Evaluation of Limit of Detection (LOD) Capability for the Analysis of Total Phosphorus

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Miscellaneous Publication PUB-SS-1091 2011

Summary: Revisions to Wisconsin's water quality standards for phosphorus discharges will require laboratories to attain a level of detection (LOD) no more than 0.01 to 0.03 mg/L (as P) for total phosphorus. We surveyed accredited labs to evaluate their potential to meet this new LOD expectation. It was determined that 41% of laboratories were unable to achieve a valid LOD of 0.03 mg/L or less. Labs that use the Hach Company's Test 'N Tube™ procedure had particular difficulty in meeting the new LOD. It was determined that if absorbance measurements were made using a single, high quality cuvette, the LOD of the Test 'N Tube™ procedure could be improved (lowered) by as much as 60%. Other procedures which can be used to improve LODs are discussed as well.

Acknowledgements

We wish to acknowledge and thank the following for their contributions to this study:

- Patrick Gorski and Graham Anderson of the Wisconsin State Laboratory of Hygiene for providing analytical and technical support.
- Jim Burke of the Hach Company for providing an instrument to assist us with our assessment of the Test 'N Tube™ method capability.
- All the labs that responded to our survey, providing the data which made this study possible.

Disclaimer

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Cover Photographs: Phosphorus testing equipment and procedures by Kay Curtin, 2006. Used with permission.

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December 2011

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Introduction

In June 2010, Wisconsin's Natural Resources Board adopted rules substantially changing the regulations related to the discharge of total phosphorus (TP) into receiving waters, with an effective date of December 1, 2010. Prior to the rule change, most municipal and industrial facilities had been operating under a permit limit of 1.0 mg/L (monthly average) for TP. With a permit limit of 1.0 mg/L, analytical limits of detection (LOD) were effectively a non-issue. Assuming that a lab needs to have its limit of quantitation (LOQ) at or below the permit limit, and that LOQs are generally a factor of 10/3 greater than the LOD, the LOD which labs were expected to meet would be on the order of 0.3 mg/L. Historically, all regulated laboratories have been able to achieve an LOD well below 0.3 mg/L. In fact, until January 2011, TP was not even a parameter for which facilities were required to report their LOD or LOQ on monthly Discharge Monitoring Reports.

Information released to regulated entities early in 2011 indicated that new permit limits for TP discharges to rivers and streams, as is the case for most wastewater treatment facilities, would be approximately 0.075 to 0.10 mg/L. Consequently, the Wisconsin Department of Natural Resources (Wisconsin DNR) Laboratory Certification and Registration Program (Lab Cert) identified a potential need for many laboratories to change the way they perform TP analyses to accommodate the new lower limits. TP analysis has always been a procedure highly subject to blank contamination, but low level contamination has generally not been of significant concern due to the 1.0 mg/L permit limits. As the permit limits are lowered an order of magnitude or more, however, blank contamination and handling of blanks has a more significant impact on a lab's ability to meet permit requirements.

While anecdotal evidence and on-site evaluation experience indicated that many labs would have difficulty meeting the new LOD expectations, Lab Cert lacked data to support such a contention. Of the TP analytical approaches available to labs, Lab Cert was most concerned with the ability of the Hach Company's Test 'N Tube™ procedure to meet the department's LOD needs. Anecdotal evidence further suggested that a significant number of labs employ the Test 'N Tube™ procedure. Again, however, Lab Cert lacked documentation to make any factual statement regarding the predominance of any one particular analytical approach.

One major difference between the Test 'N Tube™ procedure and other analytical protocols is that Test 'N Tube™ uses individual vials containing all necessary reagents. These vials serve as both the digestion vessel and the cuvette for making spectrophotometric measurements. The U.S. Environmental Protection Agency (U.S. EPA) protocol for determining method detection limits—essentially equivalent to the LOD—is based solely on the precision of multiple analyses. If individual cuvettes are not of consistent quality, variability (i.e. increased standard deviation) will result. In the determination of LOD, variability translates to an elevated LOD.

We surveyed laboratories to determine what percentage could be expected to achieve new lower LODs without changing their procedures. We also evaluated whether any improvement in the Test 'N Tube™ LOD could be gained by eliminating the suspected variability of Test 'N Tube™ tubes and using a single high quality cuvette for making spectrophotometric determinations.

Methods

Survey

We e-mailed a simple survey (Figure 1), consisting of five questions to 173 Wisconsin laboratory facilities accredited to analyze aqueous matrix samples for TP. The survey requested the data (observed concentration and absorbance measurements for LOD replicates) associated with each lab's most recent determination of LOD for TP. In addition, specific information regarding the instruments and analytical process used by the lab, basic calibration information, and results of recent blank determinations was requested.

FIGURE 1: Survey questions.

1. What instrument, method, and digestion technique are you using?
2. What is your current TP LOD and the spike concentration used to determine it?
3. What were the concentrations and absorbance responses for each of your LOD samples from your last LOD determination/study?
4. What is the concentration and corresponding absorbance response of the lowest standard in a typical TP calibration curve?
5. What is the concentration and corresponding absorbance response of the last seven (7) method blanks analyzed?

Survey Data Review

Once we received all data, we compiled a master spreadsheet. Initially, an analysis was conducted to determine whether the data submitted met the requirements of the U.S. EPA protocol for determining detection limits outlined in 40 CFR Part 136, Appendix B. Using the raw data, all calculations were verified. Corrections to a final version of the resulting spreadsheet were made as necessary.

We divided results into three groups:

(1) those with a *valid* and defensible LOD,

(2) those whose LOD did not meet one or more of the U.S. EPA requirements and were therefore deemed to be *invalid*, and

(3) those labs whose data met U.S. EPA requirements, but the LOD was deemed to be questionable in light of blank results.

For the purposes of this study, the absolute concentration and response of blanks, compared to the theoretical response which would be predicted for a sample concentration equal to the LOD, were used to determine defensibility of the reported LOD. By conventional definition, the LOD represents the concentration at which the analytical response is significantly different than that of a blank. Consequently, if supporting data suggest this is not the case, then the reported LOD is neither realistic nor defensible.

Statistical Analysis

We used a Student's *t*-test to compare the LODs determined by the three major protocols (Test 'N Tube™, Hotplate Manual Digestion/Spectrophotometry, and Autoclave Digestion/Spectrophotometry) used by laboratories. The null hypothesis for the *t*-test was that no significant statistical difference existed between the means of LODs generated by the two analytical approaches being compared.

LODs generated by conventional hotplate digestion ["Hotplate"] followed by spectrophotometric color measurement were compared to results generated using an autoclave for digestion ["Autoclave"] followed by spectrophotometric color measurement. Both Hotplate and Autoclave results were compared to LODs generated using Test 'N Tube™. A sufficient sample size was available to draw conclusions from the analysis.

Multiple vs. Single Cuvette Comparison

Lab Cert commissioned the Wisconsin State Laboratory of Hygiene (WSLH) to assess the effects of using a single cuvette to perform colorimetric measurements following analysis using the Test 'N Tube™ procedure. The study procedure, outlined in Figure 2, was to use the Hach Test 'N Tube™ protocol (Method 8190; Figure 3) to prepare a calibration, and then, on each of four separate days (*to induce typical analytical variability*), analyze 2-3 LOD spike replicates along with a continuing calibration verification (CCV) standard and a method blank. This same sequence was then repeated with all color measurements made using a single, high-quality 25-mm, round, glass cuvette (Hach catalog #249502) instead of the 13-mm Test 'N Tube™ vials. According to the Hach instructions, each individual vial is used to zero the instrument before adding the Phos-Ver 3 color reagent. This practice conflicts with Lab Cert requirements for handling blanks; consequently, in the WSLH study, the spectrophotometer was zeroed at the beginning of each day using a "zero blank", which contained only the acid, persulfate, and sodium hydroxide reagents; no Phos-Ver 3 color reagent was added.

The spectrophotometer used a single wavelength user program at 880 nm. A Vortex mixer was used for mixing samples.

The mean and standard deviation for the two LOD data sets were compared using a Student's *t*-test.

FIGURE 2: Layout for the WSLH study to evaluate whether use of a single cuvette improves the LOD attainable using Test 'N Tube.

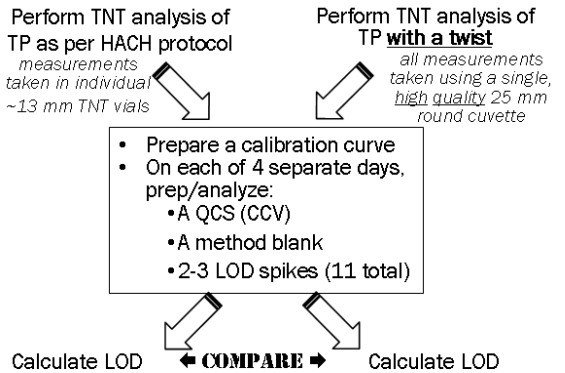
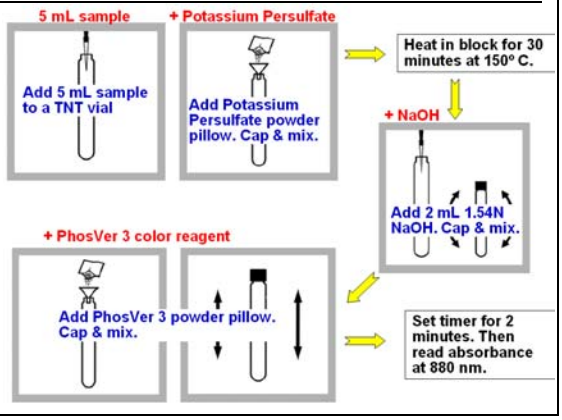


FIGURE 3: Hach Test 'N Tube™ procedure.



