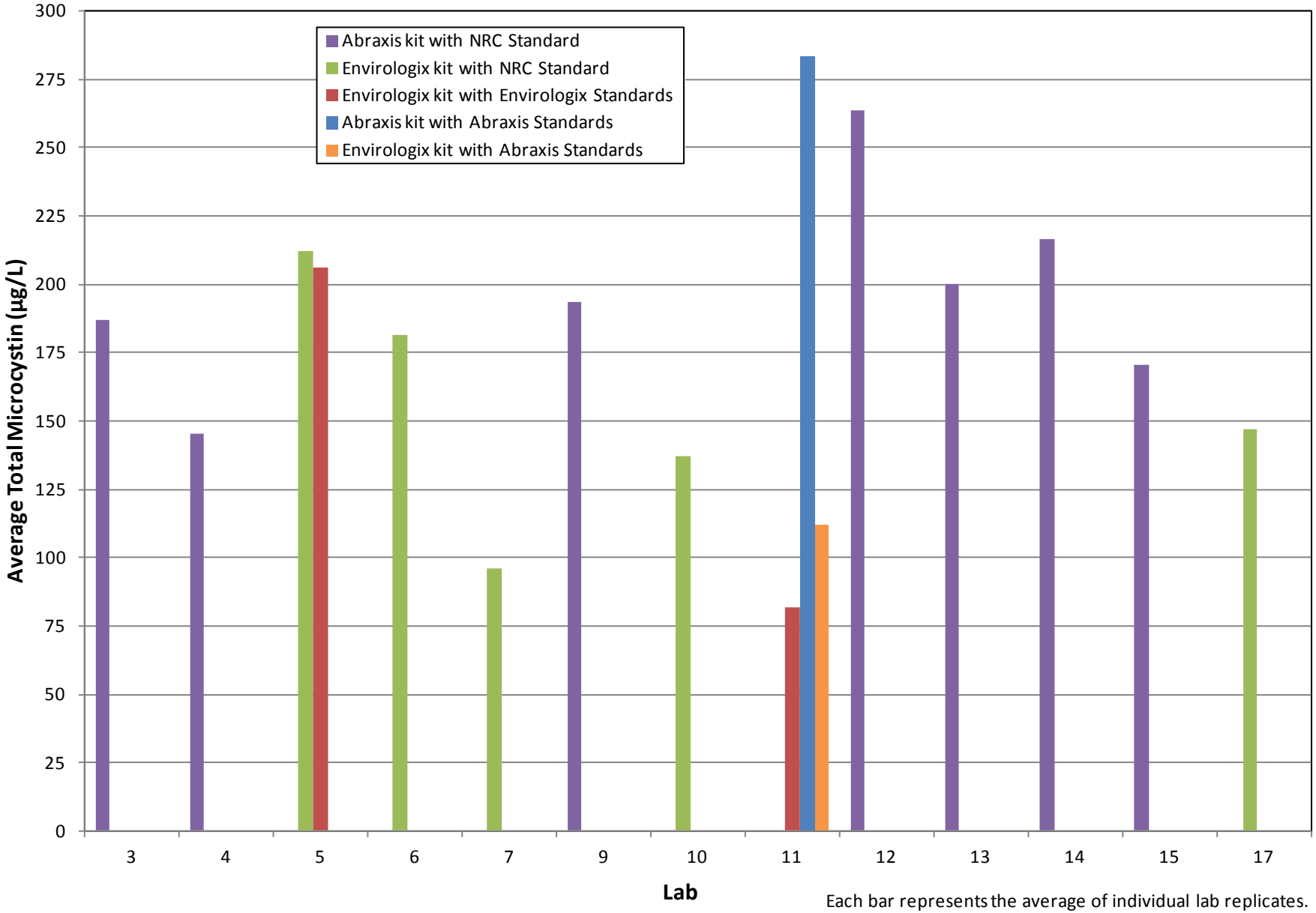
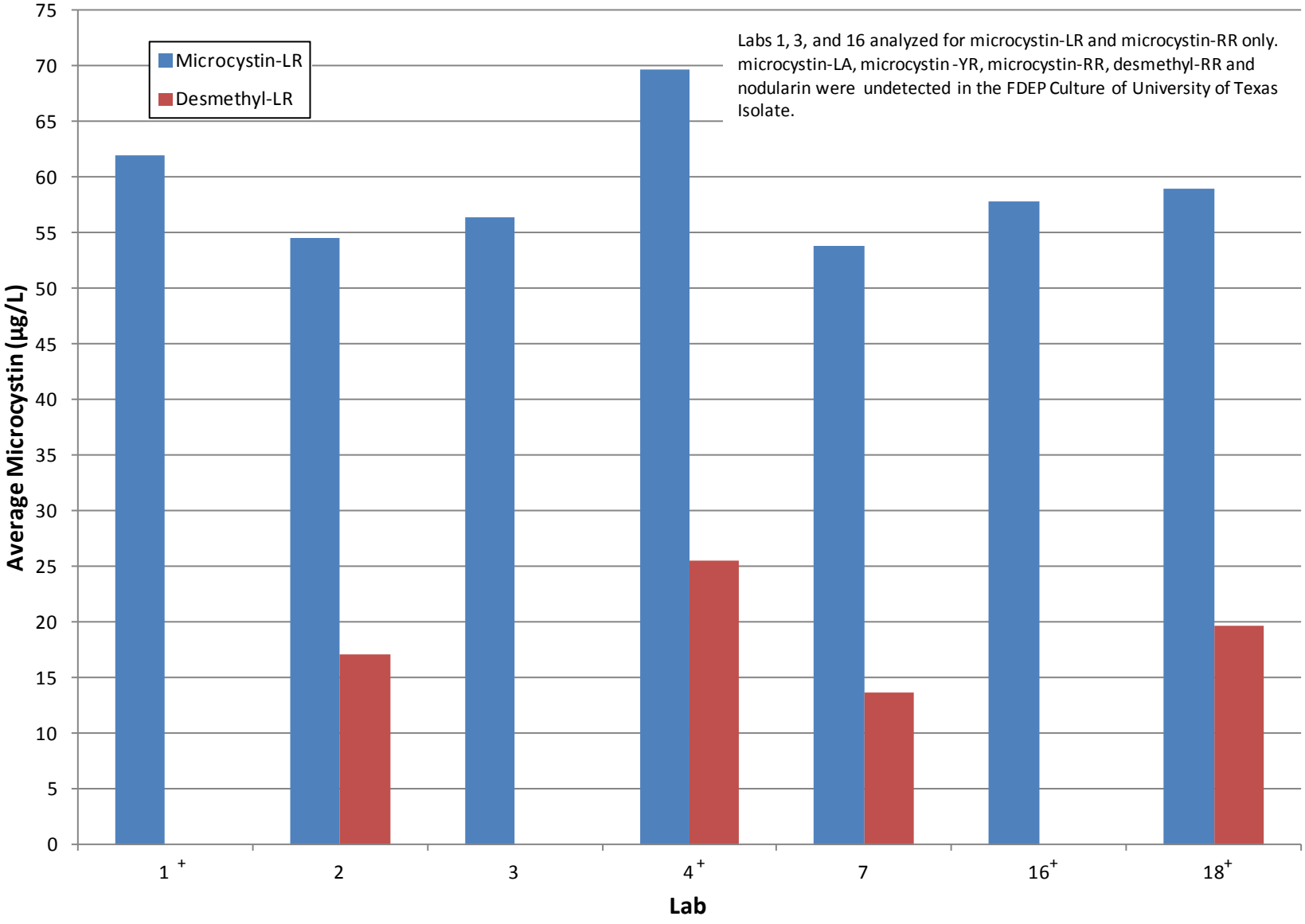


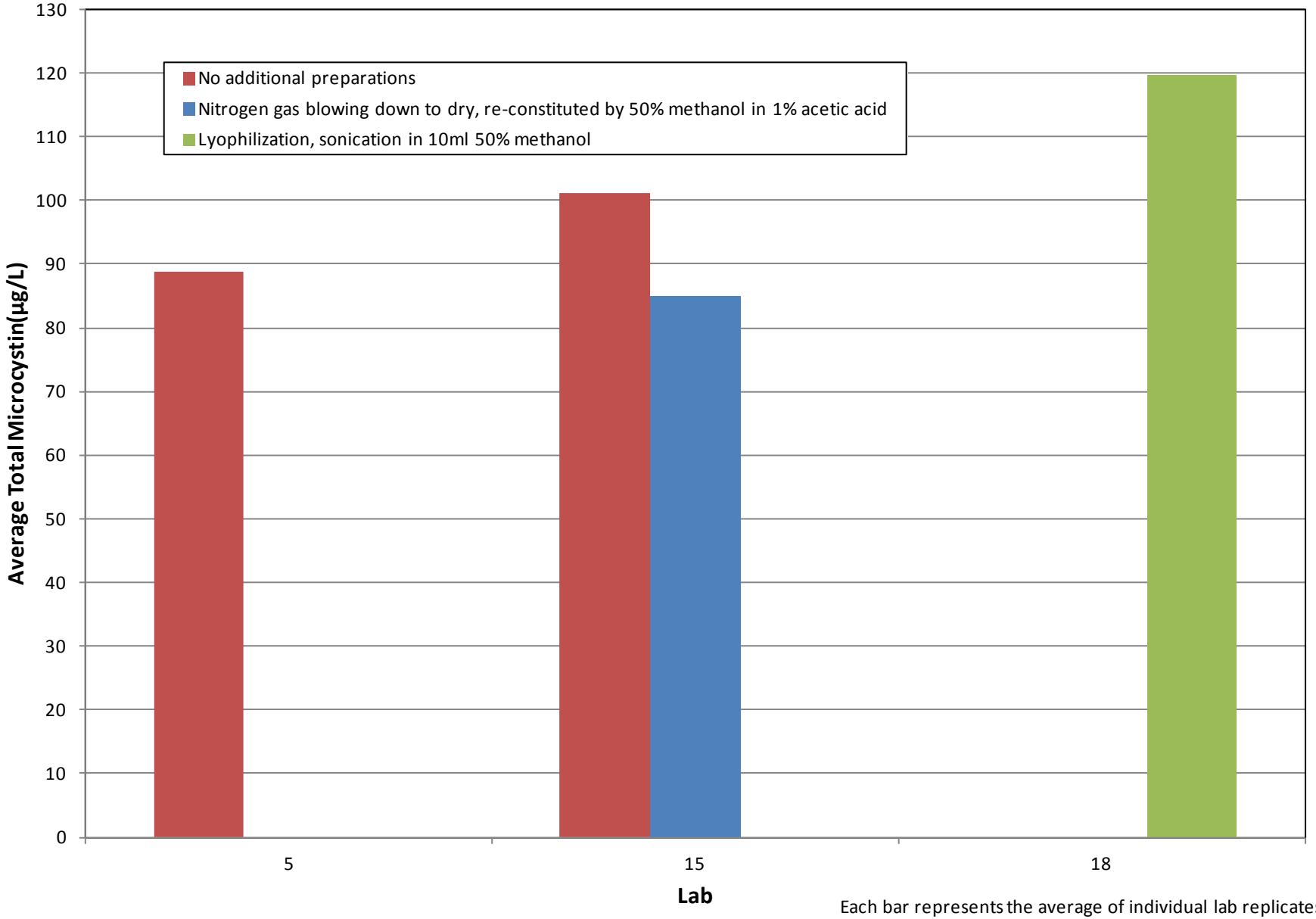
Figure 7. LCMS FDEP Culture of University of Texas *Microcystis aeruginosa* Isolate Results



+ Analyzed using LC/MS (others analyzed using LC/MS/MS)

Each bar represents the average of individual lab replicates.

Figure 8. PPIA FDEP Culture of University of Texas *Microcystis aeruginosa* Isolate Results



Each bar represents the average of individual lab replicates.

FDEP Lake Munson Culture (Munson)

ELISA Kit Results

Values reported for the Lake Munson Culture ranged from 130 to 1200 µg/L. The overall mean and % CV for all ELISA kit results was 618 µg/L and 43% respectively.

Values reported for the Lake Munson Culture using the NRC ELISA Standard ranged from 251 to 1200 µg/L. The overall mean and % CV for all ELISA kit results was 677 µg/L and 40% respectively.

Abraxis values reported for the Lake Munson Culture ranged from 406 to 1200 µg/L. The mean and % CV for the Abraxis ELISA kit results was 777µg/L and 30% respectively.

Envirologix values reported for the Lake Munson Culture isolate ranged from 130 to 718 µg/L. The mean and % CV for the Envirologix ELISA kit results was 436 µg/L and 34% respectively.

