July/August 2007 Microcystin Round Robin Study

Prepared by the

Bureau of Laboratories

Florida Department of Environmental Protection

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 (DoH) created the Cyanobacteria Bloom Sampling and Analysis Standardization Workgroup in an effort to standardize the way cyanobacterial blooms were sampled, analyzed, and reported upon. In addition to FDEP and DoH, 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 (ftp://ftp.dep.state.fl.us/pub/labs/biology/hab/cyanobacteria_sop.pdf) prior to the 2006 bloom season.

The next step was to assess the variability associated with the analyses for cyanobacterial toxins. Since the majority of the large 2005 blooms were caused by *Microcystis aeruginosa*, a 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 produced 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. On July 24, 2007 the Florida Department of Environmental Protection sent out samples to twelve laboratories throughout the United States that had volunteered to perform analyses for microcystins at no cost. Sample replicates were prepared by placing 10 liters of sample in a 20-L glass carboy on a stir table. Each replicate vial was filled 1/3 full by siphon tube while the sample was stirred. Once each replicate 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.

Each laboratory was provided with ten blind samples. Either three or four replicates were provided to each laboratory for each of the sample types. Laboratories were required to hold the samples in the dark at 4ºC for no more than one week before analyzing them. No attempts were made to standardize the

sample preparation, extraction, or analysis methods. The laboratories were asked to report their results to FDEP within one month using a standardized reporting form.

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

Results

ELISA Kit Results

Nine laboratories performed microcystin analyses using Enzyme-Linked ImmunoSorbent Assays (ELISA) using kits from two manufacturers. While trends may appear in the results that seem to be attributable to kit manufacturer, it is important to note that this was not a controlled experiment. Each manufacturer's kit was not run using each preparation method; therefore, the "kit manufacturer effect" may be confounded with preparation method. A later round robin will be conducted to control preparation method and further clarify how the kits differ. For this report, the kits from the two manufacturers are labeled as "Kit A" and "Kit B" and will not be further identified.

Four laboratories (A, G, K, and J) used the Kit A polyclonal microcystins kit and five laboratories (C, E, F, H, and I) used the Kit B polyclonal test kit. Average total microcystin values for each laboratory and sample combination are presented in Table 1 and Figure 1.

Microcystin-LR Standard

Values reported for the Microcystin-LR (MC-LR) standard samples ranged from 1.0 to 3.7 µg/L. The overall mean and %CV for all ELISA kit results was 2.07 and 26%, respectively. The standard sample was prepared by the FDEP Central Chemistry Laboratory at 2 µg/L microcystin LR.

Kit A

Kit A values reported for the Microcystin-LR (MC-LR) standard samples ranged from 1.0 to 2.5 µg/L. The mean and percent coefficient of variation (%CV) was 2.10 µg/L and 18%, respectively. The within laboratory %CV for the replicates ranged from 4-7%.

Kit B

Kit B values reported for the Microcystin-LR (MC-LR) standard samples ranged from 1.06 to 3.7 µg/L. The mean and %CV for all labs using the Kit B kit was 2.05 µg/L and 32%, respectively. The within laboratory %CV for the replicates ranged from 5-21%.

The overall mean MC-LR sample result for all of the laboratories using Kit A was essentially identical to that of the laboratories using Kit B. In the absence of additional toxins, the two kits appear to perform very comparably, although Kit B showed higher between and within laboratory variability.

As expected, the analytical variability associated with the MC-LR standard samples was the smallest of the three sample types. This reduced variability is likely the result of only one microcystin being involved and preparation method differences having less influence on the extracellular standard than on intracellular microcystins in *Microcystis* cells.

FDEP culture of University of Texas *Microcystis aeruginosa* isolate

Values reported for the University of Texas isolate samples ranged from 23.13 to 104.45 µg/L. The overall mean and %CV for all ELISA kit results was 60.62 µg/L and 39%, respectively.

Kit A

Kit A values for the University of Texas isolate samples ranged from 52 to 104.45 µg/L. The mean and %CV was 77.35 µg/L and 25%, respectively. The within laboratory %CV for the replicates ranged from 7 to 18%.

Kit B

Kit B values for the University of Texas isolate samples ranged from 23.13 to 73.5 µg/L. The mean and %CV was 47.22 µg/L and 36%, respectively. The within laboratory %CV for the replicates ranged from 3 to 23%.

The overall mean University of Texas isolate sample result for all of the laboratories using Kit A was 39% higher than the overall mean for the laboratories using Kit B. The LCMS results suggest that the University of Texas isolate produces predominantly MC-LR; however Kit A was responding to something that Kit B was not. It is quite possible that there are additional microcystins or other toxins being produced by the University of Texas isolate for which we do not currently possess standards. The laboratories using Kit B still had greater within and between laboratory variability than the laboratories that used Kit A, but the difference was not as great as with the MC-LR standard samples.

FDEP Lake Munson Culture

Values reported for the FDEP Lake Munson Culture samples ranged from 47.41 to 418.9 µg/L. The overall mean and %CV for all ELISA kit was 186.08 µg/L and 48%, respectively.

Kit A

Kit A values for the FDEP Lake Munson Culture samples ranged from 120 to 418.9 µg/L. The mean and %CV was 249.19 µg/L and 32%, respectively. The within laboratory %CV for the replicates ranged from 12 to 25%.

Kit B

Kit B values for the FDEP Lake Munson samples ranged from 47.41 to 240 µg/L. The mean and %CV was 135.58 µg/L and 46%, respectively. The within laboratory %CV for the replicates ranged from 5 to 28%.

The overall mean Lake Munson sample result for all of the laboratories using Kit A was 46% higher than the overall mean for the laboratories using Kit B. Unlike the University of Texas culture, the FDEP Lake Munson culture has shown numerous positive hits for several microcystins (e.g., MC-LR, MC-YR, and MC-RR) by LC/MS/MS. As discussed above, this may contribute to the greater variability between ELISA kit types, but it does not necessarily explain the greater variability within kit types. As with the University of Texas isolate samples, the laboratories using Kit B still had greater within and between laboratory variability than the laboratories that used Kit A, but the difference was not as great as with the MC-LR standard samples.

Sample Preparation Method

Table 4 is a summary of the preparation methods used by all the participating laboratories. Three laboratories (C, F, and G) prepared their samples by autoclaving. Two laboratories (E and J) used only sonication, while two more laboratories (H and I) used freeze-thaw and sonication, and another laboratory (K) used sonication followed by centrifugation. Only one laboratory (A) used freeze-thaw by itself as the preparation method. No discernable pattern in results could be detected based upon the preparation method used.

High Performance Liquid Chromatography Results

Four laboratories performed microcystin analyses using high performance liquid chromatography (HPLC) methods. Two laboratories (G and L) used LC/MS/MS (triple quadrupole), and another two laboratories (E and K) used LC/MS. The HPLC results are presented in Table 2. Average total microcystin values for each laboratory and sample combination are presented in Figure 2. Distinguishing meaningful differences in means and variability for each HPLC method is not possible since only two labs used LC/MS/MS and two labs used LC/MS, so only the overall results for the combined methods are being considered.

It is interesting to note that not all laboratories measured the same group of toxins (Table 2). While most laboratories reported microcystin LR and RR, some also reported microcystin YR and microcystin demethyl-LR and RR. For the standard sample, laboratories K and E reported microcystin RR in addition to LR even though RR was not added during the preparation of the standard. The total microcystins value reported for these samples is a sum of the individual toxin groups (LR, RR, YR, etc.) so, the differences in the groups analyzed has an effect on the final reported result.