Results

Of the 173 labs surveyed, 112 responses were received for a survey return rate of 65%.

Analytical Protocols Used by Labs

Figure 4 shows the breakdown of analytical protocols being used by surveyed laboratories. One lab did not identify the technology used and another has received a variance form the U.S. EPA to use a lab oven for digestion of TP samples. Flow injection analysis (FIA) and discrete analyzers (DA) are only used in about 10% of the responding labs. These are higher cost instruments that typically are used by laboratories that test large numbers of samples on a regular basis. For these labs, the capital investment is mitigated by the number of samples that can be processed.

FIGURE 4: Breakdown of analytical approach employed by labs responding to the survey.

	# of lab using each approach					
Lab Type	TNT*	AC	HP	FIA	DA	Other
Commercial	2	0	3	4	3	-
Public Health	-	-	-	2	-	-
Industrial	5	1	3	-	-	1
Large WWTP	1	5	2	2	1	-
Small WWTP	26	27	23	-	-	1
	34	**33**	**31**	**8**	**4**	**2**

**TNT* = Test 'N Tube*
***AC**= Autoclave*
***FIA**= Flow Injection Analyzer*
***HP**= HotPlate*
***DA**= Discrete Analyzer*

The analytical approaches employed were evenly distributed for smaller wastewater treatment plant (WWTP) labs. One-third each were using Test 'N Tube™, autoclave, or hotplate followed by colorimetric determination with a basic lab spectrophotometer. While the specific models of spectrophotometer used were captured as part of the survey, the results were too varied to be considered further. The commonality is that in each case, a small inexpensive (less than about $3,500) unit is used. Some are true spectrophotometers, while a fair number use what would be more accurately described as colorimeters.

Quality of LOD Data Received

One of the initial concerns was the quality of the LOD data submitted. In order to evaluate its quality, the supporting data was requested as well. In addition, typical blank results were requested to assess blank response and concentration at the theoretical LOD level.

Figure 5 shows the breakdown of assessment of the reported LODs in light of supporting documentation.

FIGURE 5: Quality of reported LOD data.

Lab Type	Valid LOD	Invalid LOD	Questionable LOD
Commercial	6 of 12	3 of 12	3 of 12
Public Health	2 of 2	-------	-------
Industrial	3 of 10	1 of 10	6 of 10
Large WWTP	4 of 11	1 of 11	6 of 11
Small WWTP	32 of 77	13 of 77	32 of 77
Total	**46 of 112**	**18 of 112**	**47 of 112**
	42%	**16%**	**42%**

Two labs reported LODs using what appeared to be absolute response (absorbance). In other words, they calculated the LOD based on the absorbance of the replicate LOD spikes rather than the observed concentration of the spikes. Their LODs could only be replicated if the absolute response (absorbance) reported—rather than concentration—of the LOD replicate spikes were used for LOD calculations.

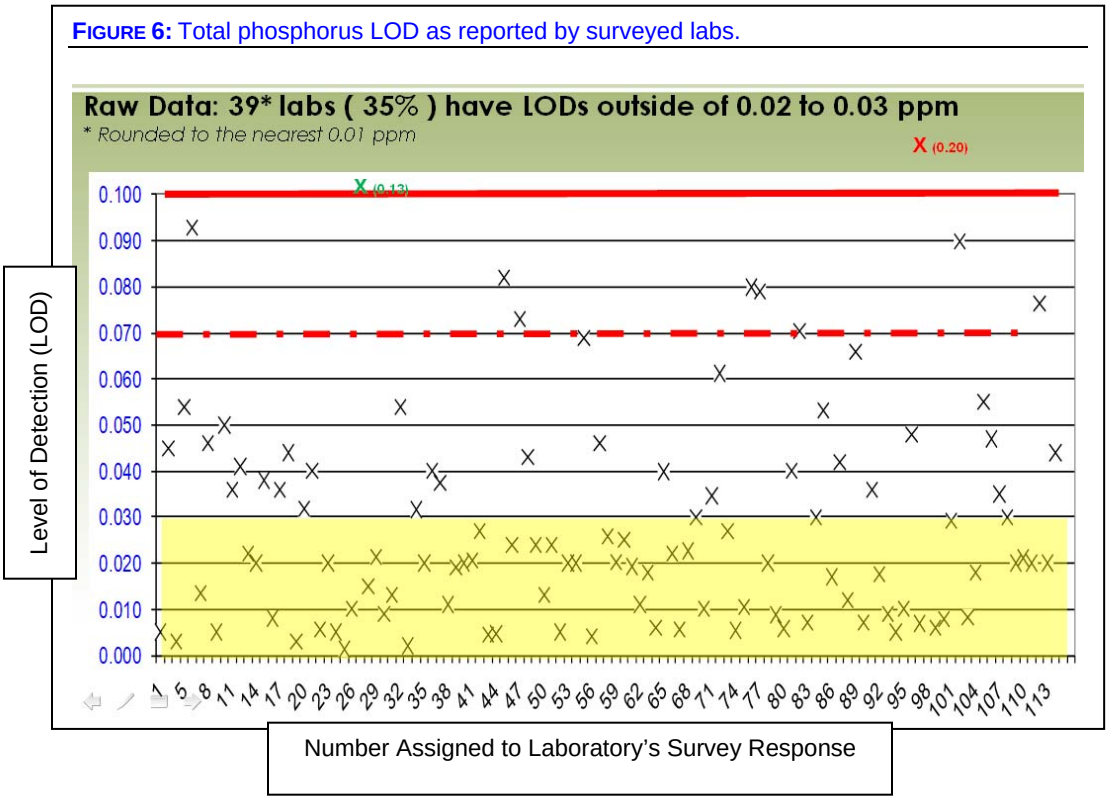
One lab reported an LOD result slightly greater than the concentration of spikes used to generate the LOD. It is impossible to accurately determine an LOD when the levels used for the determination are themselves lower than the statistically derived LOD.

Of the 61 labs whose LODs were flagged as either "invalid" or "questionable", 18 reported an LOD which was not significantly different than the concentration reported in blanks. Another 26 labs' LODs could not be substantiated because the equivalent absorbance at the LOD level was not significantly different than the absorbance typically observed in blanks.

Ability to Achieve an LOD of 0.03 mg/L

Figure 6 shows that, as reported by surveyed labs, more than one-third of regulated facilities would not be able to achieve the new desired LOD of 0.03 mg/L.

FIGURE 6: Total phosphorus LOD as reported by surveyed labs.



Ability to Achieve a Specific LOD by Lab Type

At the time of our study, Wisconsin DNR's Watershed Management program sought an LOD between 0.01 and 0.03 mg/L. Consequently, the ability of labs to achieve LODs of 0.01, 0.02, and 0.03 mg/L was evaluated. Figure 7 indicates that, even as reported by surveyed labs, more than one-third would not be able to achieve the new desired LOD of 0.03 mg/L. Only one-third of labs surveyed demonstrated the ability to achieve an LOD at the lower end of the desired LOD range (0.01). With the exception of the two of public health labs, only two-thirds of labs attained an LOD of 0.03 mg/L or below.

Even after culling those labs with invalid LODs (Figure. 8), the picture does not change appreciably. The overall percentage of labs that can reliably achieve an LOD of 0.03 or below falls to roughly 50%. With the exception of public health labs again, the percentage of labs other than small WWTP labs raises to 55-60%.

FIGURE 7: Ability to meet a specific TP LOD, based on data as reported.

Reported ability to achieve an LOD of

Lab Type	0.01 mg/L	0.02 mg/L	0.03 mg/L	
Commercial	5 of 12	9 of 12	9 of 12	**75%**
Public Health	2 of 2	2 of 2	2 of 2	**100%**
Industrial	3 of 10	4 of 10	6 of 10	**60%**
Large WWTP	4 of 11	7 of 11	7 of 11	**64%**
Small WWTP	22 of 77	41 of 77	49 of 77	**65%**
Total	36 of 112	63 of 112	73 of 112	
	32%	**56%**	**65%**	

Note: The numbers of labs that can meet 0.02 mg/L include those that can meet 0.01 mg/L. Similarly, the numbers of labs that can meet 0.03 mg/L include those that can meet 0.01 and those that can meet 0.02 mg/L.

FIGURE 8: Ability to meet a specific TP LOD, based on adjusted, "realistic" LODs.