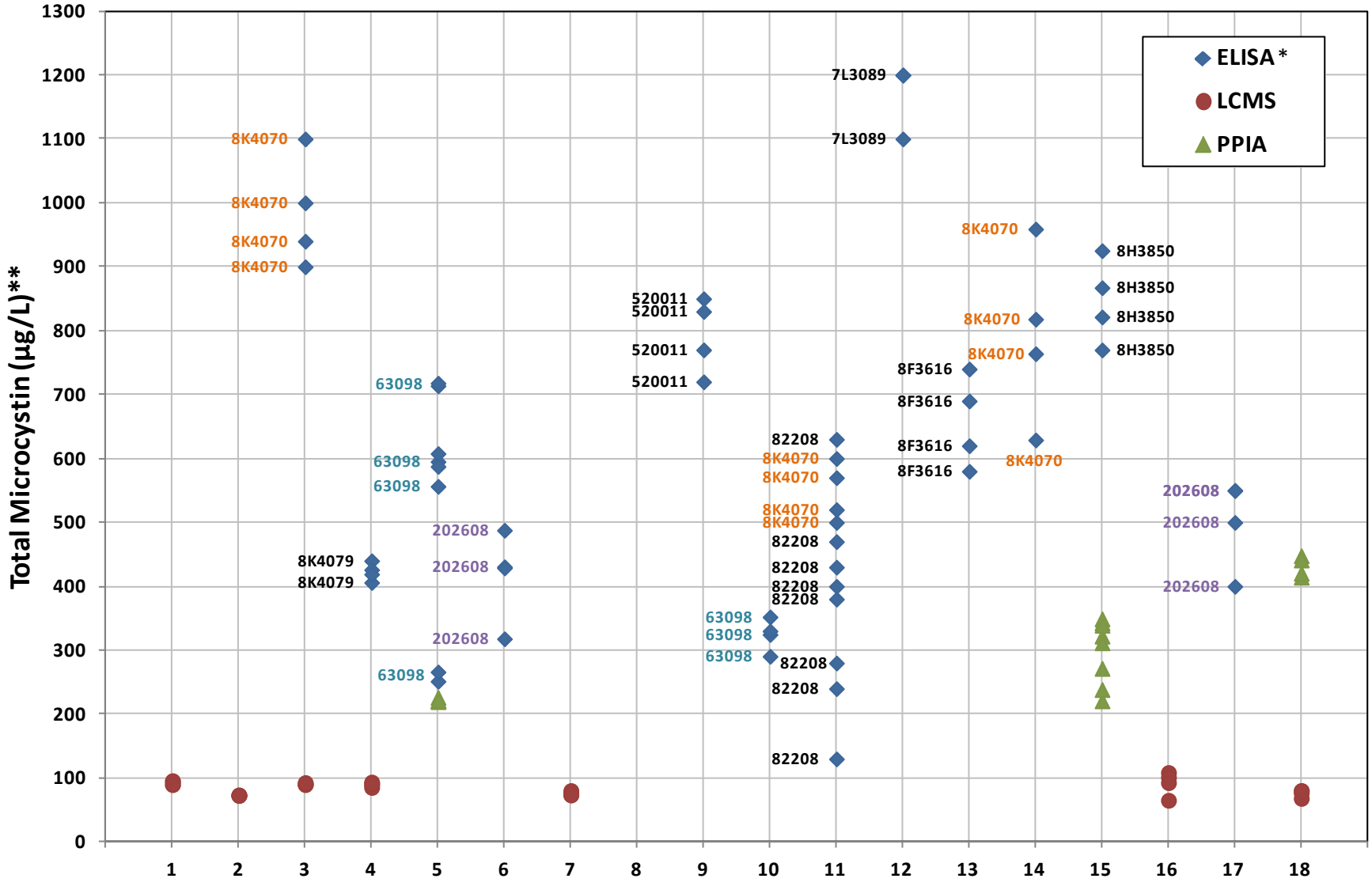
High Performance Liquid Chromatography/Mass Spectroscopy Results

Total microcystin values reported for the FDEP Lake Munson Culture samples ranged from 65 to 185 µg/L. The overall mean and % CV was 131 µg/L and 29%. The within laboratory % CV for the replicates ranged from less than 1% to 21%. Total microcystin-LR and -RR values reported ranged from 65 to 109 µg/L. The overall mean and % CV was 85 µg/L and 13%. The within laboratory % CV for the replicates ranged from less than 1% to 21%. The higher variability associated with the total microcystin values when compared with the values for total microcystin-LR and-RR can be attributed to the microcystin desmethyl-LR analyzed for and detected by four labs (Lab 2, Lab 4, Lab 7 and Lab 18). The total microcystin value was higher in these four labs when compared with their own microcystin-LR and-RR values and with the labs that did not analyze for microcystin desmethyl-LR.

Protein Phosphatase Inhibition Assay Results

Total microcystin values reported for the FDEP Lake Munson Culture samples ranged from 220 to 448 µg/L. The overall mean and % CV was 313 µg/L and 27%. The within laboratory % CV for the replicates ranged from less than 1% to 17%. Preparation methods comparisons can be made with Lab 15 for the nitrogen gas blow down/reconstitution and no additional prep method. The % CV for the nitrogen gas blow down/reconstitution was 15% and for no additional preparation method was 3%. Lab 5 also performed no additional preparations so comparisons between Lab 5 and Lab 15 can be made. The overall mean and % CV for the Lake Munson Culture-no additional preparations was 280 µg/L and 22%.

Figure 9. FDEP Lake Munson Culture Results - All Methods



*ELISA results labeled with the kit manufacturer's serial number.
**LCMS Values are Microcystin-LR+RR; Does not include other congeners or degradation products

Figure 10. ELISA FDEP Lake Munson Culture Results

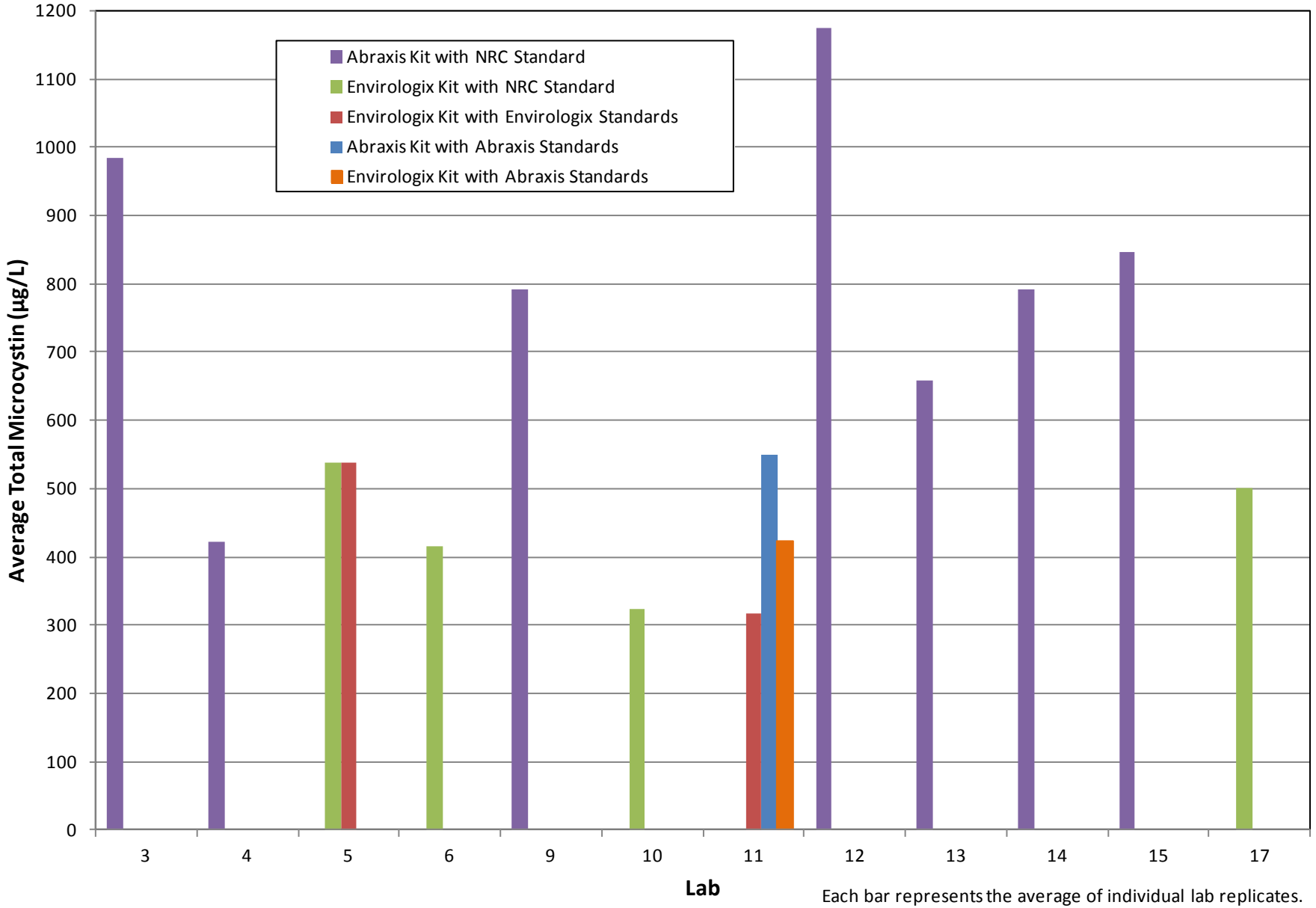
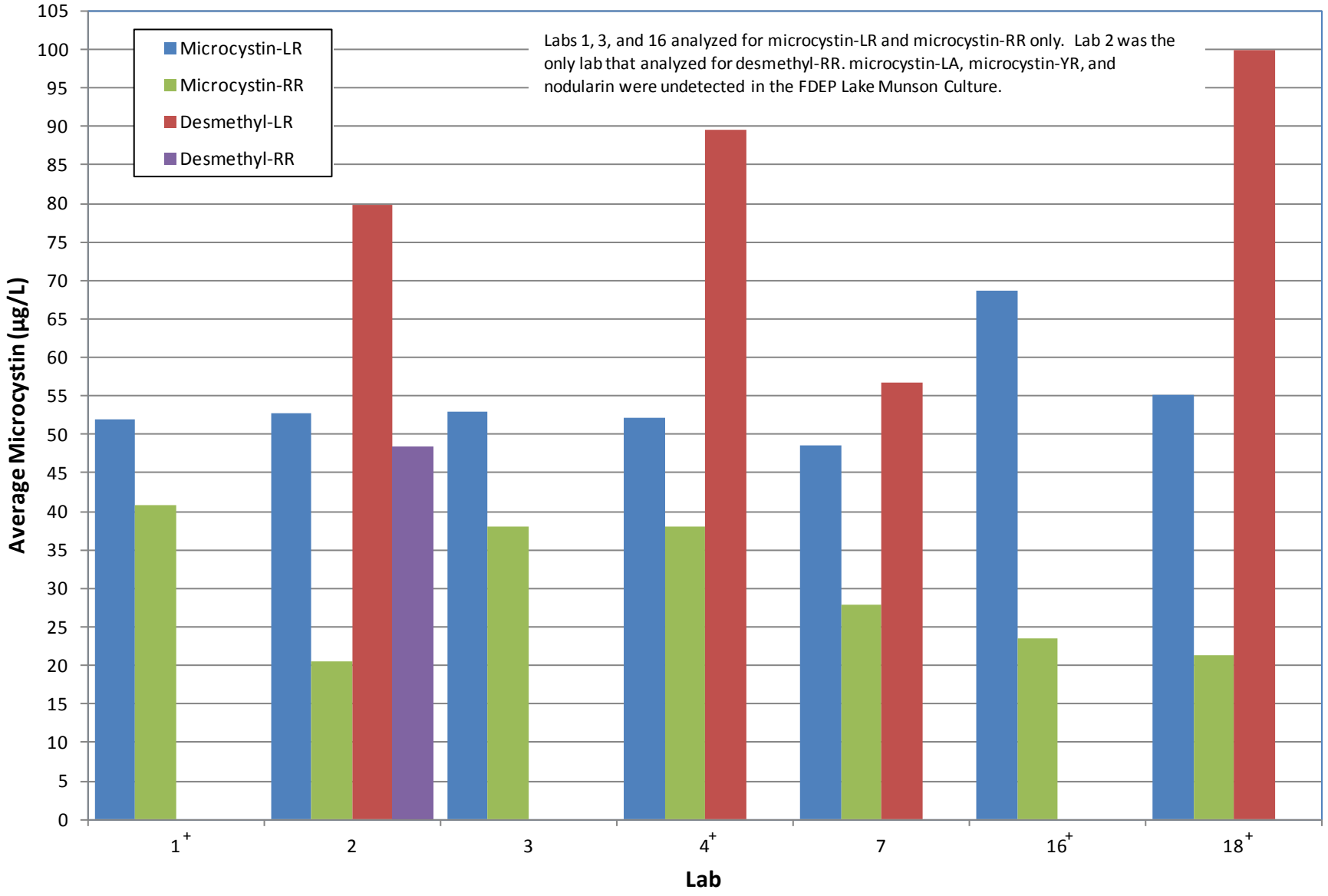


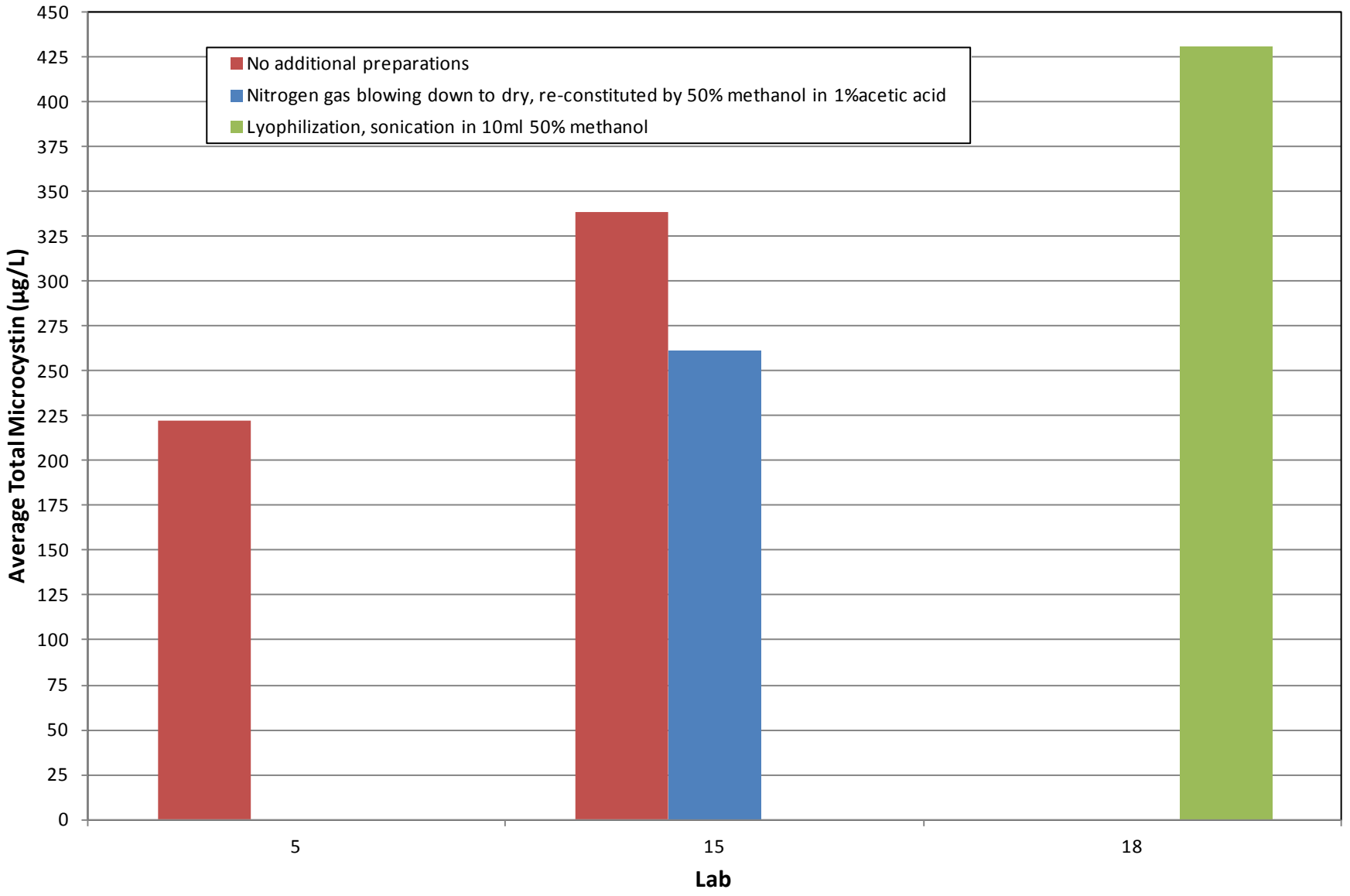
Figure 11. LCMS FDEP Lake Munson Culture Results



+ Analyzed using LC/MS (others analyzed using LC/MS/MS)

Each bar represents the average of individual lab replicates.