Microcystin-LR Standard Sample

Total microcystin values reported for the Microcystin-LR (MC-LR) standard samples ranged from 0.62 to 4.90 µg/L. The overall mean and %CV was 2.87 µg/L and 56%, respectively. The within laboratory %CV for the replicates ranged from 3.0 to 28%.

FDEP culture of University of Texas *Microcystis aeruginosa* isolate

Total microcystin values reported for the University of Texas isolate samples ranged from 16.6 to 105.0 µg/L. The overall mean and %CV was 49.58 µg/L and 66%, respectively. The within laboratory %CV for the replicates ranged from 2 to 23%.

FDEP Lake Munson Culture

Total microcystin values reported for the FDEP Lake Munson Culture samples ranged from 43.6 to 327.5 µg/L. The overall mean and %CV was 134.33 µg/L and 78%, respectively. The within laboratory %CV for the replicates ranged from 0 to 38%.

Laboratory E experienced a greater degree of variability for all three sample types than any of the other laboratories performing HPLC analyses. This may be a result of the high levels of dilution that were required to report the results (e.g., 10,000X). None of the other HPLC labs reported dilution factors greater than 10X.

Sample Preparation Method

Table 4 is a summary of the preparation methods used by all the participating laboratories. None of the HPLC labs used an identical sample preparation method; however three of the laboratories (E, K, and L) used sonication alone, sonication with centrifugation or solid phase extraction. Only one HPLC

laboratory (G) used autoclaving as the preparation method. Given the lack of replication, no discernable pattern in results could be detected based upon sample preparation method.

Protein Phosphatase Inhibition Assay Results

Two laboratories (D and F) performed microcystin analyses using the protein phosphatase inhibition assay (PPIA). The PPIA results are presented in Table 3. Average total microcystin values for each laboratory and sample combination are presented in Figure 3.

Microcystin-LR Standard Sample

Total microcystin values reported for the Microcystin-LR (MC-LR) standard samples ranged from 1.96 to 2.49 µg/L (Figure 4). The overall mean and %CV was 2.30 µg/L and 8%, respectively. The within laboratory %CV for the replicates ranged from 1 to 12%.

FDEP culture of University of Texas *Microcystis aeruginosa* isolate

Total microcystin values reported for the University of Texas isolate samples ranged from 42.56 to 60.65 µg/L (Figure 5). The overall mean and %CV was 51.71 µg/L and 15%, respectively. The within laboratory %CV for the replicates ranged from 3 to 7%.

FDEP Lake Munson Culture

Total microcystin values reported for the FDEP Lake Munson Culture samples ranged from 88.61 to 148.16 µg/L (Figure 6). The overall mean and %CV was 111.17 µg/L and 23%, respectively. The within laboratory %CV for the replicates ranged from 2 to 14%.

Sample Preparation Method

Table 4 is a summary of the preparation methods used by all the participating laboratories. The two labs performing the PPIA method used different sample preparation methods. Laboratory D used lyophilization, followed by sonication in 50% MEOH containing 1% acetic acid. Laboratory F autoclaved the samples. Despite the different sample preparation methods, the results from the two laboratories were very similar.

Data Analysis

A preliminary evaluation of the data from this *Microcystin* round robin shows a wide range of result values from one sample. This is the same situation FDEP encountered when the Total Phosphorus (TP) Round Robin Inter-laboratory Comparison Program (TP ERR) was initiated in 1995. FDEP needed to assess the comparability of phosphorus data from laboratories engaged in the analysis of water samples from the Everglades. A statistical methodology for evaluating these round robins was developed by Dr. Pi-Erh Lin and Dr. Xufeng Niu of the Florida State University Department of Statistics. The details of the method can be found at ftp://ftp.dep.state.fl.us/pub/labs/assessment/roundrobin/err-method.pdf (the results of all theTP round robins can be found at http://www.floridadep.org/labs/everglades/index.htm).

While the intent of the TP ERR is to rate laboratory comparability, the intent of this *Microcystin* round robin is to learn how variable total *Microcystin* values are when preparation and analysis method are not controlled, i.e., when everyone does what they normally do. Since the statistical method can show when a laboratory has a low or high bias, it seems reasonable that it would show if a particular kind of analysis (Kit A ELISA, Kit B ELISA, LC/MS, PPIA) has a systematic bias. Unfortunately, there was so much diversity in preparation method (Table 4) that the data could not be analyzed using that as a variable.

Here is a quick summary of how the FSU round robin statistical method works. The individual laboratory results for each sample (Munson, Texas, and Standard) are evaluated in three steps:

1) Test for outliers using three times the interquartile range of the residuals;
2) Remove highly influential observations using the Cook-Weisberg (C-W) distance;
3) Calculate the consensus mean from the non-outlier, non-influential results.

The results of this analysis method (Table 5) gave a consensus mean of 2.16 μg/L for the FDEP-prepared standard, 50.99 μg/L for the University of Texas isolate, and 136.94 μg/L for the Lake Munson culture.

A t-value for each laboratory/method/sample was calculated as a way to compare the results from different samples (equation 5.1 in the FSU method document). The t-value is a statistic that is based on the mean result for a laboratory/method/sample and the consensus mean and standard deviation for that sample (Table 6). For example, a t-value for LabA/Kit A/Munson is calculated from the mean of the three replicate values (152.5 μg/L) and the consensus mean and standard deviation for the Munson culture (136.94 μg/L, 458.87 μg/L). The resulting t-value is a standardized value which allows comparison of results between samples with different means and standard deviations.

A plot (Table 6, Figure 7) is constructed showing each laboratory/method and its t-values for all three samples. The t-value plot is not to be used as a quick guide to evaluate a laboratory/method's "performance" because the rating of a laboratory/method is based on the lack of outliers and the Cook-Weisberg distance in addition to the t-value. The t-value plot serves the purpose of identifying systematic bias (high or low) with respect to the consensus mean value. For example, Figure 7 shows that all t-values of Method/Laboratories EH and LL are above zero, which indicate that these Method/Labs are reporting values systematically higher than the consensus mean values for all three samples. Similarly, Method/Labs EI, LE, and LG tend to give systematically lower measurements than the consensus mean values.

Discussion

In any round robin, the "right" answer is never known. There was a wide variety of results from each of the samples in this round robin. For the most part, the variability within a laboratory/method/sample was smaller than that between laboratories using the same method; however, this variability was confounded by the differences in preparation method and dilution. In general, the ELISA Kit A gave higher results than Kit B. Since preparation method was not controlled, it is possible that part of the difference is due to that and not solely the kit manufacturer. The fact that a single toxin was employed in the preparation of the standard sample would explain the comparability of the two kits for that sample as opposed to dissimilar results obtained with Munson and University of Texas cultures.

The LC methods tended to give the lowest values. A partial reason for this effect may be that there were standards for only a limited number of the microcystins. Cross-reactivity of ELISA kits to other non-microcystin, biologically-derived substances may provide another explanation. Furthermore, ELISA kits are typically calibrated for their response to LR. Since the sensitivity of their response to different toxins (and cross-reacting substances) may vary, ELISA results may be subject to greater quantitative uncertainty when toxins other than LR are present.

Lessons Learned

Following review of the initial round robin results, laboratory L notified the FDEP Bureau of Laboratories that they had purchased new certified standards and determined that their previous MC-LR and MC-RR standards were low compared to the certified standards. Laboratory L believes that they overestimated the MC-LR values by a factor of 1.65 and the MC-RR values by a factor of 2.60.