Reported ability to achieve an LOD of

Lab Type	0.01 mg/L	0.02 mg/L	0.03 mg/L	
Commercial	1 of 9	5 of 92	5 of 9	**55%**
Public Health	2 of 2	2 of 2	2 of 2	**100%**
Industrial	1 of 9	3 of 9	5 of 9	**55%**
Large WWTP	2 of 10	6 of 10	6 of 10	**60%**
Small WWTP	7 of 59	18 of 59	29 of 59	**49%**
Total	13 of 89	34 of 89	47 of 89	
	15%	**38%**	**53%**	

Note: The numbers of labs that can meet 0.02 mg/L include those that can meet 0.01 mg/L. Similarly, the numbers of labs that can meet 0.03 mg/L include those that can meet 0.01 and those that can meet 0.02 mg/L.

Ability to Achieve a Specific LOD by Analytical Approach

When broken down by analytical approach (Figure 9), those labs using the Test 'N Tube™ procedure represented the lowest probability of meeting the new LODs. Only 44% of labs surveyed that use Test 'N Tube™ achieved an LOD of 0.03 mg/L or lower. That number fell more than five-fold to only 8% (2 labs) when considering only those LODs which could be substantiated by supporting information.

FIGURE 9: Ability to meet a specific TP LOD by approach, based on data as reported.

Reported ability to achieve an LOD (mg/L)

	0.01	**0.02**	**0.03**	
Test N' Tube (n=34)	3	12	15	**44%**
Autoclave (n=33)	17	24	25	**76%**
Hot Plate (n=31)	8	17	23	**71%**
Flow Injection (n=8)	5	6	6	**75%**
Discrete Analyzer(n=4)	2	2	2	**50%**
Other (n=2)	1	2	2	**100%**
Total	**36**	**63**	**73**	

Note: The numbers are cumulative. The numbers of labs that can meet 0.02 mg/L include those that can meet 0.01 mg/L. Similarly, the numbers of labs that can meet 0.03 mg/L include those that can meet 0.01 and those that can meet 0.02 mg/L

Test 'N Tube™ is *the* most popular method—and the method more and more small labs are adopting—yet, less than 10% of Test 'N Tube™ labs can meet the desired LODs.

Three quarters of labs surveyed could meet the new target LOD range when the autoclave procedure was employed. This percentage did not change significantly when only validated LODs (Figure 10) were considered.

FIGURE 10: Ability to meet a specific TP LOD by approach, based on adjusted, "realistic" LODs.

		Reported ability to achieve an LOD (mg/L)			
		0.01	**0.02**	**0.03**	
Test N' Tube	(n=25)	0	2	2	**8%**
Autoclave	(n=26)	8	16	20	**77%**
Hot Plate	(n=28)	1	8	17	**61%**
Flow Injection	(n=8)	3	6	6	**75%**
Discrete Analyzer	(n=3)	0	2	2	**67%**
Total (n=90)		**12**	**34**	**47**	

Note: The numbers are cumulative. The numbers of labs that can meet 0.02 mg/L include those that can meet 0.01 mg/L. Similarly, the numbers of labs that can meet 0.03 mg/L include those that can meet 0.01 and those that can meet 0.02 mg/L

Flow injection, as expected, looked to be the most consistent and sensitive of the techniques employed. Whether using LODs as reported or only validated LODs, 6 of 8 labs using FIA technology could meet the lower LOD.

When using LODs as reported, 71% of labs using hotplate digestion could achieve an LOD of 0.03 or less; when only validated LODs were considered, that percentage dropped to 61%.

FIGURE 11: Mean LODs (mg/L) by analytical approach.

	As Reported **LOD**	Adjusted/ Realistic **LOD**
Test 'N Tube	0.038	0.069
Autoclave	0.021	0.025
Hotplate	0.030	0.041

When the LOD means for individual analytical techniques are evaluated (Figure 11), Test 'N Tube™ not only has the highest LOD (0.038) , but the mean exceeds the new desired maximum LOD of 0.030 mg/L. The conventional hotplate digestion is also of some concern as the mean (0.030) is right at the upper limit of the target LOD range. When looking at raw, uncensored data, only the mean LOD for the autoclave digestion fell within the target LOD range. For each of the three techniques, the sample size was at least 31, a number statistically significant for making assessments using the mean.

The mean LOD for the filtered data set, after LODs were excluded or adjusted to a defensible level rose significantly (82%, an increase of 0.031 mg/L) to 0.069 mg/L for Test 'N Tube™. The mean for hotplate rose 37% to 0.041. Although still high in terms of percent increase (19%), the mean for the autoclave data only increased by 0.004 mg/L to 0.025 mg/L. Consequently, even after excluding invalid data and adjusting the remaining data based on blank responses, the autoclave approach was still capable of yielding an LOD that would meet new program expectations.

Student's *t*-Test Analysis

FIGURE 12: *t*-values at the 95% and 99% confidence levels

(df)	α=.05	α=.01
50	2.01	2.68
55	2.00	2.67
60	2.00	2.66
65	2.00	2.66
∞	1.96	2.58

We used a *t*-test to compare the LODs generated by Test 'N Tube™ vs. autoclave, Test 'N Tube™ vs. hotplate, and autoclave vs. hotplate to determine whether a statistically significant difference between any of the three techniques' means could be identified. The *t*-test was performed both before and after the raw LODs were filtered and adjusted to provide realistic, defensible values. Due to the sample sizes, the degrees of freedom for the determinations ranged from 50 to 65; subsequently (Figure 12) a *t*-value greater than 2.00 would be significant at the 95% confidence level and a *t*-value in excess of 2.70 would be significant at the 99% level.

Using the raw data as reported, only the mean LODs determined by Test 'N Tube™ and autoclave were determined to be significantly different (statistically), with a *t*-value of 3.57 (Figure 13).

FIGURE 13: Student's *t*-test results by analytical approach.

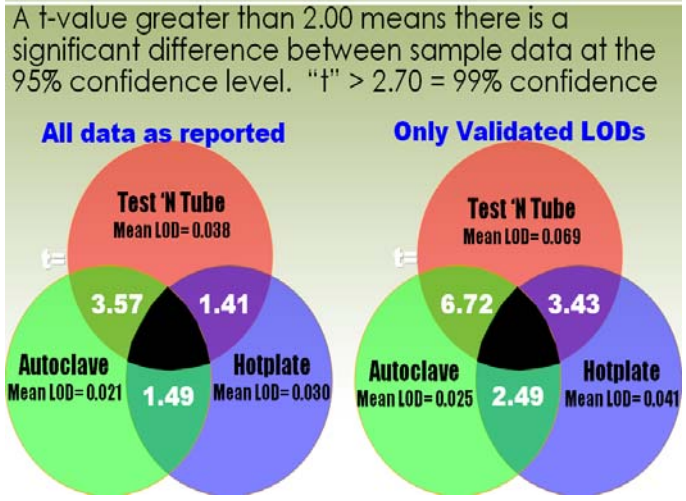
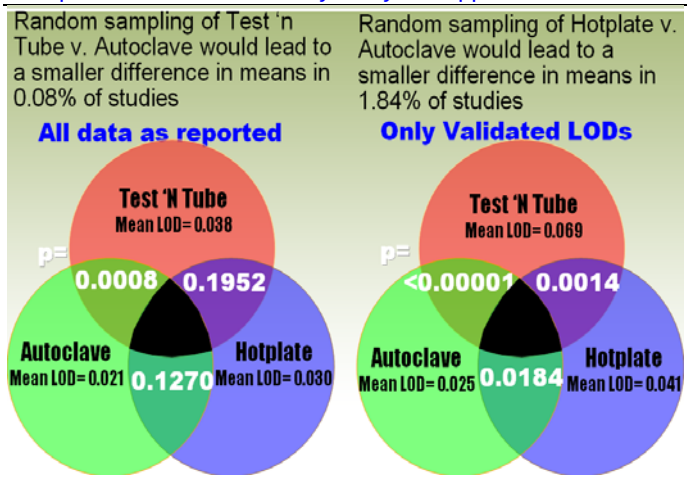


FIGURE 14: p-values associated with the Student's t-test comparison of mean LODs by analytical approach.



When the means of the adjusted LODs were compared, however, all three techniques had LODs that were statistically different at the 95% confidence level. In addition, the mean Test 'N Tube™ LOD was considered to be statistically different from the LODs determined by either autoclave or hotplate at the 99% confidence level.

The *t*-value simply indicates whether or not the null hypothesis can be rejected (i.e. that there is no difference between the LODs obtained by two different analytical approaches). In order to determine how significant the results are, we examined p-values. A p-value for the study represents the probability that one could obtain the same results assuming there is no real difference between the LODs determined by any pair of analytical techniques.

Using the original data, Figure 14 indicates that if this study were repeated, the likelihood of obtaining a mean LOD for Test 'N Tube™ that is significantly different from that determined by autoclave is 0.08%, or 8 chances in 10,000. On the other end of the spectrum, a p-value of 0.1952 was obtained for the comparison of the

mean LOD obtained from the Test 'N Tube™ and that obtained by hotplate. Assuming there is no difference between the Test 'N Tube™ and hotplate LODs, the p-value indicates that a 1 in 5 chance exists that the difference between the means could be even greater if the study were repeated.