Figure 12. PPIA FDEP Lake Munson Culture Results



Each bar represents the average of individual lab replicates.

Additional ELISA Kit Testing

Lab 5 performed testing using an Envirologix kit with the NRC ELISA Standard and with the Envirologix standards that come with each kit. The within laboratory % CV for the Microcystin-LR Standard, the University of Texas isolate and the Lake Munson Culture were similar for both types of standards used. The results are listed below.

For the Microcystin-LR Standard analyzed with the Envirologix kit using Envirologix standards, microcystin values ranged from 3.4 to 4.1 µg/L and the within laboratory % CV for the replicates was 10%. For the Microcystin-LR Standard analyzed with the Envirologix kit using NRC ELISA standards, microcystin values ranged from 3.5 to 4.3 µg/L and the within laboratory % CV for the replicates was 12%.

For the University of Texas isolate analyzed with the Envirologix kit using Envirologix standards, microcystin values ranged from 109 to 279 µg/L and the within laboratory % CV for the replicates was 42%. For the University of Texas isolate analyzed with the Envirologix kit using NRC ELISA standards, microcystin values ranged from 100 to 309 µg/L and the within laboratory % CV for the replicates was 49%.

For the Lake Munson Cultures analyzed with the Envirologix kit using Envirologix standards, microcystin values ranged from 266 to 718 µg/L and the within laboratory % CV for the replicates was 36%. For the Lake Munson Cultures analyzed with the Envirologix kit using NRC ELISA standards, microcystin values ranged from 251 to 714 µg/L and the within laboratory % CV for the replicates was 37%.

Lab 11 performed testing using an Abraxis kit with Abraxis standards, an Envirologix kit with Envirologix standards, and an Envirologix kit with Abraxis standards. The results are listed below.

For the Microcystin-LR Standard analyzed with the Abraxis kit using Abraxis standards, microcystin values ranged from 2.9 to 3.3 µg/L and the within laboratory % CV for the replicates was 7%. For the Microcystin-LR Standard analyzed with the Envirologix kit using Envirologix standards, microcystin values ranged from 5.6 to 6.1 µg/L and the within laboratory % CV for the replicates was 5%. For the Microcystin-LR Standard analyzed with the Envirologix kit using Abraxis Standards, microcystin values ranged from 4.5 to 7.9 µg/L and the within laboratory % CV for the replicates was 27%

For the University of Texas isolate analyzed with the Abraxis kit using Abraxis standards, microcystin values ranged from 240 to 310 µg/L and the within laboratory % CV for the replicates was 13%. For the University of Texas isolate analyzed with the Envirologix kit using Envirologix standards, microcystin values ranged from 60 to 120 µg/L and the within laboratory % CV for the replicates was 40%. For the University of Texas isolate analyzed with the Envirologix kit using Abraxis standards, microcystin values ranged from 96 to 140 µg/L and the within laboratory % CV for the replicates was 22%.

For the Lake Munson Cultures analyzed with the Abraxis kit using Abraxis standards, microcystin values ranged from 500 to 600 µg/L and the within laboratory % CV for the replicates was 8%. For the Lake Munson Cultures analyzed with the Envirologix kit using Envirologix standards, microcystin values ranged from 130 to 470 µg/L and the within laboratory % CV for the replicates was 50%.
For the Lake Munson Cultures analyzed with the Envirologix kit using Abraxis standards, microcystin values ranged from 280 to 630 µg/L and the within laboratory % CV for the replicates was 35%.

Several laboratories bought kits manufactured from the same batch. These results appear in Figures 13, 14, and 15.

Labs 3 and 14 analyzed the Microcystin-LR Standard with the Abraxis kit serial number 8K4070 using NRC ELISA standards. For the Microcystin-LR Standard analyzed by Lab 3, microcystin values ranged from 4.8 to 5.6 µg/L and the within laboratory % CV for the replicates was 4%. For the Microcystin-LR Standard analyzed by Lab 14, microcystin values ranged from 4.6 to 5.5 µg/L with an outlier of 18.9 µg/L and the within laboratory % CV for the replicates was 12% with the outlier excluded and 83% with the outlier included.

Labs 5 and 10 analyzed the Microcystin-LR Standard with the Envirologix kit serial number 63098 using NRC ELISA standards. For the Microcystin-LR Standard analyzed by Lab 5, microcystin values ranged from 3.5 to 4.3 µg/L and the within laboratory % CV for the replicates was 12%. For the Microcystin-LR Standard analyzed by Lab 10, microcystin values ranged from 3.1 to 5.0 µg/L and the within laboratory % CV for the replicates was 23%.

Labs 6 and 17 analyzed the Microcystin-LR Standard with the Envirologix kit serial number 202608 using NRC ELISA standards. For the Microcystin-LR Standard analyzed by Lab 6, microcystin values ranged from 2.8 to 4.6 µg/L and the within laboratory % CV for the replicates was 24%. For the Microcystin-LR Standard analyzed by Lab 17, microcystin values ranged from 5.5 to 6.5 µg/L and the within laboratory % CV for the replicates was 8%.

Labs 3 and 14 analyzed the University of Texas isolate with the Abraxis kit serial number 8K4070 using NRC ELISA standards. For the University of Texas isolate analyzed by Lab 3, microcystin values ranged from 170 to 200 µg/L and the within laboratory % CV for the replicates was 8%. For the University of Texas isolate analyzed by Lab 14, microcystin values ranged from 154 to 262 µg/L and the within laboratory % CV for the replicates was 26%.

Labs 5 and 10 analyzed the University of Texas isolate with the Envirologix kit serial number 63098 using NRC ELISA standards. For the University of Texas isolate analyzed by Lab 5, microcystin values ranged from 100 to 309 µg/L and the within laboratory % CV for the replicates was 49%. For the University of Texas isolate analyzed by Lab 10, microcystin values ranged from 99 to 158 µg/L and the within laboratory % CV for the replicates was 24%.

Labs 6 and 17 analyzed the University of Texas isolate with the Envirologix kit serial number 202608 using NRC ELISA standards. For the University of Texas isolate analyzed by Lab 6, microcystin values ranged from 155 to 201 µg/L and the within laboratory % CV for the replicates was 13%. For the University of Texas isolate analyzed by Lab 17, microcystin values ranged from 120 to 160 µg/L and the within laboratory % CV for the replicates was 16%.

Labs 3 and 14 analyzed the Lake Munson Cultures with the Abraxis kit serial number 8K4070 using NRC ELISA standards. For the Lake Munson Cultures analyzed by Lab 3, microcystin values ranged from 900 to 1100 µg/L and the within laboratory % CV for the replicates was 9%. For the Lake Munson Cultures analyzed by Lab 14, microcystin values ranged from 629 to 959 µg/L and the within laboratory % CV for the replicates was 17%.

Labs 5 and 10 analyzed the Lake Munson Cultures with the Envirologix kit serial number 63098 using NRC ELISA standards. For the Lake Munson Cultures analyzed by Lab 5, microcystin values ranged from 251 to 714 µg/L and the within laboratory % CV for the replicates was 37%. For the Lake Munson Cultures analyzed by Lab 10, microcystin values ranged from 291 to 352 µg/L and the within laboratory % CV for the replicates was 8%.

Labs 6 and 17 analyzed the Lake Munson Cultures with the Envirologix kit serial number 202608 using NRC ELISA standards. For the Lake Munson Cultures analyzed by Lab 6, microcystin values ranged from 318 to 488 µg/L and the within laboratory % CV for the replicates was 17%. For the Lake Munson Cultures analyzed by Lab 17, microcystin values ranged from 400 to 550 µg/L and the within laboratory % CV for the replicates was 14%.

Figure 13. ELISA tests with multiple laboratories using kits from the same lot, Prepared Standard.

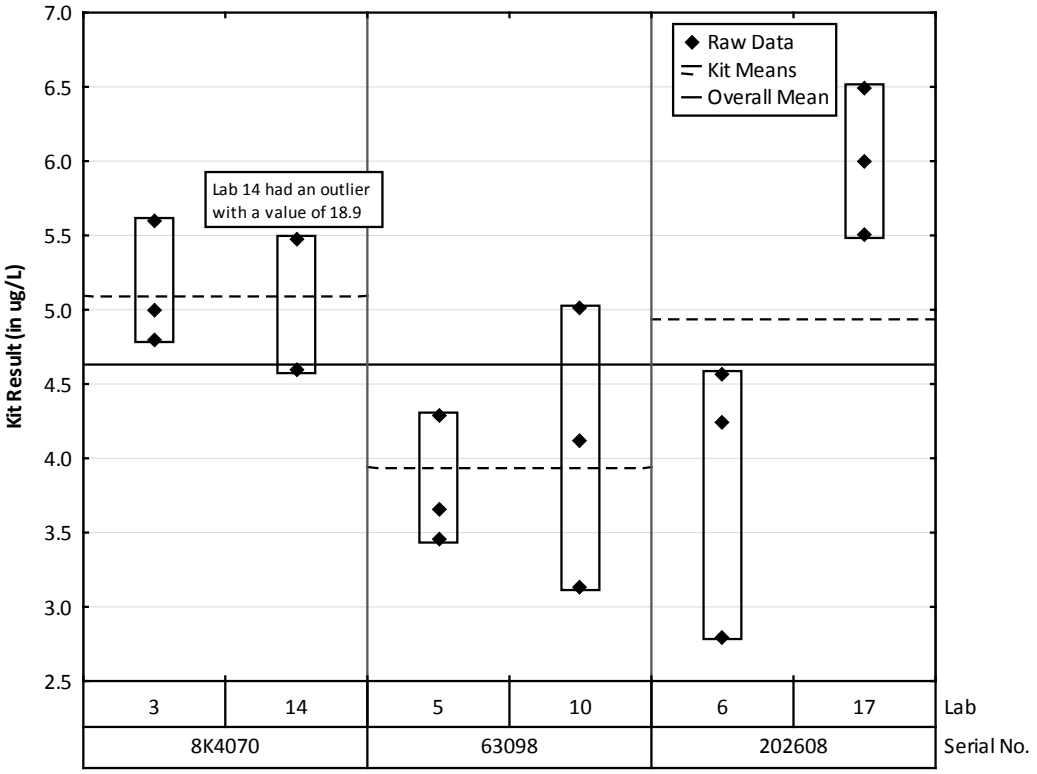


Figure 14. ELISA tests with multiple laboratories using kits from the same lot, Utex sample.