An obvious next step in the next round robin is to control preparation method to see how much it contributes to the differences in the results.

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

Agency/University/Lab
California Department of Fish & Game Fish and Wildlife Water Pollution Control Laboratory Rancho Cordova, CA
Florida Dept of Environmental Protection Bureau of Laboratories Tallahassee, FL
Florida Dept of Health Bureau of Laboratories Jacksonville, FL
Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute St Petersburg, FL
Great Lakes Research Consortium State Univ of New York Syracuse, NY
GreenWater Laboratories/CyanoLab Palatka, FL
Lake Superior State University (for SJRWMD microcystin analyses) Sault Ste. Marie, MI
Manatee County Water Plant QC Lab Bradenton, FL
South Florida Water Management District Water Quality Analysis Division West Palm Beach, FL
US EPA Region 9 Lab Richmond, CA
USGS Florida Integrated Science Center Orlando, FL
USGS,WRD Kansas Water Science Center Lawrence, KS

Figure 1. ELISA results

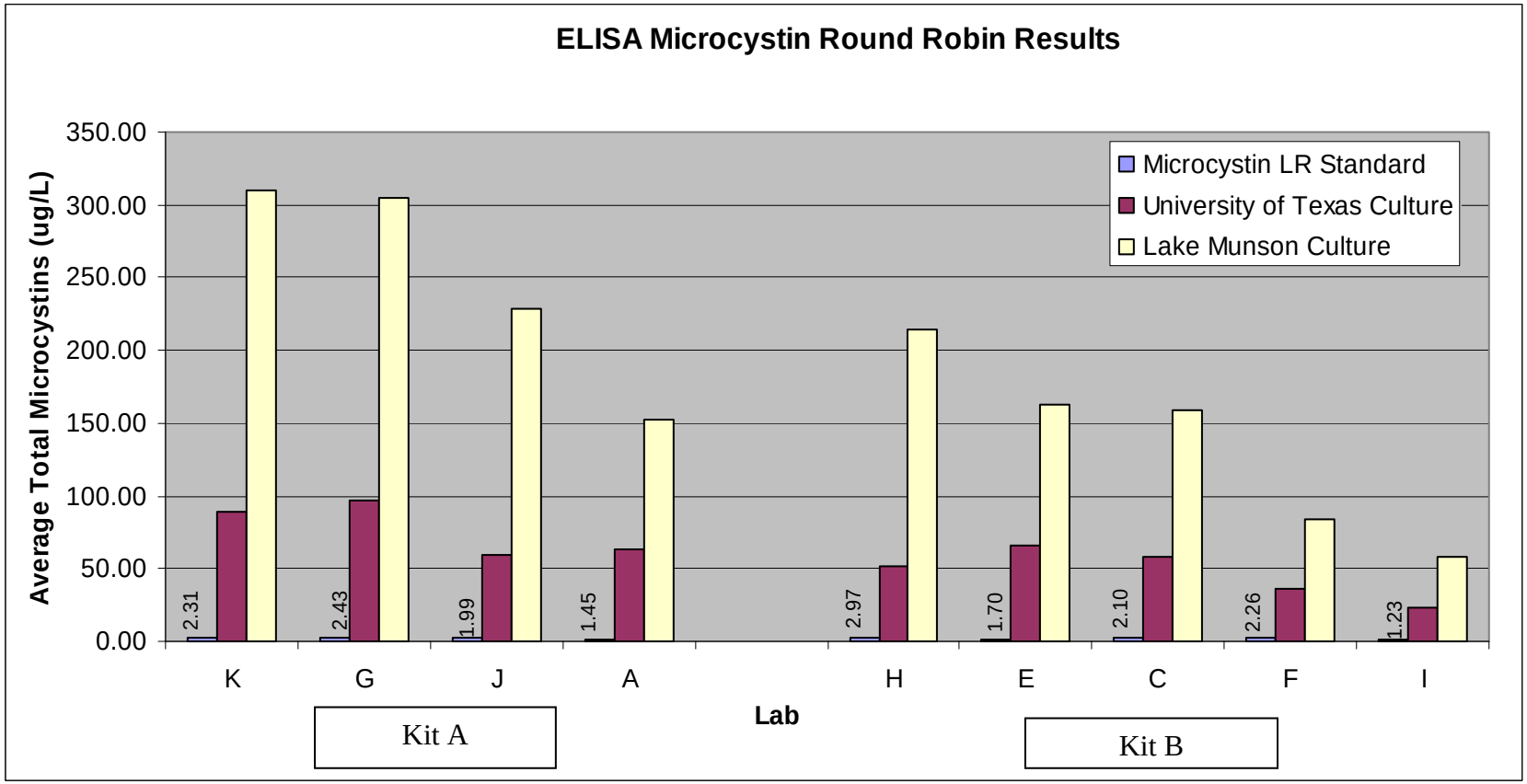

Figure 2. LC/MS and LC/MS/MS results

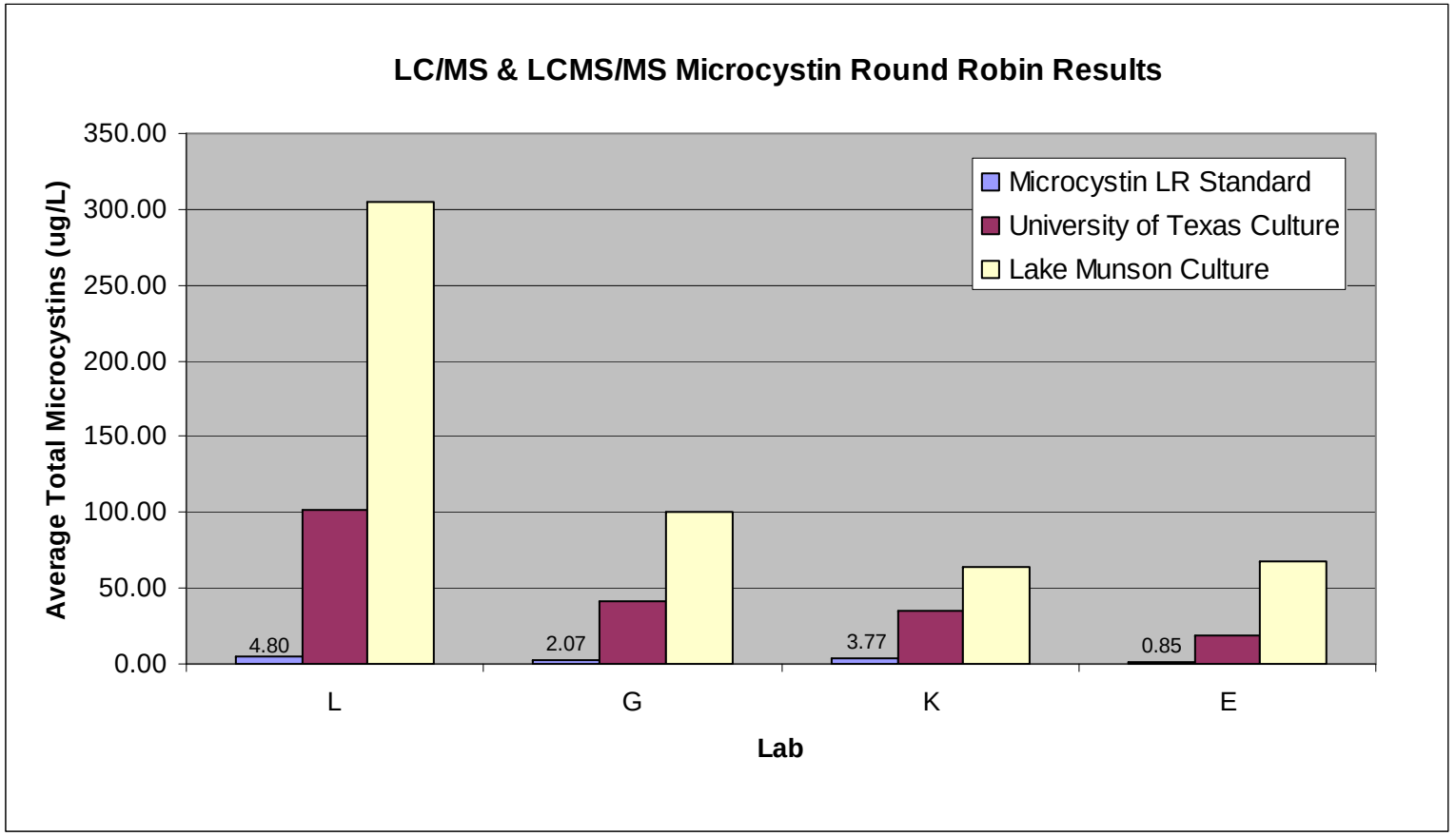

Figure 3. PPIA results

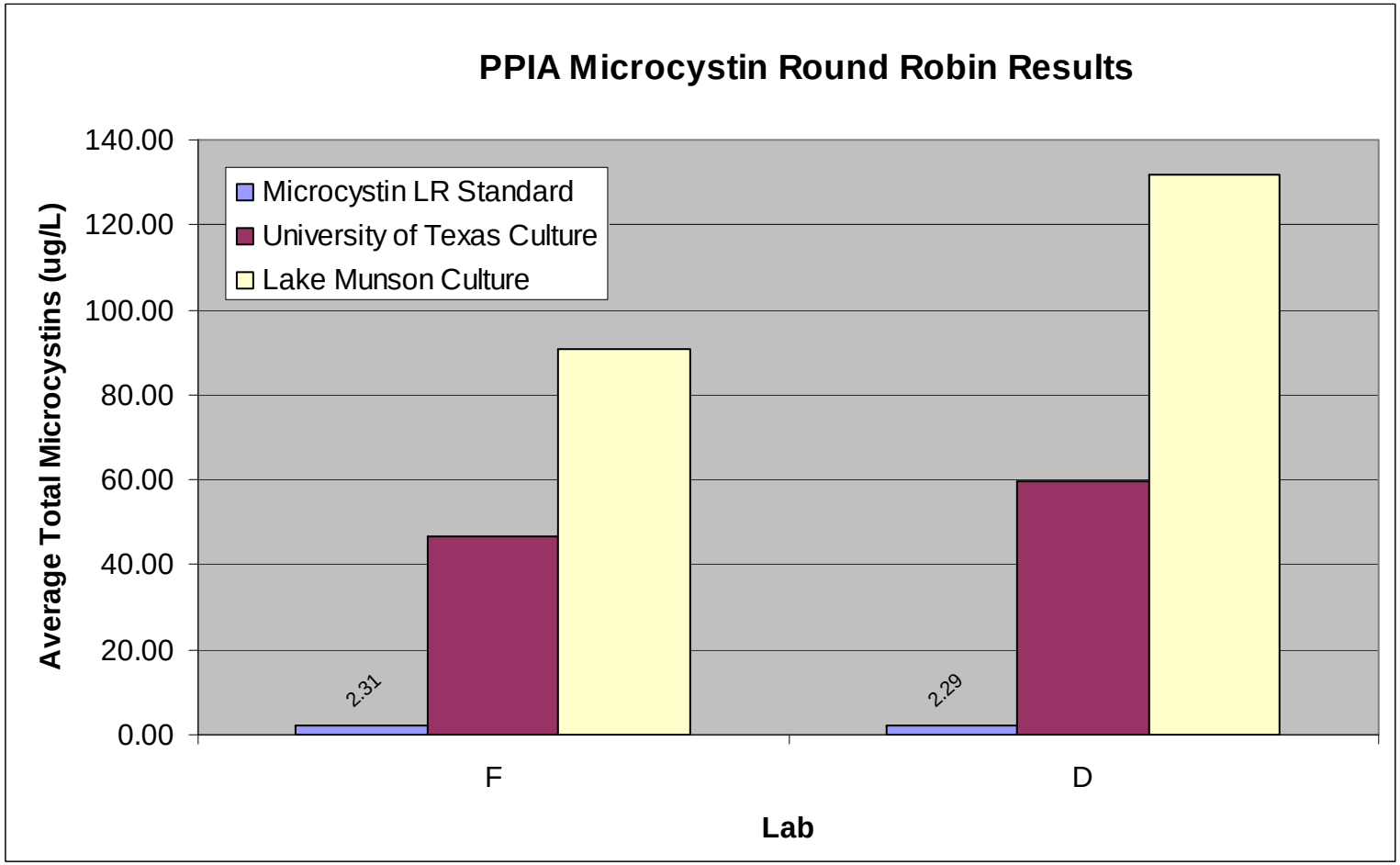

Figure 4. FDEP-Prepared Standard

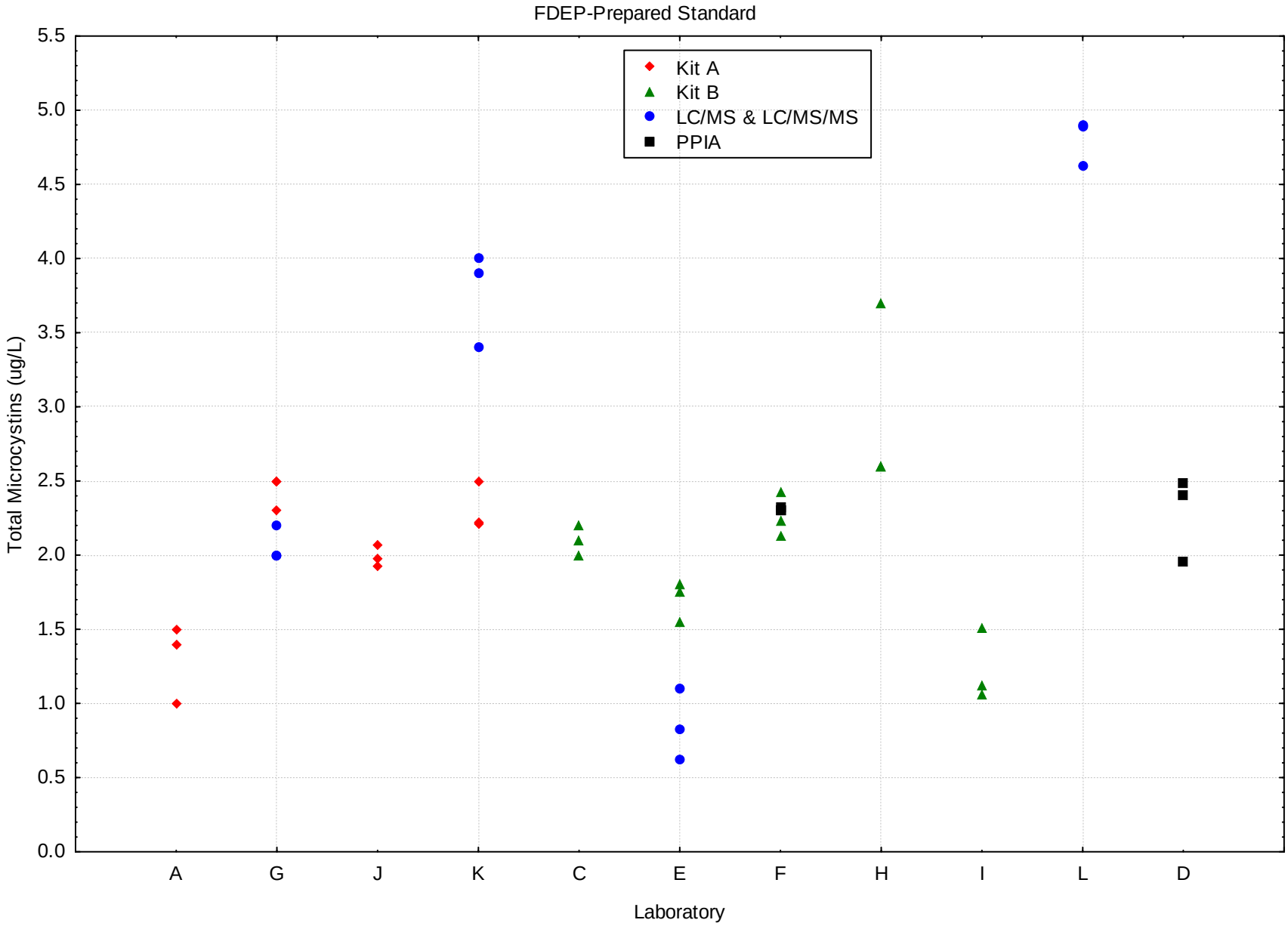

Figure 5. University of Texas Culture Results

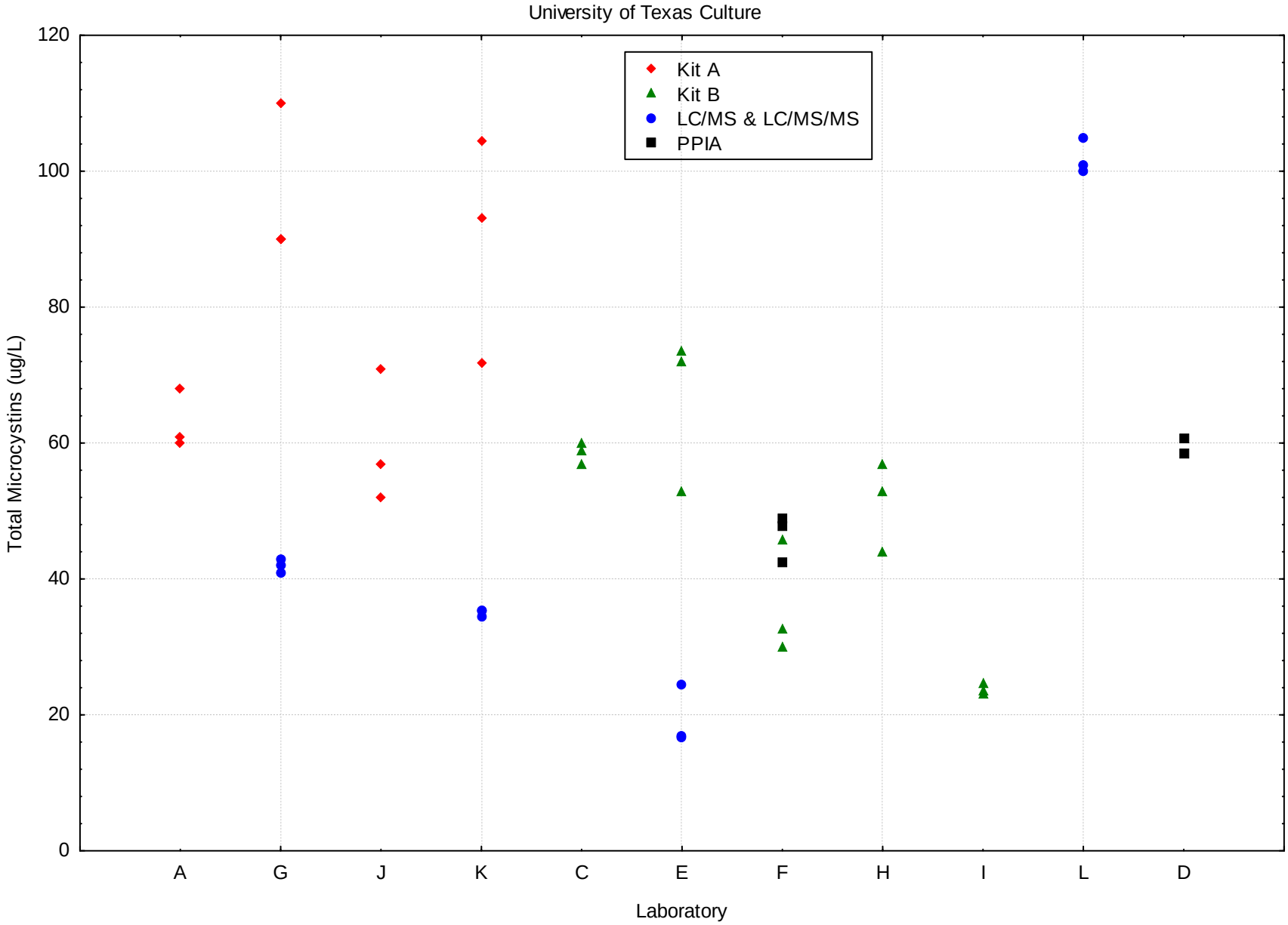