Significant changes occur in p-values when the adjusted LOD data are analyzed. Now the null hypothesis that there is no significant difference between the Test 'N Tube™ and hotplate LODs is easily rejected. Using the adjusted LOD results, there is only a 0.14% chance that a repeated study would yield a wider difference. In reviewing the statistics for the adjusted LOD data, it is now the autoclave and hotplate LODs that are least likely to be different; and even then, the effective probability that the means are the same is less than 2%.

Color Measurement in Test 'N Tube™ Vial vs. Single Cuvette

Noting the significantly higher LODs obtained using Test 'N Tube™ as compared to other techniques, one theory that was explored concerned the Test 'N Tube™ vials themselves. One of the conveniences offered by the Test 'N Tube™ procedure is that the individual vials serve as both the digestion vessel and the cuvette for colorimetric measurements. In all of the other procedures, a single cell, or cuvette, is used to make all spectrophotometric measurements. These cells are typically constructed of high optical quality glass. Our simple test determined whether any difference in LOD is obtained if the Test 'N Tube™ vials are used for digestion but all colorimetric measurements are made using a single high quality cell.

The chemist made several noteworthy observations during the testing:

- The potassium persulfate did not completely dissolve until heated in the block reactor.
- The PhosVer 3 color reagent did not completely dissolve (as stated in the method instructions). The chemist did not observe any significant sticking of it on cuvette walls. It seemed to settle to bottom in both the Test 'N Tube™ vials and the 25-mm cuvette.

The data generated during this study are provided in Appendix D. One of the initial observations made was that the response (absorbance) nearly doubled when the single 25-mm cell was used. This is what one would expect according to Beer-Lambert's law, which can be simplified to state that the absorbance of light is directly related to the path length. Therefore if path length is doubled, the absorbance of a known standard is expected to double.

Another consequence of the increased path length was that the Y-intercept of the linear regression also doubles. What does not change, however, is what is known as the "X-intercept", or the concentration where response equals zero. This is the phenomenon which causes "negative" blanks. In our study (Figures 15, 16, next page), we found that while the absorbance (response) and Y-intercept double, there is no significant change to the X-intercept.

FIGURE 15: Lower end of calibration performed using Test 'N Tube™ vials.

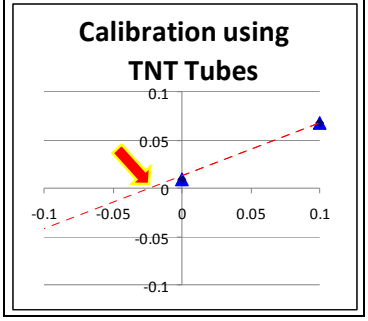
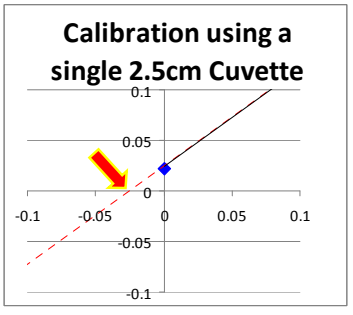


FIGURE 16: Lower end of calibration performed using single 25-mm cuvette.



Of greatest significance was the reduction in LOD observed when only a single cuvette was used for color measurement. The LOD obtained by using the Test 'N Tube™ procedure as directed was 0.03 mg/L. The only adjustment made was measuring absorbance using a single cuvette with a longer path length, yet the LOD was reduced to 0.012 mg/L.

The results (Appendix D) obtained using a single cell were much "tighter" than those obtained from individual Test 'N Tube™ tubes. The relative standard deviation (RSD) was half of that which resulted from performing colorimetric measurements in the Test 'N Tube™ vials themselves. The range of the 11 LOD replicate spikes analyzed was also half (0.014 absorbance units) of the range obtained measuring color in the Test 'N Tube™ vials (0.030).

One other observation of note was that, despite yielding approximately double the response (absorbance) as obtained using the Test 'N Tube™ vials, concentrations obtained using a single 2.5 mm cuvette were consistently about 20% less than those obtained using standard Test 'N Tube™ vials. Looking at it another way, the mean "recovery" of LOD spikes using the Test 'N Tube™ vials was 111%, while the mean recovery using a single cuvette was only 92%

Discussion

This survey and associated work should be viewed as neither an indictment nor an endorsement of the Test 'N Tube™ method for TP. In reality, many laboratories may find the Test 'N Tube method to be a convenient and cost effective approach to performing TP testing. Our results show that the Test 'N Tube™ approach has some limitations when it comes to achieving LODs that will be necessary in dealing with Wisconsin's new phosphorus regulations. Hach Method 8190 (the written method associated with the Test 'N Tube™ procedure for TP) indicates that the estimated detection limit (LOD) is 0.02 mg/L (as P). While that level of sensitivity is certainly possible in some labs, our study data support the contention that the actual LODs determined in smaller labs, and particularly WWTP labs, are significantly higher.

Test 'N Tube™ *is* different because it is the only currently recognized procedure (with U.S. EPA equivalence) for TP that uses individual sample cuvettes. Saying it is different, and that it presents some challenges in light of new LOD expectations, is not to suggest that Test 'N Tube™ is no longer acceptable. Rather, by highlighting the differences and focusing on changes which can be easily incorporated, labs should be able to consistently achieve an LOD which meets the needs of the Watershed Management program.

Analytical Protocols Used by Survey Labs

If survey results are representative of the entire Wisconsin certified/registered lab community, then approximately one-third of smaller labs are actively using the Test 'N Tube™ procedure. Consequently, there is a need to educate laboratories regarding the changes which can be made that will allow the Test 'N Tube™ method to produce results that meet the needs of the Watershed Management program.

Ability to Achieve an LOD of 0.03 mg/L

This is not a problem limited to Test 'N Tube™. Only results from labs using flow injection (FIA)—and it was a very limited dataset—could reliably achieve an LOD that meets the new Watershed Management program expectation. Is it possible to achieve an LOD of 0.03 mg/L or less with any of the typical techniques used? Yes, but only if the lab takes steps to control background contamination and other analytical aspects that affect sensitivity. Also coming in a close second in terms of consistently being able to obtain an LOD of 0.03 mg/l or less, was the autoclave method.

Ability to Achieve a Specific LOD by Analytical Approach

The answer to the question, "Are LODs obtained using Test 'N Tube™ really different from other methods?" is clearly, "yes". Results from Student's *t*-test analyses indicate that even before any of the raw data were filtered or adjusted, there was a significant difference in the data generated by Test 'N Tube™ and data generated using autoclave digestion followed by colorimetric measurement using a single cuvette.

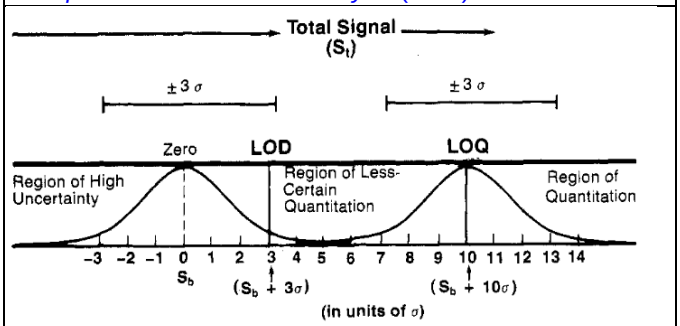
The results of Student's *t*-test indicates that the mean LODs for the three most common analytical approaches are all significantly different from one another. Survey results suggest that the lowest, most reliable LODs are obtained using an autoclave digestion, but there is rationale to support that. The pressure and temperature of the autoclave digestion is such that the sample volume is not boiled down, as is done with hotplate digestions. In addition, samples are not exposed to atmospheric contaimination during digestion as occurs when using the hotplate method, and there is less sample handling, which helps reduce background contamination.

The hotblock digestion used for Test 'N Tube™ results in a similar situation. However, the variability—and thus higher LODs—would appear to result from inconsistencies in the optical quality of individual Test 'N Tube™ vials, or from scratches and micro-abrasions due to improper handling. Anecdotal reports have been received of several facilities that actually wash and re-use Test 'N Tube™ vials, re-filling them with their own purchased or prepared reagents.

Quality of LOD Data Received

Sixteen percent of surveyed labs reported results which do not meet requirements of the U.S. EPA LOD procedure, indicating a need for additional lab training. Another 42% of labs reported an LOD which could not be substantiated due to blank responses or concentrations which approached or even exceeded the LOD or predicted reponse at the LOD. That left only 42% of data deemed to be valid.

FIGURE 17: Classic graphical representation of the LOD from *Principles of Environmental Analysis* (1983).