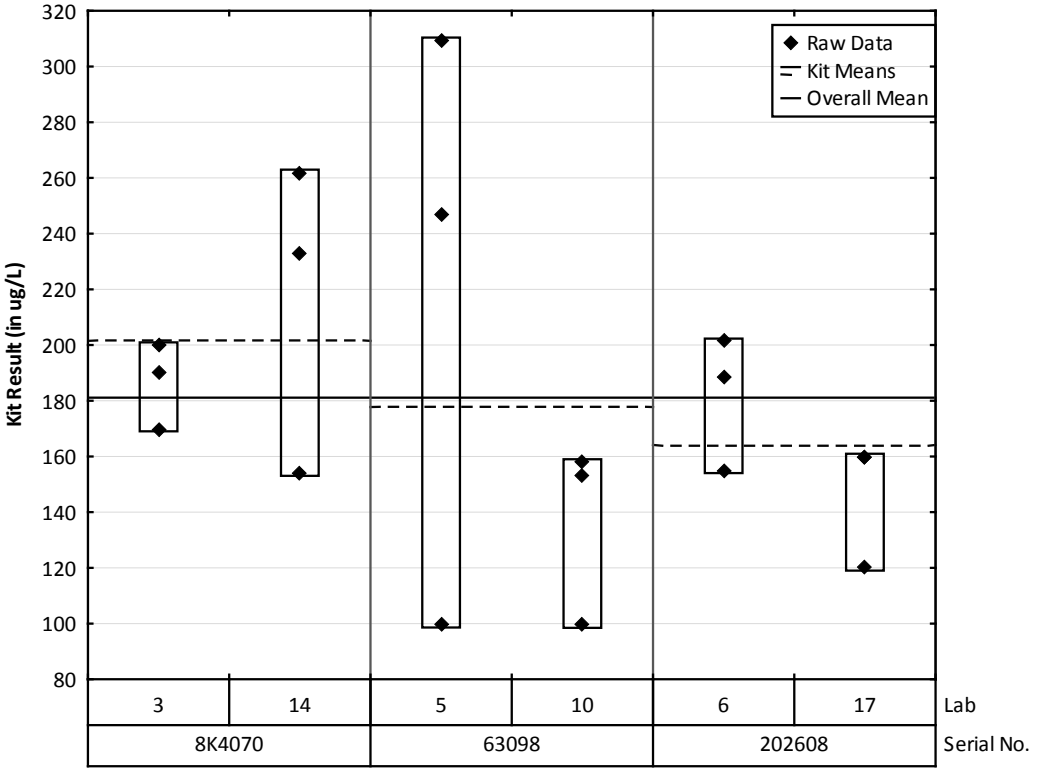
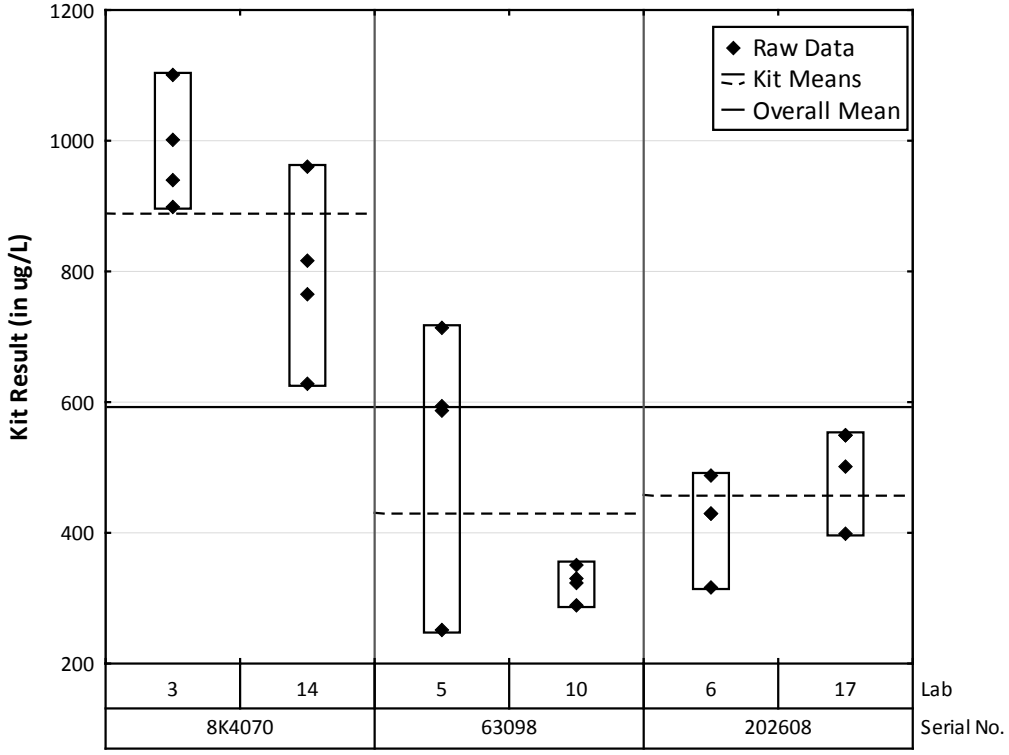


Figure 15. ELISA tests with multiple laboratories using kits from the same lot, Lake Munson sample.



Conclusions

The intent of this second round robin was to control more aspects of the microcystin analysis than in the previous round robin. The expectation was that there would be less variability in the results; however, these controls did not reduce the variability. As in the previous round robin, there was a wide variety of results from each sample and the variability between laboratories was greater than that within laboratory. The ELISA kit results continue to be the most variable. These kits are manufactured with sensitivity to specific congeners. They may also react differently to other non-microcystin substances in the water sample. The results for the LCMS method are much less variable than the other methods as a specific congener is being targeted, although some laboratories have much less variability than others. With so many potential variants in a sample, it is prohibitively expensive to buy standards for all 80 variants. Laboratories have to choose which ones to buy given their budget constraints and knowledge of the site. The PPIA results were slightly higher than the LCMS and less variable than the ELISA tests. There were only two laboratories that analyzed the samples with the same preparation prior to PPIA, therefore, it is not possible to quantitatively compare the variance estimates of the PPIA results to those of the other methods.

Since none of the laboratories reported a false positive for the blank sample, there is confidence that when a method reports a positive result, there is microcystin toxin present. The quantification of the amount of toxin is not as reliable. It is important to emphasize that none of these methods characterize the entire microcystin community. ELISA kits can be used to determine the presence of toxins, especially microcystin-LR. When reliable estimates of how much of a particular toxin is needed, LCMS or PPIA methods should be used.

Acknowledgements

All the laboratories that participated in this round robin provided their analyses including the purchase of the common standard at their own expense. The FDEP thanks them for their support and contribution of time and supplies.

The pre-release NRC Canada standards and ELISA kits were made available for purchase to the laboratories by Fernando Rubio of the Abraxis company. The FDEP thanks him for his assistance with making this part of the round robin possible.

Participating Laboratories

This list is sorted alphabetically and is not the order in which the lab letters were assigned.

Agency/University/Lab

Aquatic Ecosystem Management Research
Canada Centre for Inland Waters
Environment Canada
Burlington, Ontario

California Department of Fish and Game
Fish and Wildlife Water Pollution Control Laboratory
Rancho Cordova, CA

CH2M HILL Inc
Applied Sciences Laboratory
Corvallis, OR

Delaware Dept of Natural Resources and Env. Control
Environmental Laboratory Section
Dover, DE

Florida Dept of Environmental Protection
Bureau of Laboratories
Tallahassee, FL

Florida Dept of Health
Bureau of Labs
Jacksonville, FL

Florida Fish and Wildlife Commission
Fish and Wildlife Research Institute
St. Petersburg, FL

Grand Valley State University
Annis Water Resources Institute
Muskegon, MI

GreenWater Laboratories/CyanoLab
Palatka, FL

Lake Superior State University
Department of Chemistry
Sault Ste. Marie, MI

Manatee County Water Plant QC Lab
Bradenton, FL

South Florida Water Management District
Chemistry Lab
West Palm Beach, FL

State University of New York
College of Environmental Science and Forestry
Syracuse, NY

University of California, Davis
California Animal Health and Food Safety
Toxicology Lab
Davis, CA

US Environmental Protection Agency
Region 9 Lab
Richmond, CA

U.S. Geological Survey
Florida Integrated Science Center
Orlando, FL

U.S. Geological Survey
Kansas Water Science Center
Lawrence, Kansas

Table 1. Summary Stats Table - Overall Mean, Standard Deviation, % CV and Number of Values for Each Sample

Sample	Method	Mean (ug/L)	Std Dev	% CV	Number of values (N)
Std	ELISA	4.4†	1.4	31%	47
Std	LCMS	4.2	1.7	40%	21
Std	PPIA	2.9	0.9	30%	12
Std	All Methods	4.1†	1.5	36%	80
Utex	ELISA	177	67	38%	48
Utex	LCMS	70	15	21%	21
Utex	LCMS*	59	9	15%	21
Utex	PPIA	99	15	15%	12
Utex	All Methods	138	71	52%	81
Utex	All Methods*	135	74	55%	81
Munson	ELISA	618	263	43%	60
Munson	LCMS	131	38	29%	28
Munson	LCMS*	85	11	13%	28
Munson	PPIA	313	85	27%	16
Munson	All Methods	440	297	67%	104
Munson	All Methods*	428	310	72%	104

†-Outlier value from Lab 14 not included

*-Total LR+RR; other LCMS averages include microcystin desmethyl-LR and microcystin desmethyl-RR

Table 2. ELISA Results

Lab	Sample	Bottle Number	Standard used	Manufacturer	Serial No.	Mono- or Polyclonal (M or P)	Result (ug/L)	Qualifier	Average (ug/L)	Std Dev	%CV
3	Std	4	**NRC ELISA dil std**	Abraxis	8K4070	P	**5.0**				
3	Std	11	**NRC ELISA dil std**	Abraxis	8K4070	P	**5.6**				
3	Std	12	**NRC ELISA dil std**	Abraxis	8K4070	P	**4.8**		5.1	0.4	4%
4	Std	10	**NRC ELISA dil std**	Abraxis	8K4079	M	**6.1**				
4	Std	11	**NRC ELISA dil std**	Abraxis	8K4079	M	**6.0**				
4	Std	12	**NRC ELISA dil std**	Abraxis	8K4079	M	**6.1**		6.0	0.1	1%
5	Std	6	**Envirologix Kit Std**	Envirologix	63098	P	**3.6**				
5	Std	7	**Envirologix Kit Std**	Envirologix	63098	P	**4.1**				
5	Std	13	**Envirologix Kit Std**	Envirologix	63098	P	**3.4**		3.7	0.4	10%
5	Std	6	**NRC ELISA dil std**	Envirologix	63098	P	**3.7**				
5	Std	7	**NRC ELISA dil std**	Envirologix	63098	P	**4.3**				
5	Std	13	**NRC ELISA dil std**	Envirologix	63098	P	**3.5**		3.8	0.4	12%
6	Std	7	**NRC ELISA dil std**	Envirologix	202608	P	**2.8**				
6	Std	10	**NRC ELISA dil std**	Envirologix	202608	P	**4.6**				
6	Std	13	**NRC ELISA dil std**	Envirologix	202608	P	**4.2**		3.9	0.9	24%
7	Std	8	**NRC ELISA dil std**	Envirologix			**2.1**				
7	Std	9	**NRC ELISA dil std**	Envirologix			**2.3**				
7	Std	11	**NRC ELISA dil std**	Envirologix			**2.0**		2.1	0.1	6%
9	Std	1	**NRC ELISA dil std**	Abraxis	520011	P	**3.6**				
9	Std	5	**NRC ELISA dil std**	Abraxis	520011	P	**3.6**				
9	Std	6	**NRC ELISA dil std**	Abraxis	520011	P	**3.8**		3.7	0.1	3%
10	Std	2	**NRC ELISA dil std**	Envirologix	63098	P	**5.0**				
10	Std	9	**NRC ELISA dil std**	Envirologix	63098	P	**3.1**				
10	Std	13	**NRC ELISA dil std**	Envirologix	63098	P	**4.1**		4.1	0.9	23%
11	Std	3	**Abraxis Standards**	Abraxis	8K4070	P	**2.9**	J, C3			
11	Std	7	**Abraxis Standards**	Abraxis	8K4070	P	**3.3**	J, C3			
11	Std	13	**Abraxis Standards**	Abraxis	8K4070	P	**3.3**	J, C3	3.2	0.2	7%
11	Std	3	**Envirologix Standards**	Envirologix	82208	M	**5.6**	J, Q11			
11	Std	7	**Envirologix Standards**	Envirologix	82208	M	**6.1**				
11	Std	13	**Envirologix Standards**	Envirologix	82208	M	**5.7**		5.8	0.3	5%
11	Std	3	**Abraxis Standards**	Envirologix	82208	M	**6.7**	J, C3			
11	Std	7	**Abraxis Standards**	Envirologix	82208	M	**4.5**	J, C3			
11	Std	13	**Abraxis Standards**	Envirologix	82208	M	**7.9**	J, C3	6.4	1.7	27%
12	Std	5	**NRC ELISA dil std**	Abraxis	7L3089	P	**6.1**				
12	Std	11	**NRC ELISA dil std**	Abraxis	7L3089	P	**4.9**				
12	Std	12	**NRC ELISA dil std**	Abraxis	7L3089	P	**3.6**		4.9	1.3	26%
13	Std	3	**NRC ELISA dil std**	Abraxis	8F3616	P	**4.4**				
13	Std	10	**NRC ELISA dil std**	Abraxis	8F3616	P	**4.4**				
13	Std	11	**NRC ELISA dil std**	Abraxis	8F3616	P	**5.8**		4.9	0.8	17%
14	Std	2	**NRC ELISA dil std**	Abraxis	8K4070	P	**5.5**				
14	Std	6	**NRC ELISA dil std**	Abraxis	8K4070	P	**4.6**		5.0**	0.6**	12%**
14	Std	11	**NRC ELISA dil std**	Abraxis	8K4070	P	**18.9***		9.7	8.0	83%

*- Outlier

**-Lab 14 Outlier Value Not Included

Qualifiers:

J - The reported result for this analyte should be considered an estimated value.
Q11 - The replicate analysis of the sample did not meet QC precision critieria.
C3 – The initial calibration for this analyte did not meet calibration criteria. (R^2 < 0.985)

Table 2. ELISA Results (continued)