Figure 6. Lake Munson Culture Results

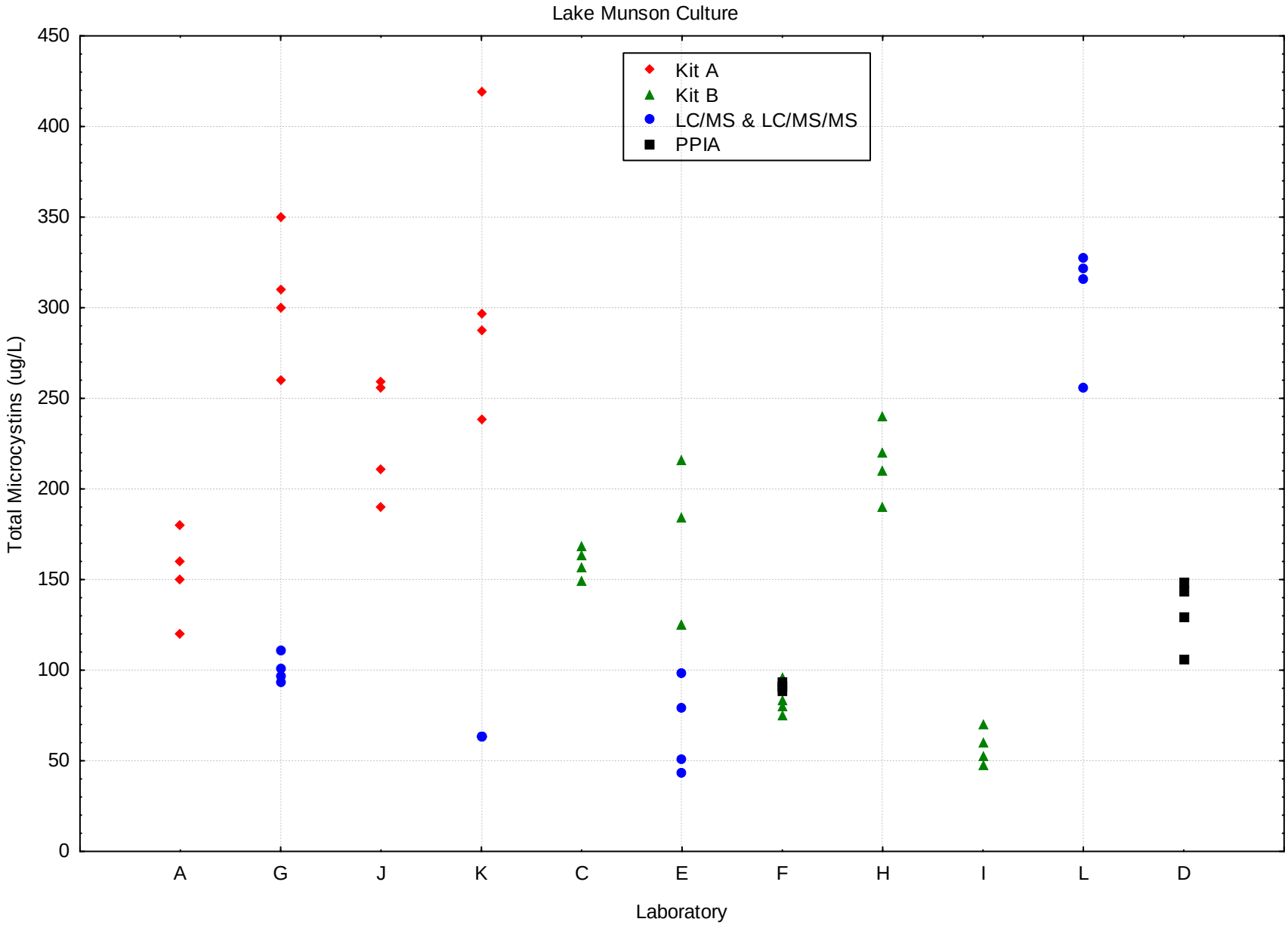

Figure 7. Scatterplot of *t*-values for three samples (Munson, Texas, Standard).

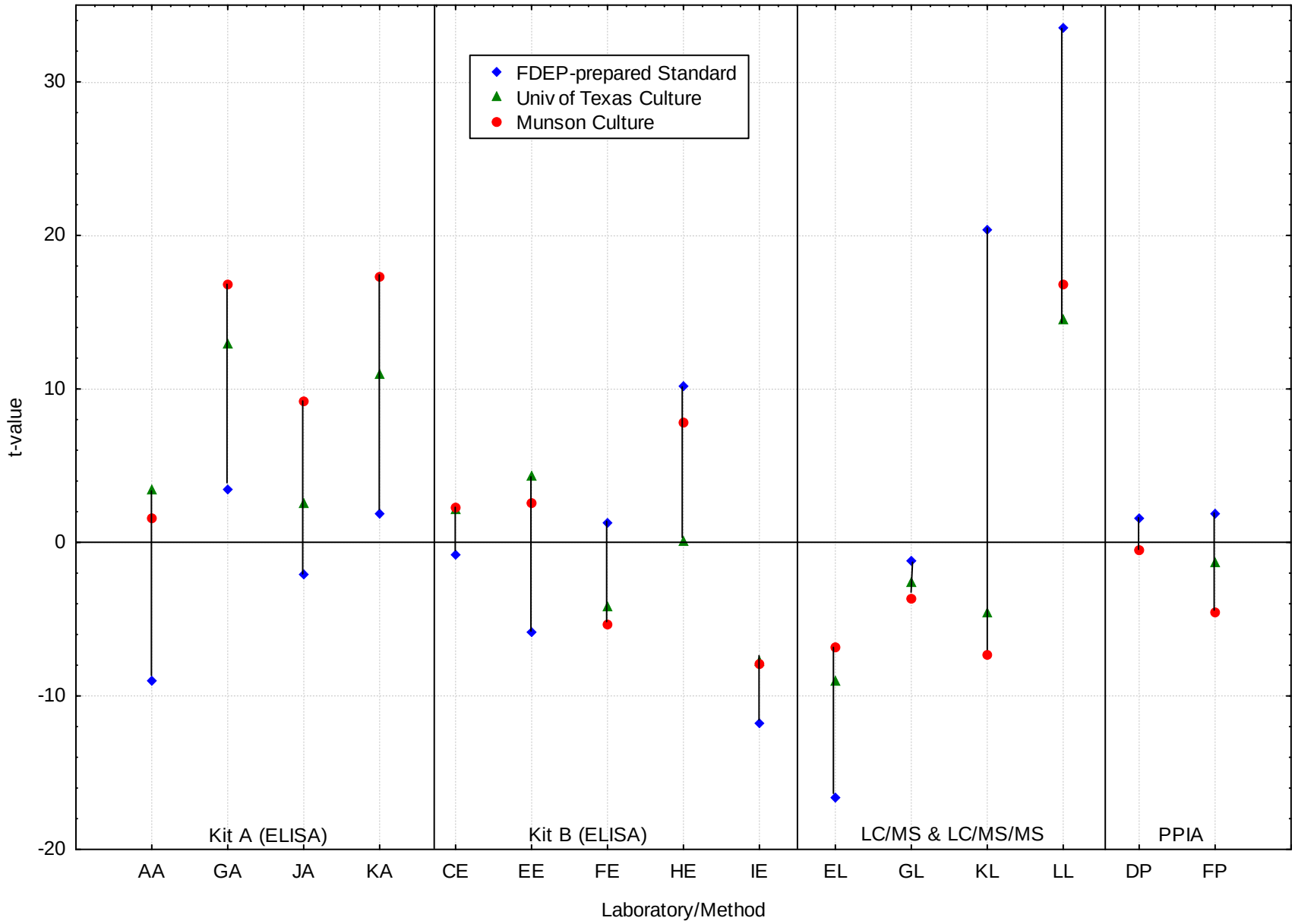

Table 1. ELISA results

Lab Letter	*Bottle*	*M=Munson culture*	*Result*	*Qual-ifier*	*Dup Result*	*Kit*	*Mean*	*Std Dev*	*%CV*
K	2	M	238.3		239.9	Kit A			
K	6	M	287.6		292.2	Kit A			
K	4	M	296.3		292	Kit A			
K	9	M	418.9		432.9	Kit A	310.28	76.79	25%
G	1	M	260		360	Kit A			
G	8	M	300		320	Kit A			
G	2	M	310		280	Kit A			
G	7	M	350		320	Kit A	305.00	36.97	12%
J	1	M	190		200	Kit A			
J	5	M	211		229	Kit A			
J	10	M	256		224	Kit A			
J	7	M	259		215	Kit A	229.00	34.03	15%
A	9	M	120			Kit A			
A	7	M	150			Kit A			
A	8	M	160			Kit A			
A	2	M	180			Kit A	152.50	25.00	16%
						All labs, Kit A	249.19	78.97	32%
F	5	M	75.02			Kit B			
F	4	M	80.28			Kit B			
F	8	M	83.17			Kit B			
F	7	M	95.55			Kit B	83.51	8.71	10%
E	5	M	184		169	Kit B			
E	1	M	216		234	Kit B			
E	7	M	125	>125*J		Kit B			
E	10	M	125	>125*J		Kit B	162.50	45.23	28%
I	5	M	47.41			Kit B			
I	8	M	52.87			Kit B			
I	7	M	59.98			Kit B			
I	1	M	70.36			Kit B	57.66	9.91	17%
C	10	M	149			Kit B			
C	1	M	157			Kit B			
C	6	M	163			Kit B			
C	8	M	168			Kit B	159.25	8.18	5%
H	9	M	190	J		Kit B			
H	2	M	210	J		Kit B			
H	3	M	220	J		Kit B			
H	1	M	240	J		Kit B	215.00	20.82	10%
						All labs, Kit B	135.58	62.27	46%
						All labs, all analysis types	186.08	89.75	48%