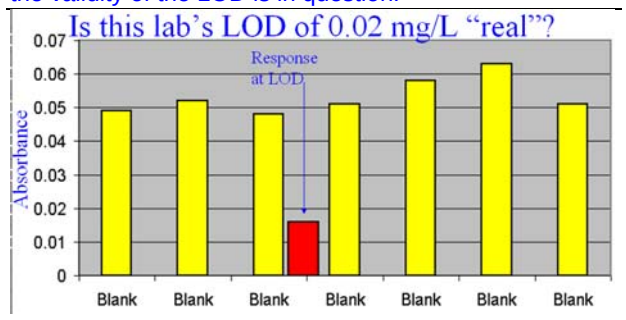
The U.S. EPA procedure for determining an LOD (40 CFR Part 136, Appendix B Rev. 1.11) is generally recognized to be flawed in that it focuses entirely on analytical precision, without giving any consideration to accuracy. In addition, the U.S. EPA procedure does not consider the basic premise of the original treatise on environmental detection (Keith et al. 1983. Principles of Environmental Analysis. *Anal. Chem*. 55:2210-2218): "*The limit of detection (LOD) is defined as the lowest concentration level that can be determined to be statistically different from a blank*." Figure 17 provides a graphical representation of the LOD and LOQ relationship.

A "reality" test should be a requisite part of the LOD process. An LOD should only be considered "realistic" if the LOD can be distinguished statistically from a blank.

Until such time as a replacement mechanism for determining LODs is promulgated, labs must use what is available. That determination, however, should be supplemented by reviewing the calculated LOD while taking into consideration the response and concentration of typical method blanks. See Figure 18 for an unrealistic LOD as compared to the blank response.

As part of the LOD determination process, labs should also review the response of a prepared standard (LCS), at a concentration equal (or very close) to the LOD, against the response typically observed for blanks. If the LOD is not significantly different than that of a blank, then the "realistic" LOD is higher than the calculated LOD. This might require an iterative process until the lab identifies a concentration that can be distinguished from a blank.

FIGURE 18: The response of typical blanks suggests that the validity of the LOD is in question.



Color Measurement in Test 'N Tube™ Vial vs. Single Cuvette

While limited in scope, the data generated make a strong case to support the concern that the variability between individual Test 'N Tube™ vials induces variability in results, which translates to a higher LOD using the U.S. EPA model. Conventional colorimetric procedures have historically employed a single cuvette which is rinsed carefully between samples. This ensures that all sample and standard measurements are made by measuring absorbance through the same optical quality glass.

One item of note is that the Hach company's new "Test 'N Tube-plus" products, which are used with their DR3900 (and newer models) spectrophotometer has incorporated a mechanism to deal with vial variability. The DR 3900, which was not evaluated as part of this study, slowly rotates the Test 'N Tube™ vial and measures absorbance at 10 positions around the vial. An internal outlier program then tests and rejects any measurements that are deemed to be outliers. The average absorbance is then reported. The only concern with the DR 3900 is that the instrument uses barcodes on Test 'N Tube™ vials to select the internal program and pre-programmed (fixed) calibration to use. Unfortunately, ch NR 149, Wis. Admin. Code, as with many state accrediting rules, does not allow the use of pre-programmed calibrations. User-generated calibrations can be performed, however, and the unique absorbance measurement system is still used.

The most prominent observation in the single vial vs. Test 'N Tube™ vials study was the bias observed in the Test 'N Tube™ vial measured samples relative to measurements made using the single cuvette. While the larger path length resulted in a doubling of instrument response and an LOD 60% lower than that obtained with Test 'N Tube™ vials, the absolute measured *concentrations* were, on average, 17% higher in the Test 'N Tube™ vials.

It would appear that the fact that the color reagent powder does not completely dissolve did not affect the Test 'N Tube™ results. The same procedure was used to "zero" the instrument before use, so that would not explain the bias. The question remains whether or not there is a bias inherent to the analysis when Test 'N Tube™ vials are used to make colorimetric measurements.

Other Considerations to Improve LOD

- *Purchase a flow-injection analysis (FIA) instrument.* FIA systems are generally closed systems which eliminate, to a great extent, background contamination. These systems are also very precise, and the current U.S. EPA procedure for determining LODs is based solely on precision. The downside of this option is cost. Even a used instrument can cost upwards of $15,000, and a brand new instrument equipped to analyze both TP and total Kjeldahl nitrogen (TKN) will cost about $30,000-$35,000. Consequently, investing in such a system is likely cost-prohibitive for most smaller industrial or municipal wastewater treatment plants.

- *Closely monitor and evaluate calibration data.* Calibration errors will also affect LOD. At the extreme range of linearity, the net change in slope (change in response per unit concentration) starts to plateau. The linear regression equation will often still result in an acceptable calibration as determined by the correlation coefficient, but the regression line will cease to pass through all calibration points. As the upper end of the calibration "drops" the linear regression line "teeters" at the calibration midpoint and the lower end of the calibration rises. This amounts to an increase in the Y-intercept, which, in turn, results in increasingly "negative" values for blanks.

  A drop, or dip, in the points at the upper end of a calibration can be caused by exceeding the linear range, a spectrophotometer bulb (lamp) which is not operating at peak performance (i.e. "going bad"), poorly prepared standards, spectrophotometer optics becoming coated with acids (e.g., HCl), or failing detector. To check for this, labs can monitor the response factors (response divided by concentration) for each of the calibration standards. In addition, attention must be paid to blanks that yield negative concentrations. A small degree of negativity can be expected, but when the absolute value of the blank concentration exceeds the LOD, corrective action should be initiated.

- *Optimize spectrophotometer performance.* Insufficient spectrophotometer maintenance can significantly degrade measurement accuracy and precision. Incorporating the following into the laboratory maintenance program may help generate consistent results at optimum sensitivity.

  - Clean up spills on or inside the spectrophotometer immediately.
  - Periodically clean cell compartment by wiping it out with soft damp cloth.
  - Avoid exposing spectrophotometers to a corrosive environment.
  - Acid vapors, dust, and moisture can coat optics and degrade performance.
  - Consider changing lamp/bulb annually (and before generating a new calibration curve).
  - Recalibrate anytime major maintenance is performed.
  - Track absorbance of CCVs to ensure sensitivity does not degrade over time.
  - Consider having wavelength accuracy and performance checked by an outside vendor every few years.

- *Issues specific to Test 'N Tube™.* Test 'N Tube™ is convenient, but the convenience comes with concerns related to using a different cuvette to measure absorbance in every sample, standard, or blank. It is reasonable to ponder whether each tube or lot of tubes are of the same optical quality. Further, handling of the tubes upon receipt in the laboratory can result in micro-abrasions that impact light scatter, absorbance, and transmittance. And, if these were not challenge enough, Lab Cert is aware of several labs that historically have cleaned and re-used Test 'N Tube™ vials, re-filling them with their own reagents.

  FIGURE 19: Pictures of new Test 'N Tube™ vials under a light microscope.

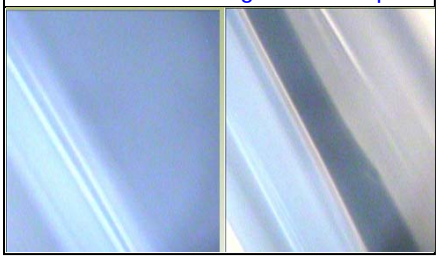
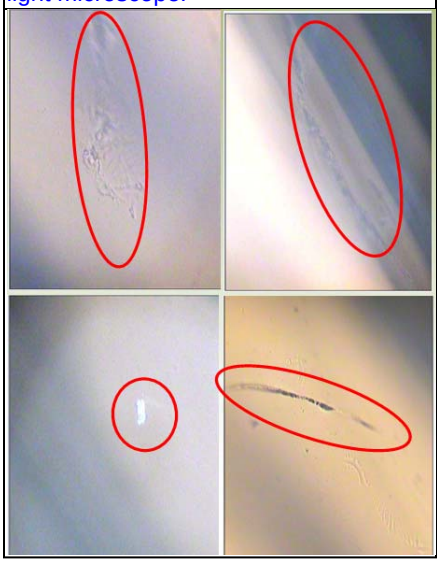
  

  FIGURE 20: Pictures of cleaned and reused Test 'N Tube™ vials under a light microscope.

  Figures 19 and 20 show the differences observed when new and re-used Test 'N Tube™ vials are compared under a microscope. Even in this limited analysis, it seems clear that re-using Test 'N Tube™ vials corresponds with an increase in surface flaws and micro-abrasions that will impact absorbance.

  While the advent of the cell rotational measurement system offered in the Hach DR 3900 and newer instruments will help to eliminate biases caused by surficial flaws, blemishes, and smudges, Lab Cert firmly contends, with the support of Hach representatives, that reusing Test 'N Tube™ vials is not an acceptable practice.

- *Cuvette care and handling.* The detector in a spectrophotometer "sees" the difference between light going in and coming out of the cuvette as absorption by the sample itself. Anything that hinders light passage through the cuvette will produce biased absorbance readings, because the detector assumes that a reduction in light coming through the sample is solely attributed to absorbance by the sample. For example, scratches on the cuvette pose a significant concern. Incoming light scatter caused by scratches reduces the amount of light which reaches the detector. The detector interprets this as light absorbed by the sample.