Lab	Sample	Bottle Number	Standard used	Manufacturer	Serial No.	Mono- or Polyclonal (M or P)	Result (ug/L)	Qualifier	Average (ug/L)	Std Dev	%CV
15	Std	3	NRC ELISA dil std	Abraxis	8H3850		**2.7**				
15	Std	4	NRC ELISA dil std	Abraxis	8H3850		**2.1**				
15	Std	10	NRC ELISA dil std	Abraxis	8H3850		**3.3**		2.7	0.6	21%
17	Std	1	NRC ELISA dil std	Envirologix	202608	P	**5.5**				
17	Std	7	NRC ELISA dil std	Envirologix	202608	P	**6.0**				
17	Std	8	NRC ELISA dil std	Envirologix	202608	P	**6.5**		6.0	0.5	8%
Overall Results	**Std**	-	-	-	**All Standard ELISA Results****				**4.4**	**1.4**	**31%**
	Std	-	NRC ELISA dil std	-	**All NRC STANDARD Results****				**4.3**	**1.3**	**30%**
	Std	-	-	**Abraxis**	**All Abraxis Results****				**4.4**	**1.2**	**28%**
	Std	-	-	**Envirologix**	**All Envirologix Results**				**4.5**	**1.5**	**35%**
	Std	-	-	-	**All Monoclonal Kit Results (2 labs/9 results)**				**6.1**	**0.9**	**15%**
	Std	-	-	-	**All Polyclonal Kit Results** (10 labs/32 results)**				**4.4**	**1.0**	**23%**
3	Utex	2	NRC ELISA dil std	Abraxis	8K4070	P	**200**				
3	Utex	5	NRC ELISA dil std	Abraxis	8K4070	P	**190**				
3	Utex	13	NRC ELISA dil std	Abraxis	8K4070	P	**170**		187	15	8%
4	Utex	3	NRC ELISA dil std	Abraxis	8K4079	M	**144**				
4	Utex	6	NRC ELISA dil std	Abraxis	8K4079	M	**143**				
4	Utex	8	NRC ELISA dil std	Abraxis	8K4079	M	**149**		145	3	2%
5	Utex	1	Envirologix Kit Std	Envirologix	63098	P	**279**				
5	Utex	3	Envirologix Kit Std	Envirologix	63098	P	**229**				
5	Utex	5	Envirologix Kit Std	Envirologix	63098	P	**109**		206	87	42%
5	Utex	1	NRC ELISA dil std	Envirologix	63098	P	**309**				
5	Utex	3	NRC ELISA dil std	Envirologix	63098	P	**247**				
5	Utex	5	NRC ELISA dil std	Envirologix	63098	P	**100**		219	108	49%
6	Utex	2	NRC ELISA dil std	Envirologix	202608	P	**201**				
6	Utex	6	NRC ELISA dil std	Envirologix	202608	P	**188**				
6	Utex	9	NRC ELISA dil std	Envirologix	202608	P	**155**		182	24	13%
7	Utex	2	NRC ELISA dil std	Envirologix			**92**				
7	Utex	5	NRC ELISA dil std	Envirologix			**97**				
7	Utex	10	NRC ELISA dil std	Envirologix			**99**		96	4	4%
9	Utex	11	NRC ELISA dil std	Abraxis	520011	P	**180**				
9	Utex	12	NRC ELISA dil std	Abraxis	520011	P	**200**				
9	Utex	13	NRC ELISA dil std	Abraxis	520011	P	**200**		193	12	6%
10	Utex	4	NRC ELISA dil std	Envirologix	63098	P	**158**				
10	Utex	6	NRC ELISA dil std	Envirologix	63098	P	**153**				
10	Utex	11	NRC ELISA dil std	Envirologix	63098	P	**99**		137	33	24%
11	Utex	5	Abraxis Standards	Abraxis	8K4070	P	**300**	J, C3			
11	Utex	9	Abraxis Standards	Abraxis	8K4070	P	**310**	J, C3			
11	Utex	10	Abraxis Standards	Abraxis	8K4070	P	**240**	J, C3	283	38	13%
11	Utex	5	Envirologix Standards	Envirologix	82208	M	**60**				
11	Utex	9	Envirologix Standards	Envirologix	82208	M	**120**				
11	Utex	10	Envirologix Standards	Envirologix	82208	M	**67**		82	33	40%
11	Utex	5	Abraxis Standards	Envirologix	82208	M	**96**	J, C3			
11	Utex	9	Abraxis Standards	Envirologix	82208	M	**140**	J, C3			
11	Utex	10	Abraxis Standards	Envirologix	82208	M	**100**	J, C3	112	24	22%
12	Utex	6	NRC ELISA dil std	Abraxis	7L3089	P	**250**				
12	Utex	7	NRC ELISA dil std	Abraxis	7L3089	P	**270**				
12	Utex	9	NRC ELISA dil std	Abraxis	7L3089	P	**270**		263	12	4%
13	Utex	6	NRC ELISA dil std	Abraxis	8F3616	P	**270**				

*- Outlier

**-Lab 14 Outlier Value Not Included

Qualifiers:

J - The reported result for this analyte should be considered an estimated value.

C3 – The initial calibration for this analyte did not meet calibration criteria. (R^2 < 0.985)

Table 2. ELISA Results (continued)

Lab	Sample	Bottle Number	Standard used	Manufacturer	Serial No.	Mono- or Polyclonal (M or P)	Result (ug/L)	Qualifier	Average (ug/L)	Std Dev	%CV
13	Utex	8	**NRC ELISA dil std**	Abraxis	8F3616	P	**190**				
13	Utex	9	**NRC ELISA dil std**	Abraxis	8F3616	P	**140**		200	66	33%
14	Utex	1	**NRC ELISA dil std**	Abraxis	8K4070	P	**154**				
14	Utex	3	**NRC ELISA dil std**	Abraxis	8K4070	P	**233**				
14	Utex	5	**NRC ELISA dil std**	Abraxis	8K4070	P	**262**		216	56	26%
15	Utex	2	**NRC ELISA dil std**	Abraxis	8H3850		**221**				
15	Utex	6	**NRC ELISA dil std**	Abraxis	8H3850		**161**				
15	Utex	7	**NRC ELISA dil std**	Abraxis	8H3850		**130**		171	46	27%
17	Utex	6	**NRC ELISA dil std**	Envirologix	202608	P	**120**				
17	Utex	11	**NRC ELISA dil std**	Envirologix	202608	P	**160**				
17	Utex	12	**NRC ELISA dil std**	Envirologix	202608	P	**160**		147	23	16%
Overall Results	**Utex**	-	**-**	**-**	**All Utex ELISA Results**				**177**	**67**	**38%**
	Utex	-	**NRC ELISA dil std**	-	**All NRC STANDARD Results**				**180**	**57**	**32%**
	Utex	-	**-**	**Abraxis**	**All Abraxis Results**				**207**	**54**	**26%**
	Utex	-	**-**	**Envirologix**	**All Envirologix Results**				**147**	**66**	**45%**
	Utex	-	**-**	**-**	**All Monoclonal Kit Results (2 labs/9 results)**				**113**	**34**	**30%**
	Utex	-	**-**	**-**	**All Polyclonal Kit Results (10 labs/33 results)**				**203**	**61**	**30%**
3	Munson	3	**NRC ELISA dil std**	Abraxis	8K4070	P	**1100**				
3	Munson	6	**NRC ELISA dil std**	Abraxis	8K4070	P	**940**				
3	Munson	7	**NRC ELISA dil std**	Abraxis	8K4070	P	**1000**				
3	Munson	9	**NRC ELISA dil std**	Abraxis	8K4070	P	**900**		985	87	9%
4	Munson	1	**NRC ELISA dil std**	Abraxis	8K4079	M	**440**				
4	Munson	2	**NRC ELISA dil std**	Abraxis	8K4079	M	**426**				
4	Munson	4	**NRC ELISA dil std**	Abraxis	8K4079	M	**406**				
4	Munson	13	**NRC ELISA dil std**	Abraxis	8K4079	M	**419**		423	14	3%
5	Munson	4	**Envirologix Kit Std**	Envirologix	63098	P	**556**				
5	Munson	9	**Envirologix Kit Std**	Envirologix	63098	P	**718**				
5	Munson	11	**Envirologix Kit Std**	Envirologix	63098	P	**608**				
5	Munson	12	**Envirologix Kit Std**	Envirologix	63098	P	**266**		537	193	36%
5	Munson	4	**NRC ELISA dil std**	Envirologix	63098	P	**595**				
5	Munson	9	**NRC ELISA dil std**	Envirologix	63098	P	**714**				
5	Munson	11	**NRC ELISA dil std**	Envirologix	63098	P	**588**				
5	Munson	12	**NRC ELISA dil std**	Envirologix	63098	P	**251**		537	199	37%
6	Munson	3	**NRC ELISA dil std**	Envirologix	202608	P	**488**				
6	Munson	4	**NRC ELISA dil std**	Envirologix	202608	P	**431**				
6	Munson	8	**NRC ELISA dil std**	Envirologix	202608	P	**429**				
6	Munson	11	**NRC ELISA dil std**	Envirologix	202608	P	**318**		416	71	17%
7	Munson	1		Envirologix							
7	Munson	3		Envirologix							
7	Munson	7		Envirologix							
7	Munson	13		Envirologix					off scale		
9	Munson	2	**NRC ELISA dil std**	Abraxis	520011	P	**850**				
9	Munson	3	**NRC ELISA dil std**	Abraxis	520011	P	**830**				
9	Munson	8	**NRC ELISA dil std**	Abraxis	520011	P	**720**				
9	Munson	10	**NRC ELISA dil std**	Abraxis	520011	P	**770**		793	59	7%
10	Munson	1	**NRC ELISA dil std**	Envirologix	63098	P	**325**				
10	Munson	5	**NRC ELISA dil std**	Envirologix	63098	P	**291**				
10	Munson	8	**NRC ELISA dil std**	Envirologix	63098	P	**330**				
10	Munson	10	**NRC ELISA dil std**	Envirologix	63098	P	**352**		324	25	8%

Table 2. ELISA Results (continued)

Lab	Sample	Bottle Number	Standard used	Manufacturer	Serial No.	Mono- or Polyclonal (M or P)	Result (ug/L)	Qualifier	Average (ug/L)	Std Dev	%CV
11	Munson	1	**Abraxis Standards**	Abraxis	8K4070	P	**500**	J, C3			
11	Munson	4	**Abraxis Standards**	Abraxis	8K4070	P	**520**	J, C2, C3			
11	Munson	11	**Abraxis Standards**	Abraxis	8K4070	P	**570**	J, C2, C3			
11	Munson	12	**Abraxis Standards**	Abraxis	8K4070	P	**600**	J, C2, C3	548	46	8%
11	Munson	1	**Envirologix Standards**	Envirologix	82208	P	**430**				
11	Munson	4	**Envirologix Standards**	Envirologix	82208	P	**470**				
11	Munson	11	**Envirologix Standards**	Envirologix	82208	P	**240**				
11	Munson	12	**Envirologix Standards**	Envirologix	82208	P	**130**		318	160	50%
11	Munson	1	**Abraxis Standards**	Envirologix	82208	P	**380**	J, C3			
11	Munson	4	**Abraxis Standards**	Envirologix	82208	P	**630**	J, C3			
11	Munson	11	**Abraxis Standards**	Envirologix	82208	P	**400**	J, C3			
11	Munson	12	**Abraxis Standards**	Envirologix	82208	P	**280**	J, C3	423	148	35%
12	Munson	1	**NRC ELISA dil std**	Abraxis	7L3089	P	**1200**				
12	Munson	2	**NRC ELISA dil std**	Abraxis	7L3089	P	**1200**				
12	Munson	4	**NRC ELISA dil std**	Abraxis	7L3089	P	**1200**				
12	Munson	13	**NRC ELISA dil std**	Abraxis	7L3089	P	**1100**		1175	50	4%
13	Munson	1	**NRC ELISA dil std**	Abraxis	8F3616	P	**690**				
13	Munson	2	**NRC ELISA dil std**	Abraxis	8F3616	P	**620**				
13	Munson	4	**NRC ELISA dil std**	Abraxis	8F3616	P	**740**				
13	Munson	7	**NRC ELISA dil std**	Abraxis	8F3616	P	**580**		658	71	11%
14	Munson	7	**NRC ELISA dil std**	Abraxis	8K4070	P	**818**				
14	Munson	8	**NRC ELISA dil std**	Abraxis	8K4070	P	**764**				
14	Munson	12	**NRC ELISA dil std**	Abraxis	8K4070	P	**959**				
14	Munson	13	**NRC ELISA dil std**	Abraxis	8K4070	P	**629**		793	137	17%
15	Munson	1	**NRC ELISA dil std**	Abraxis	8H3850		**770**				
15	Munson	5	**NRC ELISA dil std**	Abraxis	8H3850		**821**				
15	Munson	12	**NRC ELISA dil std**	Abraxis	8H3850		**867**				
15	Munson	13	**NRC ELISA dil std**	Abraxis	8H3850		**925**		846	66	8%
17	Munson	3	**NRC ELISA dil std**	Envirologix	202608	P	**550**				
17	Munson	4	**NRC ELISA dil std**	Envirologix	202608	P	**500**				
17	Munson	10	**NRC ELISA dil std**	Envirologix	202608	P	**400**				
17	Munson	13	**NRC ELISA dil std**	Envirologix	202608	P	**550**		500	71	14%
Overall Results	**Munson**	-	**-**	**-**	**All Munson ELISA Results**				**618**	**263**	**43%**
	Munson	-	**NRC ELISA dil std**	-	**All NRC STANDARD Results**				**677**	**269**	**40%**
	Munson	-	**-**	**Abraxis**	**All Abraxis Results**				**777**	**236**	**30%**
	Munson	-	**-**	**Envirologix**	**All Envirologix Results**				**436**	**150**	**34%**
	Munson	-	**-**	**-**	**All Monoclonal Kit Results (1 labs/4 results)**				**423**	**14**	**3%**
	Munson	-	**-**	**-**	**All Polyclonal Kit Results (10 labs/52 results)**				**616**	**269**	**44%**

Qualifiers:

J - The reported result for this analyte should be considered an estimated value.
C2 - The reported concentration of this analyte is above the calibration range of the instrument.
C3 – The initial calibration for this analyte did not meet calibration criteria. (R^2 < 0.985)

Table 3. LCMS Results

Lab	Sample	Bottle	MC-LR (ug/L)	desmethyl LR (ug/L)	MC-RR (ug/L)	desmethyl-RR (ug/L)	Total Result (ug/L)	Average (ug/L)	Std Dev	%CV	Total LR+RR (ug/L)	Average (ug/L)	Std Dev	%CV
1	Std	9	5.60		1.0 U		5.6				5.6			
1	Std	12	4.90		1.0 U		4.9				4.9			
1	Std	13	4.80		1.0 U		4.8	5.1	0.4	9%	4.8	5.1	0.4	9%
2	Std	2	3.82	0.5U	0.5U	0.5U	3.82				3.82			
2	Std	8	4.02	0.5U	0.5U	0.5U	4.02				4.02			
2	Std	10	4.11	0.5U	0.5U	0.5U	4.11	4.0	0.1	4%	4.11	4.0	0.1	4%
3	Std	4	4.30				4.3				4.3			
3	Std	11	4.40				4.4				4.4			
3	Std	12	4.50				4.5	4.4	0.1	2%	4.5	4.4	0.1	2%
4	Std	10	6.40				6.4				6.4			
4	Std	11	6.60				6.6				6.6			
4	Std	12	6.40				6.4	6.5	0.1	2%	6.4	6.5	0.1	2%
7	Std	8	3.10	ND	ND		3.1				3.1			
7	Std	9	3.50	ND	ND		3.5				3.5			
7	Std	11	3.40	ND	ND		3.4	3.3	0.2	6%	3.4	3.3	0.2	6%
16	Std	5	5.20		2.5 U		5.2				5.2			
16	Std	9	5.60		2.5 U		5.6				5.6			
16	Std	11	3.80		2.5 U		3.8	4.9	0.9	19%	3.8	4.9	0.9	19%
18	Std	2	0.45				0.5				0.5			
18	Std	7	1.63				1.6				1.6			
18	Std	12	1.04				1.0	1.0	0.6	56%	1.0	1.0	0.6	56%
-	**Std**	**All Standard LCMS Results**						**4.2**	**1.7**	**40%**	**-**	**4.2**	**1.7**	**40%**
1	Utex	5	60.00		1.0 U		60				60			
1	Utex	7	60.00		1.0 U		60				60			
1	Utex	10	66.00		1.0 U		66	62	3	6%	66	62	3	6%
2	Utex	1	54.60	17.00	0.5U	0.5U	71.6				54.6			
2	Utex	3	54.70	17.30	0.5U	0.5U	72.0				54.7			
2	Utex	9	54.40	17.10	0.5U	0.5U	71.5	72	0	0.4%	54.4	55	0	0%
3	Utex	2	59.00				59				59			
3	Utex	5	55.00				55				55			
3	Utex	13	55.00				55	56	2	4%	55	56	2	4%
4	Utex	3	72.00	25.40			97.4				72			
4	Utex	6	65.00	25.80			90.8				65			
4	Utex	8	72.00	25.20			97.2	95	4	4%	72	70	4	6%
7	Utex	2	54.90	11.90	ND		66.8				54.9			

Qualifiers:

J-Estimated value; value not accurate.