Lab Letter	Bottle	S=Standard sample	Result	Qual-ifier	Dup Result	Kit	Mean	StdDev	%CV
K	8	S	2.21		2.36	Kit A			
K	1	S	2.221		2.151	Kit A			
K	5	S	2.5		2.551	Kit A	2.31	0.16	7%
G	10	S	2.3			Kit A			
G	4	S	2.5		2.6	Kit A			
G	6	S	2.5		2.8	Kit A	2.43	0.12	5%
J	4	S	1.93		2.06	Kit A			
J	2	S	1.98		2.10	Kit A			
J	3	S	2.07		2.10	Kit A	1.99	0.07	4%
A	10	S	1.0			Kit A			
A	5	S	1.4			Kit A			
A	1	S	1.5			Kit A	1.45	0.07	5%
						All labs, Kit A	2.10	0.38	18%
F	9	S	2.13			Kit B			
F	10	S	2.23			Kit B			
F	2	S	2.42			Kit B	2.26	0.15	7%
E	2	S	1.55		1.85	Kit B			
E	3	S	1.75		2.00	Kit B			
E	9	S	1.80		2.2	Kit B	1.70	0.13	8%
I	3	S	1.06			Kit B			
I	6	S	1.12			Kit B			
I	10	S	1.51			Kit B	1.23	0.24	20%
C	7	S	2			Kit B			
C	2	S	2.1			Kit B			
C	4	S	2.2			Kit B	2.10	0.10	5%
H	8	S	2.6	J		Kit B			
H	10	S	2.6	J		Kit B			
H	6	S	3.7	J		Kit B	2.97	0.64	21%
						All labs, Kit B	2.05	0.66	32%
						All labs, all analysis types	2.07	0.55	26%

Lab Letter	Bottle	T=Univ of Texas culture	Result	Qual-ifier	Dup Result	Kit	Mean	Std Dev	%CV
K	7	T	71.8		75.2	Kit A			
K	10	T	93		91.2	Kit A			
K	3	T	104.45		98.8	Kit A	89.75	16.57	18%
G	3	T	90		140	Kit A			
G	9	T	90		90	Kit A			
G	5	T	110		120	Kit A	96.67	11.55	12%
J	6	T	52		46	Kit A			
J	8	T	57		44	Kit A			
J	9	T	71		63	Kit A	60.00	9.85	16%
A	6	T	60			Kit A			
A	4	T	61			Kit A			
A	3	T	68			Kit A	63.00	4.36	7%
						All labs, Kit A	77.35	19.42	25%
F	6	T	30.04			Kit B			
F	3	T	32.75			Kit B			
F	1	T	45.75			Kit B	36.18	8.40	23%
E	6	T	53		74	Kit B			
E	4	T	72		144	Kit B			
E	8	T	73.5		65	Kit B	66.17	11.43	17%
I	2	T	23.13			Kit B			
I	4	T	23.5			Kit B			
I	9	T	24.7			Kit B	23.78	0.82	3%
C	9	T	57			Kit B			
C	5	T	59			Kit B			
C	3	T	60			Kit B	58.67	1.53	3%
H	7	T	44			Kit B			
H	4	T	53			Kit B			
H	5	T	57			Kit B	51.33	6.66	13%
						All labs, Kit B	47.22	16.97	36%
						All labs, all analysis types	60.62	23.40	39%

Table 2. LC/MS and LC/MS/MS results.

Lab Letter	*Bottle*	*M=Munson T=U Texas S=Standard*	MC-LR	MC-LA	MC-YR	MC-RR	MC-Demethyl-LR	MC-Demethyl-RR	Nodularin	Total Result in ug/L	Mean	Std Dev	%CV
L	1	M	105	0.5U	1.80	149	90.4J	156J		255.8			
L	9	M	130	0.5U	1.90	184	110J	189J		315.9			
L	8	M	131	0.5U	1.50	189	109J	193J		321.5			
L	4	M	139	0.5U	1.50	187	116J	193J		327.5	305.18	33.26	11%
K	9	M	37.80	U	NS	25.50				63.3			
K	4	M	38.60	U	NS	24.90				63.5			
K	2	M	39.30	U	NS	24.40				63.7			
K	6	M	37.60	U	NS	26.20				63.8	63.58	0.22	0%
G	8	M	67.00	0.5U	1.20	25.00			0.5UJ	93.2			
G	7	M	69.00	0.5U	1.30	26.00			0.5UJ	96.3			
G	2	M	75.00	0.5U	1.10	25.00			0.5UJ	101.1			
G	1	M	85.00	0.5U	0.92	25.00			0.5UJ	110.9	100.38	7.74	8%
E	5	M	43.60	NA	NA	present*				43.6			
E	10	M	51.00	NA	NA	ND				51.0			
E	7	M	79.40	NA	NA	ND				79.4			
E	1	M	98.80	NA	NA	present*				98.8	68.20	25.58	38%
										All Labs	134.33	104.67	78%
L	3	S	4.62	0.5U	0.5U	0.5U	0.5U	0.5U		4.62			
L	6	S	4.89	0.5U	0.5U	0.5U	0.5U	0.5U		4.89			
L	5	S	4.90	0.5U	0.5U	0.5U	0.5U	0.5U		4.90	4.80	0.16	3%
K	1	S	2.90	U	NS	0.50				3.40			
K	5	S	3.10	U	NS	0.80				3.90			
K	8	S	3.00	U	NS	1.00				4.00	3.77	0.32	8%
G	4	S	2.00	0.5U	0.5U	0.5U			0.5U	2.00			
G	10	S	2.00	0.5U	0.5U	0.5U			0.5U	2.00			
G	6	S	2.20	0.5U	0.5U	0.5U			0.5U	2.20	2.07	0.12	6%
E	2	S	0.62	NA	NA	present*				0.62			
E	9	S	0.83	NA	NA	present*				0.83			
E	3	S	1.10	NA	NA	ND				1.10	0.85	0.24	28%
										All Labs	2.87	1.6	56%

Lab Letter	*Bottle*	*M=Munson T=U Texas S=Standard*	MC-LR	MC-LA	MC-YR	MC_RR	MC-Demethyl-LR	MC-Demethyl-RR	Nodularin	Total Result in ug/L	Mean	Std Deviation	%CV
L	7	T	100	0.5U	0.5U	0.5U	26.4J	14.7J		100.0			
L	10	T	101	0.5U	0.5U	0.5U	28.9J	19.8J		101.0			
L	2	T	105	0.5U	0.5U	0.5U	27.1J	16.1J		105.0	102.00	2.65	3%
K	3	T	33.30	U	NS	1.10				34.4			
K	7	T	34.30	U	NS	1.10				35.4			
K	10	T	34.90	U	NS	0.50				35.4	35.07	0.58	2%
G	9	T	41.00	0.5U	0.5U	0.5U			0.5UJ	41.0			
G	5	T	42.00	0.5U	0.5U	0.5U			0.5UJ	42.0			
G	3	T	43.00	0.5U	0.5U	0.5U			0.5UJ	43.0	42.00	1	2%
E	4	T	16.60	NA	NA	ND				16.6			
E	6	T	16.80	NA	NA	ND				16.8			
E	8	T	24.40	NA	NA	ND				24.4	19.27	4.45	23%
										All Labs	49.58	32.84	66%