  To avoid scratches, cuvettes should always be hand-washed (the jostling that occurs in the glassware tubs is damaging) using a cotton swab dipped in a non-phosphate detergent solution. After the cuvette is scrubbed inside and out, the soap should be removed by rinsing with tap water and then distilled water.

- *Cuvette shape.* While not evaluated as part of this study, the shape of cuvettes for spectrophotometric measurements can also impact results. Test 'N Tube™ vials are generally 13-mm, round tubes (approximately ½ inch). The diameter corresponds to the path length through which light from the spectrophotometer must travel

through the sample and to the detector. Most other labs use round cuvettes that range from 13 mm to 25 mm. Generally speaking, round cuvettes are used because they are less expensive than square or rectangular cuvettes.

Studies have shown that square (or rectangular) cuvettes are more precise than round ones, and thus yield more accurate results. One reason for this is that as incident light hits the rounded surface, diffraction or scatter eliminates some of the light from ever reaching the detector, which reduces potential absorbance. While there is certainly some degree of diffraction even from a flat surface (square cells), it understandably occurs to a much lesser extent than with round cuvettes. Labs wishing to further improve the quality of their data may wish to consider using square (or rectangular) cuvettes.

Summary/Conclusions

- New administrative rules for phosphorus will mean labs need to achieve a valid LOD of at least 0.03 mg/L.
- In general, labs need to review LOD protocols.
- The LOD determination process must be focused on obtaining a valid LOD which is both realistic and defensible, rather than simply meeting the minimum U.S. EPA criteria for LOD.
- It will be very difficult to achieve a realistic, defensible LOD of 0.03 mg/L or less when using Test 'N Tube™ procedures without making some adjustments.
- It is strongly recommended that Test 'N Tube™ users adopt the single quality cuvette approach.

Appendix A: Survey Summary Data

		Question 1	Question 2		Question 4	
Type	#	Technique	LOD (mg/L)	Spike (mg/L)	Low std mg/L	Low std abs
CO	93	Discrete Analyzer	0.009	0.05	0.0625	0.004
CO	102	Discrete Analyzer	0.090	0.4	0.25	0.006
CO	98	Discrete Analyzer	0.200	0.4	0.4	0.027
CO	19	Flow Injection (FIA)	0.003	0.025	0.025	0.341
CO	43	Flow Injection (FIA)	0.005	0.1	0.2	0.787
CO	94	Flow Injection (FIA)	0.005	0.015	0.005	0.163
CO	76	Flow Injection (FIA)	0.080	0.1	0.1	0.809
CO	99	Hotplate	0.006	0.0375	0.015	0.022
CO	39	Hotplate	0.019	0.05	0.05	0.022
CO	113	Hotplate	0.020	0.1		
CO	23	TNT (Test N' Tube)	0.020	0.1	0.1	0.074
CO	78	TNT (Test N' Tube)	0.020	0.2	0.1	0.119
IN	38	Autoclave	0.011	0.1	0.1	0.078
IN	88	Hotplate	0.012	0.05	0.1	0.061
IN	84	Hotplate	0.030	0.01	0.05	0.032
IN	20	Hotplate	0.032	0.1	0.1	0.174
IN	41	TNT (Test N' Tube)	0.021	0.1	0.2	0.126
IN	21	TNT (Test N' Tube)	0.040	0.1	0.2	0.109
IN	85	TNT (Test N' Tube)	0.053	0.3	0.1	0.080
IN	89	TNT (Test N' Tube)	0.066	0.2	0.1	0.075
IN	82	TNT (Test N' Tube)	0.070	0.1	0.3	0.105
IN	103		0.008	0.025	0.5	0.028
lg MU	56	Autoclave	0.004	0.015	0.05	0.037
lg MU	44	Autoclave	0.005	0.1	0.1	0.059
lg MU	59	Autoclave	0.020	0.1		
lg MU	49	Autoclave	0.024	0.1	0.1	0.074
lg MU	10	Autoclave	0.050	0.05	0.05	0.035
lg MU	83	Discrete Analyzer	0.007	0.01	0.01	0.009
lg MU	53	Flow Injection (FIA)	0.020	0.08	0.4	0.030
lg MU	37	Flow Injection (FIA)	0.038	0.1	0.1	2.342
lg MU	9	Hotplate	0.005	0.04	0.02	0.023
lg MU	27	Hotplate	0.130	0.2	0.2	0.012
lg MU	32	TNT (Test N' Tube)	0.054	0.5	0.5	0.300
MU	33	Autoclave	0.002	0.05	0.2	0.055
MU	1	Autoclave	0.005	0.025	0.1	0.072
MU	52	Autoclave	0.005	0.015	0.1	0.068
MU	74	Autoclave	0.005	0.05	0.1	0.080

		Question 1	Question 2		Question 4	
Type	#	Technique	LOD (mg/L)	Spike (mg/L)	Low std mg/L	Low std abs
MU	80	Autoclave	0.006	0.05	0.05	0.040
MU	97	Autoclave	0.007	0.1	0.1	0.070
MU	90	Autoclave	0.007	0.15	0.1	0.072
MU	100	Autoclave	0.008	0.02	0.05	0.031
MU	16	Autoclave	0.008	0.05	0.1	0.072
MU	26	Autoclave	0.010	0.1	0.2	0.145
MU	70	Autoclave	0.010	0.1	0.1	0.036
MU	62	Autoclave	0.011	0.1		
MU	31	Autoclave	0.013	0.1	0.1	0.069
MU	50	Autoclave	0.013	0.2	0.1	0.067
MU	86	Autoclave	0.017	0.05	0.05	0.040
MU	63	Autoclave	0.018	0.1	0.1	0.082
MU	61	Autoclave	0.019	0.1	0.2	0.072
MU	40	Autoclave	0.020	0.03	0.12	0.172
MU	68	Autoclave	0.023	0.15	0.1	0.077
MU	42	Autoclave	0.027	0.1	0.2	0.132
MU	11	Autoclave	0.036	0.2	0.2	0.158
MU	81	Autoclave	0.040	0.4	0.2	0.229
MU	12	Autoclave	0.041	0.1	0.25	0.041
MU	3	Autoclave	0.045	0.24	0.12	0.030
MU	57	Autoclave	0.046	0.2	0.1	0.033
MU	5	Autoclave	0.054	0.18	0.1	0.190
MU	77	Autoclave	0.079	0.5	0.1	0.079
MU	4	Hotplate	0.003	0.1	0.2	0.141
MU	22	Hotplate	0.006	0.15	0.1	0.078
MU	67	Hotplate	0.006	0.1	0.2	0.165
MU	64	Hotplate	0.006	0.05	0.1	0.015
MU	30	Hotplate	0.009	0.05		
MU	92	Hotplate	0.018	0.1	0.1	0.167
MU	14	Hotplate	0.020	0.1	0.2	0.150
MU	54	Hotplate	0.020	0.1	0.2	0.126
MU	110	Hotplate	0.021	0.2	0.2	0.154
MU	29	Hotplate	0.021	0.1	0.1	0.076
MU	46	Hotplate	0.024	0.2	0.1	0.028
MU	51	Hotplate	0.024	0.1	0.1	0.057
MU	101	Hotplate	0.029	0.1	0.1	0.072
MU	69	Hotplate	0.030	0.2	0.2	0.140

		Question 1	Question 2		Question 4	
Type	#	Technique	LOD (mg/L)	Spike (mg/L)	Low std mg/L	Low std abs
MU	108	Hotplate	0.030	0.1	0.2	0.143
MU	34	Hotplate	0.032	0.1	0.1	0.123
MU	71	Hotplate	0.035	0.05	0.125	0.077
MU	107	Hotplate	0.035	0.1	0.1	0.048
MU	17	Hotplate	0.036	0.1		
MU	91	Hotplate	0.036	0.1	0.2	0.342
MU	105	Hotplate	0.055	0.1	0.1	0.099
MU	112	Hotplate	0.076	0.5	0.05	0.025
MU	45	Hotplate	0.082	0.1	0.1	0.032
MU	104	Oven (250C)	0.018	0.1	0.1	0.080
MU	95	TNT (Test N' Tube)	0.010	0.1	0.2	0.118
MU	75	TNT (Test N' Tube)	0.011	0.2	0.2	0.130
MU	7	TNT (Test N' Tube)	0.014	0.2	0.2	0.136
MU	28	TNT (Test N' Tube)	0.015	0.05	0.15	0.281
MU	35	TNT (Test N' Tube)	0.020	0.1	0.1	0.078
MU	109	TNT (Test N' Tube)	0.020	0.2	0.1	0.032
MU	111	TNT (Test N' Tube)	0.020	0.7	0.1	0.077
MU	13	TNT (Test N' Tube)	0.022	0.05	0.1	0.043
MU	66	TNT (Test N' Tube)	0.022	0.2	0.484	0.147
MU	60	TNT (Test N' Tube)	0.025	0.1	0.1	0.071
MU	58	TNT (Test N' Tube)	0.026	0.2	0.1	0.100
MU	73	TNT (Test N' Tube)	0.027	0.1	0.2	0.152
MU	15	TNT (Test N' Tube)	0.038	0.1	0.1	0.122
MU	65	TNT (Test N' Tube)	0.040	0.3		
MU	36	TNT (Test N' Tube)	0.040	0.2	0.1	0.090
MU	87	TNT (Test N' Tube)	0.042	0.2	0.2	0.146
MU	48	TNT (Test N' Tube)	0.043	0.2	0.3	0.165
MU	18	TNT (Test N' Tube)	0.044	0.1	0.1	0.139
MU	114	TNT (Test N' Tube)	0.044	0.1		
MU	8	TNT (Test N' Tube)	0.046	0.2	0.1	0.069
MU	106	TNT (Test N' Tube)	0.047	0.1	0.01	0.027
MU	96	TNT (Test N' Tube)	0.048	0.1	0.1	0.102
MU	72	TNT (Test N' Tube)	0.061	0.2	0.1	0.056
MU	55	TNT (Test N' Tube)	0.069	0.2	0.2	0.136
MU	47	TNT (Test N' Tube)	0.073	0.2	0.2	0.090
MU	6	TNT (Test N' Tube)	0.093	0.2	0.2	0.188
PH	24	Flow Injection (FIA)	0.005	0.016	0.016	0.530
PH	79	Flow Injection (FIA)	0.009	0.025	0.2	0.735