ND - Not Detected

U - Compound was analyzed for but not detected.

Table 3. LCMS Results (continued)

Lab	Sample	Bottle	MC-LR (ug/L)	desmethyl LR (ug/L)	MC-RR (ug/L)	desmethyl-RR (ug/L)	Total Result (ug/L)	Average (ug/L)	Std Dev	%CV	Total LR+RR (ug/L)	Average (ug/L)	Std Dev	%CV
7	Utex	5	52.90	16.10	ND		69.0				52.9			
7	Utex	10	53.60	12.90	ND		66.5	67	1	2%	53.6	54	1	2%
16	Utex	2	34.00		2.5 U		34.0				34			
16	Utex	12	70.80		2.5 U		70.8				70.8			
16	Utex	13	68.60		2.5 U		68.6	58	21	36%	68.6	58	21	36%
18	Utex	1	55.67	19.22			74.9				55.66884			
18	Utex	4	58.99	19.77			78.8				58.98605			
18	Utex	6	62.43	19.97			82.4	79	4	5%	62.43481	59	3	6%
-	**Utex**	**All Utex LCMS Results**						**70**	**15**	**21%**	-	**59**	**9**	**15%**
1	Munson	1	51.00		44		95				95			
1	Munson	3	51.00		39		90				90			
1	Munson	6	55.00		41		96	93	3	3%	96	93	3	3%
1	Munson	11	51.00		39		90				90			
2	Munson	4	52.70	77.20	20.7	48.5J	151				73.4			
2	Munson	6	52.20	79.60	20.9	51.3J	153				73.1			
2	Munson	12	53.10	79.40	20.5	47.3J	153				73.6			
2	Munson	13	52.90	83.20	19.9	46.3J	156	153	2	1%	72.8	73	0	0.5%
3	Munson	3	53.00		37		90				90			
3	Munson	6	53.00		37		90				90			
3	Munson	7	52.00		39		91				91			
3	Munson	9	54.00		39		93	91	1	2%	93	91	1	2%
4	Munson	1	51.20	89.60	38.30		179.1				89.5			
4	Munson	2	49.60	87.20	35.80		172.6				85.4			
4	Munson	4	54.80	90.80	39.00		184.6				93.8			
4	Munson	13	52.80	90.80	38.90		182.5	180	5	3%	91.7	90	4	4%
7	Munson	1	48.50	53.90	25.4		127.8				73.9			
7	Munson	3	49.60	58.50	30.9		139.0				80.5			
7	Munson	7	49.20	61.40	28.1		138.7				77.3			
7	Munson	13	46.80	53.30	26.9		127.0	133	7	5%	73.7	76	3	4%
16	Munson	1	50.80		14.5		65.3				65.3			
16	Munson	3	78.10		30.6		108.7				108.7			
16	Munson	4	74.50		27.0		101.5				101.5			
16	Munson	8	71.30		21.6		92.9	92	19	21%	92.9	92	19	21%
18	Munson	3	53.60	98.85	23.19		175.6				76.79402			
18	Munson	9	57.39	99.76	23.22		180.4				80.60614			
18	Munson	10	48.40	104.09	19.87		172.4				68.27956			
18	Munson	13	61.39	96.95	18.82		177.2	176	3	2%	80.2142	76	6	7%
-	**Munson**	**All Munson LCMS Results**						**131**	**38**	**29%**	-	**85**	**11**	**13%**

Qualifiers:

J-Estimated value; value not accurate.

ND - Not Detected

U - Compound was analyzed for but not detected.

Table 4. PPIA Results

Lab	Sample	Bottle Number	Result (ug/L)	Duplicate Result in ug/L as rpt	Average (ug/L)	Std Dev	% CV
5	Std	6	**4.22**	3.61			
5	Std	7	**4.18**	4.07			
5	Std	13	**4.22**	4.36	4.2	0.0	1%
15	Std	3	**1.831168**	3.2			
15	Std	3	**2.496904**	3.1			
15	Std	4	**2.001316**	3.7			
15	Std	4	**2.652884**	2.5			
15	Std	10	**2.343644**	3.0			
15	Std	10	**2.474596**	2.9	2.3	0.3	14%
18	Std	2	**2.40655**	2.32			
18	Std	7	**2.889263**	2.77			
18	Std	12	**3.622758**	3.76	3.0	0.6	21%
-	**Std**	**All Standard PPIA Results**			**2.9**	**1**	**30%**
5	Utex	1	**89.9**	112.93			
5	Utex	3	**79.66**	112.83			
5	Utex	5	**97.01**	119.22	89	9	10%
15	Utex	2	**101.8066**	122			
15	Utex	2	**73.98859**	113			
15	Utex	6	**102.3609**	107			
15	Utex	6	**94.66748**	100			
15	Utex	7	**99.64858**	112			
15	Utex	7	**86.72578**	97	93	11	12%
18	Utex	1	**120.0768**	118.90			
18	Utex	4	**119.8675**	117.52			
18	Utex	6	**119.0305**	126.83	120	1	0%
-	**Utex**	**All Utex PPIA Results**			**99**	**15**	**15%**
5	Munson	4	**221.33**	267.23			
5	Munson	9	**226.33**	222.61			
5	Munson	11	**219.61**	218.89			
5	Munson	12	**221.33**	228.77	222	3	1%
15	Munson	1	**342.8981**	375			
15	Munson	1	**271.6145**	299			
15	Munson	5	**349.0638**	362			
15	Munson	5	**220.8592**	318			
15	Munson	12	**322.1096**	396			
15	Munson	12	**238.4989**	293			
15	Munson	13	**339.1758**	315			
15	Munson	13	**311.7048**	301	299	50	17%
18	Munson	3	**414.4023**	497.25			
18	Munson	9	**420.5003**	545.63			
18	Munson	10	**447.8091**	491.88			
18	Munson	13	**441.202**	517.29	431	16	4%
-	**Munson**	**All Munson PPIA Results**			**313**	**85**	**27%**

Table 5. Microcystin Round Robin Analyses and Method Variations Performed by Laboratory

	ELISA					LCMS											PPIA		
	Kit		Standard			Extraction Method				Congeners and Degradation Products							Preparation Method		
LAB	Abraxis	Envirologix	NRC	Abraxis	Envirologix	Sonication	Lyophilization, sonication in 10mL 50% methanol	Solid Phase Extraction	None	Microcystin-LR	Desmethyl-LR	Microcystin-LA	Microcystin-YR	Microcystin-RR	Desmethyl-RR	Nodularin	N2 blowing down to dry, re-constituted by 50%MEOH in 1%Acetic Acid	Lyophilization, sonication in 10mL 50% methanol	None
1									✓	✓		✓	✓	✓					
2						✓				✓	✓	✓	✓	✓	✓	✓			
3	✓		✓						✓	✓				✓					
4	✓		✓						✓	✓	✓			✓					
5		✓	✓		✓														✓
6		✓	✓																
7									✓	✓	✓	✓	✓	✓					
8																			
9	✓		✓																
10		✓	✓																
11	✓	✓		✓	✓														
12	✓		✓																
13	✓		✓																
14	✓		✓																
15	✓		✓														✓		
16								✓		✓				✓					
17		✓	✓																
18							✓			✓	✓			✓				✓	

Table 6. Calibration Ranges and Dilutions

All values are µg/L unless otherwise specified. NR=Not Reported

		ELISA			LCMS			PPIA		
LAB	SAMPLE	MIN	MAX	Dilution	MIN	MAX	Dilution	MIN	MAX	Dilution
1	Jax Pond				1	200	2			
	Std				1	200	2			
	Utex				1	200	2			
	Munson				1	200	2			
2	Jax Pond				1	200	NR			
	Std				1	200	NR			
	Utex				1	200	NR			
	Munson				1	200	NR			
3	Jax Pond	0	5	No	0.5	50	No			
	Std	0	5	Yes	0.5	50	No			
	Utex	0	5	Yes	0.5	50	Yes			
	Munson	0	5	Yes	0.5	50	Yes			
4	Jax Pond	NR	NR	NR	NR	NR	NR			
	Std	NR	NR	NR	NR	NR	NR			
	Utex	NR	NR	NR	NR	NR	NR			
	Munson	NR	NR	NR	NR	NR	NR			
5	NRC Standard									
	Jax Pond	0.15	5	1				0.4	2.0 & 2.4	1
	Std	0.15	5	4				0.4	2.0 & 2.4	10
	Utex	0.15	5	200 & 250				0.4	2.0 & 2.4	100
	Munson	0.15	5	500 & 1000				0.4	2.0 & 2.4	200
	Envirologix Standard									
	Jax Pond	0.16	2.5	1						
	Std	0.16	2.5	4						
	Utex	0.16	2.5	200 & 250						
	Munson	0.16	2.5	500 & 1000						
6	Jax Pond	0.15	2	1						
	Std	0.15	5	10						
	Utex	0.15	5	100						
	Munson	0.15	5	100						
7	Jax Pond	0	2.5	1	1	1000	1			
	Std	0	2.5	1	1	1000	1			
	Utex	0	2.5	100	1	1000	1			
	Munson	0	2.5	>100	1	1000	1			
8	Jax Pond									
	Std									
	Utex									
	Munson									
9	Jax Pond	0.15	3	1						
	Std	0.15	3	2						
	Utex	0.15	3	100						
	Munson	0.15	3	1000						
10	Jax Pond	0.15	2	1						
	Std	0.15	2	10 & 1000						
	Utex	0.15	2	100						
	Munson	0.15	2	10 & 1000						

Table 6. Calibration Ranges and Dilutions (continued)

All values are µg/L unless otherwise specified. NR=Not Reported

		ELISA			LCMS			PPIA		
LAB	SAMPLE	MIN	MAX	Dilution	MIN	MAX	Dilution	MIN	MAX	Dilution
11	Envirologix Kit with Envirologix Standards									
	Jax Pond	0	2.5	1						
	Std	0	2.5	4						
	Utex	0	2.5	200						
	Munson	0	2.5	400						
	Abraxis Kit with Abraxis Standards									
	Jax Pond	0.15	5	1						
	Std	0.15	5	1						
	Utex	0.15	5	100						
	Munson	0.15	5	100						
	Envirologix Kit with Abraxis Standards									
	Jax Pond	0.15	5	1						
	Std	0.15	5	4						
	Utex	0.15	5	200						
	Munson	0.15	5	400						
12	Jax Pond	0.15	5	1						
	Std	0.15	5	2						
	Utex	0.15	5	100						
	Munson	0.15	5	500						
13	Jax Pond	0.15	5	1						
	Std	0.15	5	10						
	Utex	0.15	5	100						
	Munson	0.15	5	1000						
14	Jax Pond	0.18	4.49	1						
	Std	0.15, 0.18	4.49, 4.68 & 4.84	1, 10 & 100						
	Utex	0.18	4.68	100						
	Munson	0.18	4.49	1000						
15	Jax Pond	0.15	5	1				1.5	40	0.3 & 1.0
	Std	0.15	5	10				1.5	40	0.5 & 1.0
	Utex	0.15	5	100				1.5	40	5 & 10
	Munson	0.15	5	200 & 300				1.5	40	10 & 20
16	Jax Pond				0.1	10				
	Std				0.1	10				
	Utex				0.1	10				
	Munson				0.1	10				
17	Jax Pond	0	2.5	1						
	Std	0	2.5	5						
	Utex	0	2.5	200						
	Munson	0	2.5	500						
18	Jax Pond				1000	NR	1.1	6	40	1.1
	Std				1000	NR	1.1	6	40	1.1
	Utex				1000	NR	1.1	6	40	5
	Munson				1000	NR	1.1	6	40	34

Appendix A.
Description of standards purchased from the National Research Council Canada (NRC)

NRC CRM-dmMCLR – CERTIFIED REFERENCE MATERIAL – PRE-RELEASE
This is a standard solution of [7]desmethyl microcystin-LR (dmMCLR) isolated from a laboratory culture of the algae *Microcystis sp. (cf. flos-aquae)*. The dmMCLR concentration is 9.6 µmol/L in filtered methanol/ water (1:1 v/v). Each ampoule contains 0.5 mL of the standard solution. The ampoules were flame sealed and placed in a small carton. Storage Requirements: Store in the dark in a refrigerator at 4 ^{o}C Solutions are also stable when stored in a good freezer, one that does not undergo a periodic freeze-thaw cycle preferably <-20°C).