Table 3. PPIA results

Lab Letter	*Bottle Number*	*M=Munson T=U Texas S=standard*	*Result in ug/L*	*Duplicate Result in ug/L*	*Mean*	*Std Dev*	*%CV*
F	5	M	88.61	96.28			
F	4	M	90.50	93.37			
F	8	M	90.50	97.41			
F	7	M	93.45	100.66	90.77	1.999	2%
D	4	M	105.92	109.27			
D	6	M	143.41	121.65			
D	7	M	128.84	131.48			
D	5	M	148.16	142.99	131.58	18.98	14%
				All Labs	111.17	25.14	23%
F	9	S	2.30	2.33			
F	10	S	2.30	2.37			
F	2	S	2.32	2.52	2.31	0.012	1%
D	9	S	2.41	2.18			
D	3	S	1.96	2.25			
D	1	S	2.49	2.68	2.29	0.285	12%
				All Labs	2.30	0.181	8%
F	1	T	42.56	42.31			
F	3	T	47.82	44.50			
F	6	T	48.98	45.23	46.45	3.421	7%
D	8	T	58.54	54.74			
D	10	T	60.65	60.65			
D	2	T	Sample lost		59.59	1.491	3%
				All Labs	51.71	7.63	15%

Table 4. Preparation Methods (Lab B did not return results)

Prep Method / Lab	3 Freeze/thaw cycle and 20 minute Sonication	Freeze/ Sonicate	Freeze/ Thaw	Lyophilization and Sonication in 50% MeOH containing 1% acetic acid	Sonication	Sonication & SPE	Sonication followed by Centrifugation	Autoclave
A			Y					
C								Y
D				Y				
E					Y	Y		
F								Y
G								Y
H		Y						
I	Y							
J					Y			
K							Y	
L					Y			

Table 5. Results of FSU Statistical Method.

FDEP-prepared Standard					Univ of Texas Culture					Lake Munson Culture				
Lab/ Method	Lab/ Method Mean	C-W Distance	t-value	Score	Lab/ Method	Lab/ Method Mean	C-W Distance	t-value	Score	Lab/ Method	Lab/ Method Mean	C-W Distance	t-value	Score
AA	1.45	3.839	Highly Influential	2	AA	63.00	0.219	3.41	4	AA	152.50	0.000	1.55	5
CE	2.10	0.141	-0.78	5	CE	58.67	0.025	2.18	4	CE	159.25	0.030	2.23	4
DP	2.29	0.006	1.58	5	DP - lost a rep					DP	131.58	0.217	-0.53	5
EE	1.70	1.823	-5.85	3	EE	66.17	0.483	4.31	3	EE	162.50	0.058	2.55	4
EL	0.85	11.705	Extremely Influential	1	EL	19.27	7.077	Highly Influential	2	EL	68.20	3.731	Highly Influential	2
FE	2.26	0.000	1.24	5	FE	36.18	2.103	-4.20	3	FE	83.51	2.489	-5.33	3
FP	2.31	0.016	1.84	5	FP	46.45	0.512	-1.29	5	FP	90.77	1.987	-4.61	3
GA	2.43	0.191	3.44	4	GA	96.67	8.277	Highly Influential	2	GA	305.00	12.566	Extremely Influential	1
GL	2.07	0.208	-1.21	5	GL	42.00	1.069	-2.55	4	GL	100.38	1.410	-3.65	4
HE	2.97	Outlier	Outlier	0	HE	51.33	0.134	0.10	5	HE	215.00	2.142	7.79	3
IE	1.23	6.227	Highly Influential	2	IE	23.78	5.464	Highly Influential	2	IE	57.66	4.732	Highly Influential	2
JA	1.99	0.404	-2.14	4	JA	60.00	0.064	2.56	4	JA	229.00	3.194	Highly Influential	2
KA	2.31	0.019	1.88	5	KA	89.75	5.675	Highly Influential	2	KA	310.28	Withdrawn	Outlier	0
KL	3.77	13.586	Extremely Influential	1	KL	35.07	2.341	-4.52	3	KL	63.58	4.156	Highly Influential	2
LL	4.80	38.589	Extremely Influential	1	LL	102.00	10.618	Highly Influential	2	LL	305.18	12.595	Extremely Influential	1

	Residual stats to determine outliers	
MSE for C-W	QL	-0.110
0.03608905	QU	0.100
MSE for t-	IQR	0.210
values	OL	-0.740
0.021008893	OU	0.730

	Residual stats to determine outliers	
MSE for C-W	QL	-3.000
58.00148333	QU	3.000
MSE for t-	IQR	6.000
values	OL	-21.000
41.86810741	OU	21.000

	Residual stats to determine outliers	
MSE for C-W	QL	-10.248
575.2933787	QU	11.514
MSE for t-	IQR	21.761
values	OL	-75.531
458.8695002	OU	76.797

Final Consensus Mean
2.16
9 Labs in Final Consensus
3 Reps/LabMethod

Final Consensus Mean
50.99
9 Labs in Final Consensus
3 Reps/LabMethod

Final Consensus Mean
136.94
8 Labs in Final Consensus
4 Reps/LabMethod

Table 6. t-values for all laboratory/method/samples.

Sample	Lab/Method Mean	t-value	Method/Lab
Std	1.45	-9.02	AA
Utex	63.00	3.41	AA
Munson	152.50	1.55	AA
Std	2.43	3.44	AG
Utex	96.67	12.97	AG
Munson	305.00	16.77	AG
Std	1.99	-2.14	AJ
Utex	60.00	2.56	AJ
Munson	229.00	9.19	AJ
Std	2.31	1.88	AK
Utex	89.75	11.01	AK
Munson	310.28	17.30	AK
Std	2.10	-0.78	EC
Utex	58.67	2.18	EC
Munson	159.25	2.23	EC
Std	1.70	-5.85	EE
Utex	66.17	4.31	EE
Munson	162.50	2.55	EE
Std	2.26	1.24	EF
Utex	36.18	-4.20	EF
Munson	83.51	-5.33	EF
Std	2.97	10.20	EH
Utex	51.33	0.10	EH
Munson	215.00	7.79	EH
Std	1.23	-11.81	EI
Utex	23.78	-7.73	EI
Munson	57.66	-7.91	EI
Std	0.85	-16.63	LE
Utex	19.27	-9.01	LE
Munson	68.20	-6.86	LE
Std	2.07	-1.21	LG
Utex	42.00	-2.55	LG
Munson	100.38	-3.65	LG
Std	3.77	20.34	LK
Utex	35.07	-4.52	LK
Munson	63.58	-7.32	LK
Std	4.80	33.48	LL
Utex	102.00	14.48	LL
Munson	305.18	16.79	LL
Std	2.29	1.58	PD
Utex			PD
Munson	131.58	-0.53	PD
Std	2.31	1.84	PF
Utex	46.45	-1.29	PF
Munson	90.77	-4.61	PF