Appendix B: Survey LOD Replicate Data (1 of 3)

			Question 1	Question 2	Question 3 (LOD data)																	
					1		2		3		4		5		6		7		8			
Type	#	Technique	LOD (mg/L)	Spike (mg/L)	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs
CO	93	Discrete Analyzer	0.009	0.05	0.05	0.003	0.05	0.003	0.05	0.003	0.05	0.002	0.05	0.003	0.05	0.003	0.05	0.003				
CO	102	Discrete Analyzer	0.09	0.4	0.40	0.012	0.43	0.013	0.38	0.011	0.39	0.012	0.45	0.014	0.44	0.014	0.38	0.011				
CO	98	Discrete Analyzer	0.2	0.4	0.35	0.025	0.34	0.025	0.35	0.026	0.35	0.025	0.34	0.025	0.29	0.022	0.35	0.025				
CO	19	FIA	0.003	0.025	0.02	0.340	0.02	0.347	0.02	0.341	0.03	0.355	0.03	0.370	0.02	0.333	0.02	0.344	0.03	0.359		
CO	43	FIA	0.005	0.1	0.06	0.434	0.06	0.432	0.06	0.441	0.06	0.435	0.06	0.426	0.06	0.432	0.06	0.425	0.05	0.418		
CO	94	FIA	0.005	0.015	0.01	0.404	0.01	0.392	0.01	0.396	0.01	0.361	0.01	0.344	0.01	0.409	0.02	0.463	0.02	0.446		
CO	76	FIA	0.08	0.1	0.13	1.070	0.13	1.110	0.12	1.030	0.12	1.030	0.13	1.090	0.13	1.090	0.13	1.120	0.11	0.935		
CO	99	Hotplate	0.006	0.0375	0.04	0.061	0.04	0.062	0.04	0.064	0.04	0.060	0.04	0.060	0.04	0.062	0.04	0.061				
CO	39	Hotplate	0.019	0.05	0.05	0.019	0.03	0.014	0.03	0.014	0.04	0.016	0.04	0.016	0.04	0.015	0.04	0.017				
CO	113	Hotplate	0.02	0.1																		
CO	23	TNT (Test N' Tube)	0.020	0.1	0.10	0.080	0.12	0.089	0.10	0.081	0.11	0.088	0.11	0.083	0.11	0.086	0.10	0.078				
CO	78	TNT (Test N' Tube)	0.02	0.2	0.19	0.160	0.18	0.155	0.19	0.161	0.20	0.164	0.19	0.160	0.19	0.160	0.19	0.159				
IN	38	Autoclave	0.011	0.1	0.11	0.078	0.10	0.075	0.10	0.073	0.10	0.072	0.11	0.078	0.10	0.074	0.10	0.071				
IN	88	Hotplate	0.012	0.05	0.05	0.020	0.05	0.021	0.06	0.022	0.05	0.019	0.05	0.019	0.05	0.020	0.04	0.017				
IN	84	Hotplate	0.03	0.01	0.01	0.008	0.01	0.009	0.01	0.008	0.01	0.008	0.01	0.008	0.01	0.008	0.01	0.008				
IN	20	Hotplate	0.032	0.1	0.09		0.11		0.10		0.10		0.09		0.11		0.12					
IN	41	TNT (Test N' Tube)	0.021	0.1	0.10	0.073	0.10	0.076	0.11	0.077	0.10	0.072	0.10	0.076	0.11	0.078	0.09	0.068	0.11	0.079		
IN	21	TNT (Test N' Tube)	0.040	0.1	0.12	0.069	0.13	0.076	0.14	0.080	0.12	0.069	0.15	0.084	0.11	0.066	0.13	0.077				
IN	85	TNT (Test N' Tube)	0.0531	0.3	0.32	0.201	0.31	0.186	0.30	0.180	0.32	0.190	0.34	0.200	0.29	0.172	0.34	0.199	0.34	0.198		
IN	89	TNT (Test N' Tube)	0.066	0.2		0.145		0.143		0.123		0.161		0.139		0.146		0.148				
IN	82	TNT (Test N' Tube)	0.0704	0.1	0.16	0.063	0.13	0.057	0.11	0.051	0.10	0.047	0.09	0.046	0.12	0.052	0.09	0.044				
IN	103		0.0083	0.025	0.02	0.016	0.02	0.016	0.02	0.017	0.02	0.016	0.02	0.014	0.02	0.014	0.03	0.019				
lg MU	56	Autoclave	0.004	0.015	0.01	0.012	0.01	0.012	0.01	0.012	0.01	0.012	0.02	0.013	0.02	0.013	0.02	0.013	0.01	0.011		
lg MU	44	Autoclave	0.005	0.1	0.11	0.063	0.11	0.062	0.10	0.061	0.10	0.061	0.10	0.061	0.10	0.061	0.10	0.061	0.11	0.062		
lg MU	59	Autoclave	0.0202	0.1	0.09	0.066	0.09	0.065	0.10	0.073	0.10	0.074	0.10	0.073	0.09	0.066	0.09	0.066	0.10	0.073		
lg MU	49	Autoclave	0.024	0.1	0.09	0.042	0.08	0.038	0.07	0.036	0.08	0.039	0.09	0.042	0.08	0.037	0.07	0.036				
lg MU	10	Autoclave	0.050	0.05	0.06	0.010	0.06	0.012	0.05	0.009	0.04	0.008	0.04	0.008	0.05	0.009	0.08	0.015	0.08	0.015		
lg MU	83	Discrete Analyzer	0.007	0.01	0.01	0.010	0.01	0.010	0.01	0.010	0.01	0.010	0.01	0.010	0.01	0.009	0.01	0.009				
lg MU	53	FIA	0.020	0.08	0.09	0.007	0.08	0.007	0.09	0.007	0.09	0.007	0.08	0.007	0.09	0.008	0.09	0.007				
lg MU	37	FIA	0.038	0.1	0.07		0.06		0.07		0.08		0.06		0.09		0.09					
lg MU	9	Hotplate	0.005	0.04	0.04	0.041	0.04	0.042	0.04	0.041	0.04	0.043	0.04	0.043	0.04	0.041	0.04	0.042				
lg MU	27	Hotplate	0.130	0.2	0.16	0.057	0.20	0.060	0.25	0.063	0.23	0.062	0.27	0.064	0.20	0.060	0.17	0.058				
lg MU	32	TNT (Test N' Tube)	0.054	0.5	0.55	0.324	0.48	0.290	0.46	0.279	0.49	0.296	0.50	0.299	0.55	0.323	0.48	0.291				
MU	33	Autoclave	0.002	0.05	0.06	0.030	0.06	0.030	0.06	0.031	0.06	0.030	0.06	0.030	0.06	0.031	0.06	0.031				
MU	1	Autoclave	0.005	0.025	0.03		0.03		0.03		0.03		0.02		0.03		0.03					
MU	52	Autoclave	0.005	0.015	0.01	0.006	0.01	0.006	0.00	0.005	0.00	0.005	0.00	0.007	0.01	0.006	0.01	0.004	0.01	0.006		
MU	74	Autoclave	0.0054	0.05	0.041		0.040		0.042		0.039		0.043		0.044		0.042					

Appendix B: Survey LOD Replicate Data (2 of 3)