NRC CRM-MCLR – CERTIFIED REFERENCE MATERIAL – PRE-RELEASE
This is a standard solution of microcystin-LR (MCLR) isolated from a laboratory culture of the algae *Microcystis aeruginosa*. The MCLR concentration is 10.0 µmol/L in filtered methanol/ water (1:1 v/v). Each ampoule contains 0.5 mL of the standard solution. The ampoules were flame sealed and placed in a small carton. Storage Requirements: Store in the dark in a refrigerator at 4 ^{o}C Solutions are also stable when stored in a good freezer, one that does not undergo a periodic freeze-thaw cycle (preferably <-20°C).

NRC CRM-MCRR – CERTIFIED REFERENCE MATERIAL – PRE-RELEASE
This is a standard solution of microcystin-RR (MCRR) isolated from a laboratory culture of the algae *Anabaena sp.* The MCRR concentration is 9.83 µmol/L in filtered methanol/ water (1:1 v/v). Each ampoule contains 0.5 mL of the standard solution. The ampoules were flame sealed and placed in a small carton. Storage Requirements: Store in the dark in a refrigerator at 4 ^{o}C Solutions are also stable when stored in a good freezer, one that does not undergo a periodic freeze-thaw cycle (preferably <-20°C).

NRC CRM-NODR – CERTIFIED REFERENCE MATERIAL – PRE-RELEASE
This is a standard solution of nodularin-R (NODR) isolated from a laboratory culture of the algae *Nodularia sp.* The NODR concentration is 12.1 µmol/L in filtered methanol/ water (1:1 v/v). Each ampoule contains 0.5 mL of the standard solution. The ampoules were flame sealed and placed in a small carton. Storage Requirements: Store in the dark in a refrigerator at 4 ^{o}C Solutions are also stable when stored in a good freezer, one that does not undergo a periodic freeze-thaw cycle (preferably <-20°C).

Appendix B.

Standard Operating Procedure - Preparation of BG-11 Medium Stock Solutions, Medium and Agar Plates

Effective Date: 01/11/2012
Revision Date: 01/10/2012
Author: C. Swanson
AB-12-1.4

Preparation of BG-11-Medium Stock Solutions, Medium, and Agar Plates

(Based on BG-11 medium recipe from the John C. Meeks Laboratory at the University of California, Davis)

1. SCOPE AND APPLICATION

   1.1 This SOP describes procedures for the preparation of BG-11 stock solutions, medium, and agar plates used for culturing *Microcystis aeruginosa*. (freshwater aqueous).

2. SUMMARY OF METHOD

   2.1 Materials are gathered and placed on lab table and cart.

   2.2 Chemicals are weighed for each stock solution. Chemicals are placed in labeled flasks and dissolved in water.

   2.3 Stock solutions are stored for up to one year in walk-in refrigerator.

   2.4 When BG-11 medium is needed stock solutions are measured and added to water to make medium, which can be stored up to 3 months in walk-in refrigerator.

   2.5 Medium is poured into plates to create agar. Agar can be stored up to 6 months in walk-in refrigerator.

   2.6 Preparation information is recorded in *Algal Biology Section Solution Log*book.

3. APPARATUS AND EQUIPMENT

   3.1 **All Stock Solutions**
   - 3.1.1 OHAUS 250D balance (calibrate per SOP TA-06.15)
   - 3.1.2 Weigh boats, 9 cm x 7.5 cm (use plastic or aluminum weigh boats.)
   - 3.1.3 Volumetric flasks with stoppers, 1 L
   - 3.1.4 Metal spatula
   - 3.1.5 Magnetic stirrer
   - 3.1.6 Stir bar, 2.5-cm
   - 3.1.7 Stir-bar retriever

   3.2 **BG-11 Medium**
   - 3.2.1 OHAUS 250D balance (calibrate per SOP TA-06.15)
   - 3.2.2 Weigh boats, 9 cm x 7.5 cm (use plastic or aluminum weigh boats.)
   - 3.2.3 Autoclaved Deionized (DI) water
   - 3.2.4 Volumetric flasks with stoppers, 1 L
   - 3.2.5 Graduated cylinder, 1L
   - 3.2.6 Magnetic stirrer
   - 3.2.7 Stir bar, 2.5-cm
   - 3.2.8 Stir-bar retriever
   - 3.2.9 Disposable plastic pipettes, 10 mL
   - 3.2.10 Pipettor

   3.3 **BG-11 Medium Agar Plates**
   - 3.3.1 Mettler Toledo PB403-S Balance (calibrate per SOP TA-06.15)
   - 3.3.2 Metal spatula

3.3.3 Weigh boat, 14 cm X 12.5 cm
3.3.4 Erlenmeyer flask, 2 L
3.3.5 Hotplate/stirrer
3.3.6 Stir bar, 7 cm
3.3.7 Stir bar retriever
3.3.8 Plastic Petri dishes
3.3.9 Beaker, 250 mL

4. REAGENTS AND CHEMICALS

Minimum purity of compounds should be ACS-certified reagent grade or equivalent. Purer compounds can be used. Do not throw away empty containers purchased prior to July 2006 until the lot analyses label is pulled off, copied, and filed. Analyses data (for chemicals purchased through Fisher) is now available at www.fishersci.com.

4.1 Stock Solution 1

4.1.1 Autoclaved Deionized (DI) water
4.1.2 Wash bottle of autoclaved DI water
4.1.3 Disodium (Ethylenedinitrilo) tetraacetate (EDTA)($NA_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$) – ACS-certified reagent
Note: UC-Davis recipe calls for Na_2Mg EDTA
4.1.4 Ferric ammonium citrate ($C_6H_8O_7 \cdot nFe \cdot nNH_3$) – Laboratory Grade
4.1.5 Citric acid, monohydrate ($C_6H_8O_7 \cdot H_2O$) – ACS-certified reagent
4.1.6 Calcium chloride ($CaCl_2 \cdot 2H_2O$) – ACS-certified reagent

4.2 Stock Solution 2

4.2.1 Autoclaved DI water
4.2.2 Wash bottle of autoclaved DI water
4.2.3 Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) – ACS-certified reagent

4.3 Stock Solution 3

4.3.1 Autoclaved DI water
4.3.2 Wash bottle of autoclaved DI water
4.3.3 Potassium phosphate dibasic anhydrous (K_2HPO_4) – ACS-certified reagent

4.4 Stock Solution 4 (Microelements)

4.4.1 Autoclaved DI water
4.4.2 Wash bottle of autoclaved DI water
4.4.3 Boric acid (H_3BO_3) – ACS-certified reagent
4.4.4 Manganese chloride ($MnCL_2 \cdot 4H_2O$) – ACS-certified reagent
4.4.5 Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) – ACS-certified reagent
4.4.6 Copper sulfate ($CuSO_4 \cdot 5H_2O$) – ACS-certified reagent
4.4.7 Cobalt chloride ($CoCl_2 \cdot 6H_2O$) – ACS-certified reagent
4.4.8 Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) – meets ACS specifications

4.5 BG-11 Medium

4.5.1 Autoclaved DI water
4.5.2 Wash bottle of autoclaved DI water
4.5.3 Stock Solution 1
4.5.4 Stock Solution 2
4.5.5 Stock Solution 3

4.5.6 Sodium Carbonate, anhydrous (Na_2CO_3) – ACS-certified reagent
4.5.7 Stock Solution 4
4.5.8 Sodium Nitrate ($NaNO_3$) – ACS-certified reagent
4.5.9 1.0N Hydrochloric Acid (HCl) (prepared per SOP TA-10.10; stored in Microbiology Laboratory, B212)

4.6 **BG-11 Medium Agar Plates**
4.6.1 BG-11 Medium
4.6.2 Difco®-Bacto Agar (stored in Microbiology Laboratory, B212)

3.3.10 Parafilm®, cut into 2 cm strips

6. SAMPLE PREPARATION

6.1 **Stock Solution 1**

6.1.1 Label the 1L volumetric flask with salts and the amount of each (see Step 6.1.4) it will contain, "BG-11 Stock Solution 1," the date, batch #, and your initials. Place an expiration date of one year from the preparation date.

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

6.1.2 Fill the flask approximately half full with autoclaved DI water.

6.1.3 Use the OHAUS 250D balance to weigh the listed amount of each of the salts listed in Step 6.1.4. Use separate weigh boats for each and place each in the volumetric flask. Use the autoclaved DI wash bottle to rinse clinging salts into the flask. Clean the spatula before and after each salt.

Note: Weigh per SOP TA-06.27.

6.1.4

Na_2 EDTA	0.090g/1000mL
Ferric ammonium citrate	0.600g/1000mL
Citric acid, monohydrate	0.600g/1000mL
$CaCl_2 \cdot 2H_2O$	3.600g/1000mL

6.1.5 Fill the flask to the 1,000-mL mark with autoclaved DI water.

6.1.6 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt cannot be seen (has dissolved).

Note: The salts and chemicals contained in BG-11 Stock Solution 1 are not easily dissolved.

6.1.7 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill flask to the 1000-mL mark to account for DI water displaced by the stir bar.

6.1.8 Record the stock preparation batch information in the *Algal Biology Section Solution logbook.*

6.1.9 Store the stock solution in the walk-in refrigerator.

Note: Stock solutions may be kept for 1 year at 4 °C.

6.2 **Stock Solution 2**

6.2.1 Label the 1L volumetric flask "BG-11 Stock Solution 2," the date, batch #, and your initials, "$MgSO_4 \cdot 7H_2O$" and the amount of the single salt it will contain (see Step 6.2.4). Place an expiration date of one year from the preparation date.

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

6.2.2 Fill the flask approximately half full with autoclaved DI water.

6.2.3 Use the OHAUS 250D balance to weigh the listed amount magnesium sulfate listed in Step 6.2.4. Place the salt into the flask. Use the autoclaved DI wash bottle to rinse clinging salt into the flask. Clean the spatula before and after each salt.

Note: Weigh per SOP TA-06.27.

6.2.4 $MgSO_4 \cdot 7H_2O$ 7.500g/1000mL

6.2.5 Fill the flask to the 1,000-mL mark with autoclaved DI water.

6.2.6 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt cannot be seen (has dissolved).

6.2.7 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill the flask to the 1000-mL mark to account for the DI water displaced by the stir bar.

6.2.8 Record the stock preparation batch information in the *Algal Biology Section Solution logbook.*

6.2.9 Store the stock solution in the walk-in refrigerator.

Note: Stock solutions may be kept for 1 year at 4 °C.

6.3 **Stock Solution 3**

6.3.1 Label the 1L volumetric flask "BG-11 Stock Solution 3," the date, batch #, and your initials, "K_2HPO_4" and the amount of the single salt it will contain (see Step 6.3.4). Place an expiration date of one year from the preparation date.

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

6.3.2 Fill the flask approximately half full with autoclaved DI water.

6.3.3 Use the OHAUS 250D balance to weigh the amount of dipotassium phosphate listed in Step 6.3.4. Place the salt into the flask. Use the autoclaved DI wash bottle to rinse clinging salt into the flask.

Note: Weigh per SOP TA-06.27.

6.3.4 K_2HPO_4 3.050g/1000mL

6.3.5 Fill the flask to the 1,000-mL mark with autoclaved DI water.

6.3.6 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt cannot be seen (has dissolved).

6.3.7 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill the flask to the 1000-mL mark to account for the DI water displaced by the stir bar.

6.3.8 Record the stock preparation batch information in the *Algal Biology Section Solution logbook.*

6.3.9 Store the stock solution in the walk-in refrigerator.

Note: Stock solutions may be kept for 1 year at 4 °C.

6.4 **Stock Solution 4 (Microelements)**

6.4.1 Label the 1L volumetric flask with the Microelements and the amounts of each (see Step 6.4.4) it will contain, "BG-11 Stock Solution 4," the date, batch #, and your initials. Place an expiration date of one year from the preparation date.

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

6.4.2 Fill the flask approximately half full with autoclaved DI water.

6.4.3 Use the OHAUS 250D balance to weigh the listed amount of each of the salts listed in Step 6.4.4. Use separate weigh boats for each and place each in the volumetric flask. Use the autoclaved DI wash bottle to rinse clinging salts into the flask. Clean the spatula before and after each salt.

Note: Weigh per SOP TA-06.27.

6.4.4

H_3BO_3	2.860g/1000mL
$MnCl_2 \cdot 4H_2O$	1.810g/1000mL
$ZnSO_4 \cdot 7H_2O$	0.222g/1000mL
$CuSO_4 \cdot 5H_2O$	0.079g/1000mL
$CoCl_2 \cdot 6H_2O$	0.050g/1000mL
$NaMoO_4 \cdot 2H_2O$	0.391g/1000mL

6.4.5 Fill the flask to the 1,000-mL mark with autoclaved DI water.

6.4.6 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt cannot be seen (has dissolved).

6.4.7 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill the flask to the 1000-mL mark to account for the DI water displaced by the stir bar.

6.4.8 Record the stock preparation batch information in the *Algal Biology Section Solution logbook.*

6.4.9 Store the stock solution in the walk-in refrigerator.

Note: Stock solutions may be kept for 1 year at 4 °C.

6.5 **BG-11 Medium**

6.5.1 Label the 1L volumetric flask "BG-11 Medium," the date, batch #, and your initials. Place an expiration date of three months from the preparation date.

Note: To make a larger volume of media, use a 2L volumetric flask and double the amounts of the stock solutions, sodium carbonate and sodium nitrate.

6.5.2 Fill the flask approximately half full with autoclaved DI water.

6.5.3 Use the Mettler PB403-S to weigh the listed amount of each of the salts listed in Step 6.5.4. Use separate weigh boats for each and place each in the volumetric flask. Use the autoclaved DI wash bottle to rinse clinging salts into the flask. Clean the spatula before and after each salt.

Note: Weigh per SOP TA-06.15

6.5.4

Na2CO3	0.020g/1000mL
NaNO3	1.500g/1000mL

6.5.5 Using a 10mL disposable pipette, transfer the amount specified in Step 6.5.6 of each of the stock solutions into the volumetric flask. Use a clean, disposable 10 mL pipette for each of the stock solutions.

6.5.6 BG-11 Stock Solution 1 10mL/1000mL
BG-11 Stock Solution 2 10mL/1000mL
BG-11 Stock Solution 3 10mL/1000mL
BG-11 Stock Solution 4 1mL/1000mL

6.5.7 Fill the flask to the 1,000-mL mark with autoclaved DI water.

6.5.8 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on.

6.5.9 After the solution is homogenous, continue to stir. Adjust the pH to 7.1 by adding small amounts (3-5 drops) of 1.0N HCl to the solution. Check the pH frequently with the pH meter (per SOP TA-06.23).

6.5.10 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill the flask to the 1000-mL mark to account for the DI water displaced by the stir bar.

6.5.11 Record the stock preparation batch information in the *Algal Biology Section Solution logbook.*

6.6 **BG-11 Media Agar Plates**

6.6.1 Transfer 1L of BG-11 media from the volumetric flask into a 2L Erlenmeyer flask.

6.6.2 Use the Mettler PB403-S to weigh 10.0g of the Difco®-Bacto agar.

6.6.3 Pour the 10.0g of agar into the 2L flask. Place the stir bar in the flask and place the flask on the hotplate/stirrer in the hood in B246.

6.6.4 Slowly heat the mixture to just below boiling while stirring until the agar is dissolved. The agar is dissolved when agar particles are no longer swirling, and the mixture becomes translucent and amber in appearance.

6.6.5 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water.

6.6.6 Allow the media to cool for 10-15 minutes before pouring into plates.

6.6.7 Fill the 250 mL beaker with the media from the 2L flask. The spout on the beaker will make the media easier to pour. Clear a large, flat, and even surface. Set out several Petri dishes and slowly pour media into the dishes. Replace the lid as soon as the media is poured. Take care not to introduce air bubbles into the media. Fill the dish so that media covers the bottom. Continue filling the beaker and pouring the media into dishes until all the media has been poured.

6.6.8 Allow the media to solidify in the dishes. The media takes about an hour to solidify.

6.6.9 Place Parafilm® around the edges of the Petri dish to seal it. Store face down in the walk-in refrigerator across from the Algal Lab and label their storage container with"BG-11 Media Agar Plates," the date, your initials, the batch number, the expiration date (six months from the date of preparation.)

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

Note: Each batch of plates can be stored in a white, plastic bin, which can be found in the Invert Lab or in the Algal Lab.

6.6.10 Record the stock preparation batch information in the *Algal Biology Section Solution logbook.*

7. QUALITY CONTROL AND DOCUMENTATION

7.1 Use a pen with black ink for all logbook and benchsheet entries.

7.2 *Algal Biology Section Solution logbook*

7.3 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error and initial the correction. If the correction requires an explanation, write a comment and reference the error.

7.4 See Biology Quality Manual http://www.floridadep.org/labs/library/progplan.htm

8. SAFETY

8.1 Lab coat, latex or vinyl gloves, and eye protection. Proper laboratory protection is required when working with chemicals and unprocessed samples.

8.2 Avoid breathing fumes or exposing co-workers to them. Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of this chemical. Work in a well ventilated area.

8.3 Refer to the Material Safety Data Sheet (MSDS) for information about reagents and chemicals in this SOP.

8.4 Review Laboratory Safety Manual. See http://depnet/burlabs/safety.htm

9. POLLUTION PREVENTION AND WASTE MANAGEMENT

9.1 Review Laboratory Safety Manual. See http://depnet/burlabs/safety.htm

10. REFERENCES

10.1 SOP TA-06.15 Calibration and Use of Mettler® PC440 Balance
10.2 SOP TA-10.10 Preparation of Stock HCl (Acid) Solution
10.3 SOP TA-06.27 OHAUS 250D Balance Calibration and Weighing

Appendix of Changes

1/10/2011 Reformatted the layout following SOP LB-001.

Effective Date: 02/03/2012
Revision Date: 02/03/2012
Revision Author: C. Swanson
AB-13-1.4

Culture of *Microcystis aeruginosa* Stocks

(Based on and modified from EPA-600/9-78-018, *The* Selenastrum capricornutum *Printz Algal Assay Bottle Test*)

1. SCOPE AND APPLICATION

1.1 This SOP describes the maintenance of pure cultures of *Microcystis aeruginosa* under laboratory conditions.

2. SUMMARY OF METHOD

2.1 Cultures are maintained on rotary shakers at 24 ± 3 °C under 24-hour light conditions.

2.2 Flasks of fresh BG-11 medium are inoculated (4 flasks/culture) with 7 day old culture each Tuesday and the three week old culture is discarded.

2.3 Plates of fresh BG-11 agar are inoculated with 7 day old culture once a month for reserve stock.

3. APPARATUS AND EQUIPMENT

3.1 Erlenmeyer flasks with foam stoppers (4 per culture, 250 mL, autoclaved)
3.2 Disposable pipettes (10 mL and 1 mL)
3.3 Pipettor (10 mL & 1 mL)
3.4 Graduated cylinder (100 mL)
3.5 Labeling tape, 3/4-in wide or waterproof label sheet for computer printing
3.6 Black, fine-tip, permanent marker
3.7 UV sterilizer -- Located in Microbiology Lab (B212). Operate per instruction manual.
3.8 *Microcystis Culture Logbook*

4. REAGENTS AND CHEMICALS

4.1 BG-11 media, approximately 400 mL per culture (prepared in advance per SOP AB-12)
4.2 BG-11 media agar plates (prepared in advance per SOP AB-12)
4.3 *Microcystis aeruginosa* cultures from different sources (Shaker Racks D, E and F in B246A).

5. SAMPLE COLLECTION AND PRESERVATION

5.1 Samples used for isolating a *Microcystis aeruginosa* culture are collected from blooms where it has been confirmed that the algal toxin, microcystin, is being produced.

5.2 Samples should be carried directly to the laboratory or shipped overnight as chemical preservation would kill the algal cells. Even ice preservation has the potential to damage the algal cells and halt toxin production.

6. CULTURE MAINTENANCE PROCEDURES

The culture flasks are maintained on Shaker Racks D, E and F in Room B246A. Use the youngest or most recently dated culture from each source, which should be 7±1 days old. Every 2 months, a slide prepared from each culture should be examined under a microscope to verify that the culture is not contaminated before transferring.

6.1 **Flask Culture Maintenance Methods**

Precautions must be taken to avoid cross-contamination of the cultures. (Examples: Do the daily steps for one culture before doing the daily steps for the other culture. Perform the transfers in a separate place in the lab, discard the pipettes used for one culture before using a pipette for the other culture, etc.).

6.1.1 Each Tuesday, autoclave foam-plug-stoppered flasks at 15 psi and 121 °C for 15 minutes. (Day can be changed due to holidays, weather events, etc., and flasks can be cleaned ahead of time.). Autoclave enough flasks to allow four flasks per culture.

Note: Flasks must be cleaned per the standard Biology Section glassware procedures (BG-05), which include washing with a non-phosphate detergent, an acid rinse, and a deionized water rinse. A tap-water rinse is given after each procedure except not after the DI water rinse. Phosphate detergents are avoided because of the potential of skewed test results from phosphate-contaminated glassware.

6.1.2 Remove the flasks from the autoclave and let cool to room temperature.

6.1.3 Label flasks with algal name, date, medium batch number, and your initials. To save time, labels can be printed on waterproof labels. Stick one label on each flask.

Note: Four flasks are labeled per culture. The algal "name" is a code assigned to the culture that designates the origin of the culture (e.g. "UTEX" for the University of Texas culture, "MUNSON" for the Lake Munson culture, etc.). Fold about 1/4 inch under at each end of the tape before applying label to help later removal.

6.1.4 Use a graduated cylinder to pour 100 mL of BG-11 medium into each of the flasks.

6.1.5 Place the flasks containing media in the UV sterilizer in the Microbiology Lab (B212), three at a time, and sterilize each set of three flasks for two minutes. Operate UV sterilizer per instruction manual.

6.1.6 In the Microbiology Lab hood (B212), inoculate each of the flasks for the first culture with 1 mL of concentrated cells from the previous week's culture using a disposable pipette. Repeat with each of the remaining cultures. Complete the transfer for all flasks of one culture before proceeding to the next culture.

Note: The 6–8 day old cultures are generally in a rapid-growth phase (log phase) and the algae that are transferred to fresh media during the culture transfer are viable and stay in the log phase. Cultures that are 2-3 weeks old have usually depleted most of the nutrients from the culture media and are dying off. Algae in depleted media are generally more susceptible to physiological changes and may form a type that differs from the original culture (Personal communication, D. Schultz retired, U.S. EPA, Athens, Ga.).

6.1.7 Remove the oldest culture flasks (≥ 14 days) from their respective maintenance areas to make room for the newly inoculated culture flasks. There should be three weeks worth of cultures on the shaker at any given time.

Note: The contents of old culture flasks can be discarded down the sink or the main drain in the Tox lab. Rinse the flasks with tap water and scrub with a brush to remove any algae stuck to the sides of the flask. The algal cells tend to stick to the sides of the flask and if they remain dry until they are washed, it is very difficult to get them properly cleaned. The foam stoppers from the discarded cultures can be autoclaved in aluminum foil at 15 psi and 121°C for 15 minutes and kept for reuse.

6.1.8 Place the newly inoculated flasks on the lower right shakers D, E and F in the Algal Assay room (B246A). Turn the shaker rack on to low speed (100 ±10 rpm).

Note: Specific shaker rack location may vary. Shaker-racks are controlled by a wall-mounted electronic timer. The switch on the shaker-rack should be in the on position.

6.1.9 Record the culture transfers in the *Microcystis Culture logbook.*

6.1.10 Maintain 24-hour lighting over the algae cultures.

Note: Light intensity of 360-440 foot-candles. Monitor light intensity quarterly per SOP TA-06-26.

6.1.11 Monitor the temperature of the "Algal Assay" room (B246A) daily and note any sustained deviation of more than ±2 °C from the preferred temperature of 24 ±3 °C (acceptable range 21 – 27 °C).

Note: Sustained deviation means more than 2 hours at one time or 4 total hours during a 24-hour period.

Note: Tox lab staff monitors temperature, but temperature deviations should be noted in the *Microcystis Culture logbook.*

6.2 Agar Plates Culture Maintenance Methods

6.2.1 Once a month, get two previously prepared BG-11 agar-media plates per culture from the Biology walk-in refrigerator (B227). Place plates bottom up in the hood in the Microbiology Lab (B212).

6.2.2 Under the hood remove lid and shake off excess condensate. Replace lid.

6.2.3 Using a black permanent marker and labeling tape, label the bottom of the BG-11 agar plate with the following: "*Microcystis aeruginosa*," culture name, transfer date, "BG-11," agar prep date, expiration date (3-4 months from agar prep date), and analyst initials.

Note: Agar prep and expiration dates have been written on the container in which the agar plates are stored in the walk-in refrigerator.

6.2.4 Place two of the 1 week old flasks of each algal culture in the hood beside the agar plates.

6.2.5 For each of the flasks, pour (or pipette with sterile 10 mL pipette) 10 mL of the appropriate algae on to the agar dish labeled for the appropriate culture. Do not allow the algae to overflow the agar plate. Replace the plate lid.

6.2.6 Allow the agar plates with culture additions to sit undisturbed for two hours.

6.2.7 After two hours, slowly pour off most of the liquid in the agar plate into a beaker. Leave a thin layer of liquid on the agar. Replace the plate lid and seal the rims of inoculated plates with Parafilm®.

Note: Warning! Be careful not to cross-contaminate cultures. Leaving a thin layer of liquid on the agar ensures the presence of the algae. Settled algae should be visible as a green-colored thin mass.

6.2.8 Place the inoculated agar plates on paper towels between Shakers E and F in the Algal Assay room (B246A). Do not stack the newly inoculated plates on the old plates (or vice versa). Discard the previous months plates in the Biohazard trash located in the Microbiology Laboratory (B212).

Note: Stacking the inoculated plates increased the chance of cross-contamination and creates less than optimal growing conditions by blocking light from the lower agar plates.

6.2.9 Record the agar plate inoculation in the *Microcystis Culture Logbook.*

7. QUALITY CONTROL AND DOCUMENTATION

7.1 Use a pen with black ink for all logbook entries.

7.2 *Microcystis Culture Logbook*

7.3 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error and initial the correction. If the correction requires an explanation, write a comment and reference the error.

8. SAFETY

8.1 Lab coat, latex or vinyl gloves, and eye protection. Proper laboratory protection is required when working with chemicals and unprocessed samples.

8.2 Avoid breathing fumes or exposing co-workers to them. Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of this chemical. Work in a well ventilated area.

8.3 Refer to the Material Safety Data Sheet (MSDS) for information about reagents and chemicals in this SOP.

8.4 Review Laboratory Safety Manual. See http://depnet/burlabs/safety.htm

9. POLLUTION PREVENTION AND WASTE MANAGEMENT

9.1 Review Laboratory Safety Manual. See http://depnet/burlabs/safety.htm

10. REFERENCES

10.1 The Selenastrum capricornutum Printz Algal Assay Bottle Test (EPA-600/9-78-018)
10.2 SOP AB-12 Preparation of BG-11 Medium, Medium Stock Solutions, and Agar Plates
10.3 SOP BG-05 Biology Wash Party Procedures

Appendix of Changes

1/10/11 Reformatted the layout following SOP LB-001. In step 6, added the "every 2 month" recurrence for examining a slide of the culture. In step 6.2.3, changed the expiration date from 6 months to 3-4 months.