Type	#	Technique	(mg/L)	(mg/L)																			
MU	**80**	Autoclave	0.0058	0.05	0.05	0.021	0.06	0.022	0.05	0.021	0.06	0.022	0.06	0.022	0.05	0.021	0.06	0.023	0.06	0.022
MU	**97**	Autoclave	0.00696	0.1	0.09	0.062	0.09	0.065	0.09	0.066	0.10	0.068	0.10	0.068	0.10	0.068	0.10	0.067		
MU	**90**	Autoclave	0.007	0.15	0.16		0.16		0.16		0.15		0.15		0.16		0.16			
MU	**100**	Autoclave	0.0079	0.02	0.02	0.011	0.02	0.014	0.02	0.014	0.02	0.015	0.03	0.016	0.02	0.014	0.02	0.014	0.02	0.015
MU	**16**	Autoclave	0.008	0.05		0.031		0.029		0.030		0.028		0.028		0.030		0.029		0.020
MU	**26**	Autoclave	0.010	0.1	0.11	0.080	0.11	0.080	0.10	0.075	0.11	0.080	0.11	0.080	0.10	0.075	0.11	0.080		
MU	**70**	Autoclave	0.01	0.1	0.10	0.036	0.10	0.038	0.10	0.036	0.10	0.037	0.11	0.040	0.11	0.039	0.11	0.040		
MU	**62**	Autoclave	0.011	0.1	0.10	0.076	0.09	0.071	0.09	0.071	0.10	0.073	0.10	0.076	0.09	0.070	0.10	0.072		
MU	**31**	Autoclave	0.013	0.1	0.09	0.073	0.09	0.074	0.09	0.071	0.10	0.077	0.10	0.078	0.10	0.077	0.10	0.079		
MU	**50**	Autoclave	0.013	0.2	0.20		0.21		0.21		0.20		0.20		0.20		0.20			
MU	**86**	Autoclave	0.017	0.05	0.04	0.032	0.05	0.042	0.06	0.044	0.06	0.042	0.05	0.043	0.06	0.042	0.06	0.042		
MU	**63**	Autoclave	0.018	0.1	0.10	0.084	0.10	0.081	0.10	0.083	0.10	0.084	0.09	0.077	0.09	0.074	0.09	0.077	0.09	0.075
MU	**61**	Autoclave	0.01924	0.1	0.07	0.037	0.07	0.040	0.07	0.037	0.07	0.035	0.07	0.040	0.07	0.035	0.07	0.038		
MU	**40**	Autoclave	0.020	0.03	0.02	0.022	0.03	0.025	0.03	0.025	0.03	0.028	0.03	0.029	0.04	0.036	0.04	0.033	0.04	0.031
MU	**68**	Autoclave	0.0227	0.15	0.14	0.112	0.14	0.107	0.15	0.116	0.16	0.124	0.15	0.114	0.14	0.111	0.14	0.112	0.12	0.117
MU	**42**	Autoclave	0.027	0.1	0.11	0.076	0.09	0.069	0.09	0.066	0.08	0.060	0.08	0.060	0.09	0.066	0.09	0.067	0.09	0.066
MU	**11**	Autoclave	0.036	0.2	0.22	0.180	0.18	0.148	0.20	0.162	0.19	0.156	0.18	0.148	0.19	0.156	0.20	0.158	0.19	0.153
MU	**81**	Autoclave	0.04	0.4	0.45		0.42		0.45		0.41		0.43		0.43		0.43			
MU	**12**	Autoclave	0.041	0.1	0.11	0.021	0.10	0.019	0.12	0.022	0.12	0.022	0.10	0.019	0.10	0.019	0.09	0.018	0.08	0.016
MU	**3**	Autoclave	0.045	0.24	0.22	0.071	0.24	0.079	0.244	0.080	0.27	0.088	0.241	0.079	0.23	0.075	0.23	0.076		
MU	**57**	Autoclave	0.046	0.2	0.21	0.040	0.18	0.036	0.19	0.037	0.19	0.037	0.22	0.041	0.18	0.036	0.20	0.039		
MU	**5**	Autoclave	0.054	0.18	0.18	0.176	0.17	0.159	0.19	0.178	0.17	0.163	0.18	0.174	0.21	0.199	0.16	0.153		
MU	**77**	Autoclave	0.079	0.5	0.36		0.37		0.38		0.38		0.37		0.37		0.37		0.38	
MU	**4**	Hotplate	0.003	0.1	0.10	0.070	0.10	0.071	0.10	0.070	0.10	0.069	0.10	0.069	0.10	0.069	0.10	0.068		
MU	**22**	Hotplate	0.006	0.15	0.02	0.016	0.02	0.017	0.02	0.017	0.02	0.018	0.02	0.019	0.02	0.019	0.03	0.021	0.02	0.018
MU	**67**	Hotplate	0.0057	0.1	0.12	0.094	0.11	0.093	0.11	0.092	0.12	0.096	0.12	0.095	0.11	0.093	0.12	0.094		
MU	**64**	Hotplate	0.006	0.05	0.05		0.05		0.05		0.05		0.05		0.05		0.05			
MU	**30**	Hotplate	0.009	0.05	0.06	0.018	0.06	0.018	0.06	0.021	0.06	0.018	0.06	0.019	0.06	0.018	0.06	0.018		
MU	**92**	Hotplate	0.0175	0.1	0.09	0.167	0.09	0.160	0.09	0.166	0.09	0.162	0.10	0.178	0.10	0.182	0.09	0.158		
MU	**14**	Hotplate	0.020	0.1	0.12	0.098	0.11	0.090	0.11	0.092	0.11	0.088	0.11	0.085	0.11	0.088	0.10	0.080		
MU	**54**	Hotplate	0.020	0.1	0.11	0.069	0.12	0.075	0.12	0.076	0.12	0.073	0.11	0.071	0.12	0.076	0.12	0.077		
MU	**110**	Hotplate	0.0212	0.2	0.20		0.21		0.21		0.19		0.21		0.21		0.21			
MU	**29**	Hotplate	0.021	0.1	0.09	0.071	0.09	0.070	0.09	0.072	0.09	0.071	0.11	0.081	0.10	0.080	0.09	0.067	0.09	0.070
MU	**46**	Hotplate	0.024	0.2	0.22	0.061	0.21	0.060	0.23	0.063	0.23	0.064	0.21	0.059	0.21	0.060	0.21	0.059		
MU	**51**	Hotplate	0.024	0.1		0.116		0.123		0.106		0.112		0.118		0.101		0.123		0.107
MU	**101**	Hotplate	0.0292	0.1	0.11	0.083	0.10	0.079	0.12	0.088	0.08	0.065	0.09	0.071	0.11	0.080	0.11	0.081	0.11	0.084
MU	**69**	Hotplate	0.03	0.2	0.20		0.20		0.21		0.22		0.21		0.21		0.22			

Appendix B: Survey LOD Replicate Data (3 of 3)

			Question 1	Question 2	Question 3 (LOD data)															
					1		2		3		4		5		6		7		8	
Type	#	Technique	LOD (mg/L)	Spike (mg/L)	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs
MU	108	Hotplate	0.03	0.1	0.12	0.027	0.13	0.031	0.14	0.036	0.13	0.032	0.13	0.031	0.14	0.033	0.14	0.035		
MU	34	Hotplate	0.032	0.1	0.09		0.09		0.09		0.09		0.11		0.11		0.10			
MU	71	Hotplate	0.0346	0.05	0.05		0.06		0.07		0.07		0.08		0.08		0.06			
MU	107	Hotplate	0.035	0.1	0.09	0.056	0.08	0.054	0.10	0.064	0.11	0.072	0.10	0.061	0.09	0.060	0.08	0.051		
MU	17	Hotplate	0.036	0.1	0.06	0.049	0.06	0.049	0.06	0.045	0.05	0.037	0.06	0.045	0.06	0.043	0.06	0.046		
MU	91	Hotplate	0.036	0.1	0.12	0.202	0.09	0.142	0.09	0.164	0.13	0.218	0.11	0.195	0.12	0.207	0.11	0.166	0.10	0.148
MU	105	Hotplate	0.055	0.1	0.11	0.022	0.11	0.022	0.07	0.019	0.08	0.020	0.08	0.020	0.12	0.023	0.08	0.020		
MU	112	Hotplate	0.0763	0.5	0.45	0.186	0.46	0.190	0.48	0.198	0.53	0.217	0.48	0.199	0.48	0.197	0.47	0.196		
MU	45	Hotplate	0.082	0.1	0.11	0.036	0.11	0.036	0.09	0.028	0.10	0.031	0.10	0.032	0.10	0.032	0.11	0.034		
MU	104	Oven (250C)	0.018	0.1	0.10		0.10		0.10		0.10		0.11		0.10		0.10			
MU	95	TNT (Test N' Tube)	0.01	0.1	0.11	0.068	0.11	0.066	0.11	0.066	0.12	0.070	0.11	0.067	0.11	0.064	0.11	0.065		
MU	75	TNT (Test N' Tube)	0.0105	0.2	0.20	0.124	0.20	0.125	0.20	0.125	0.20	0.128	0.20	0.127	0.21	0.132	0.20	0.127		
MU	7	TNT (Test N' Tube)	0.014	0.2	0.20	0.122	0.20	0.125	0.19	0.119	0.20	0.122	0.20	0.125	0.19	0.120	0.20	0.124		
MU	28	TNT (Test N' Tube)	0.015	0.05	0.05	0.095	0.06	0.102	0.07	0.119	0.05	0.093	0.05	0.094	0.05	0.091	0.05	0.092	0.05	0.090
MU	35	TNT (Test N' Tube)	0.020	0.1	0.09	0.072	0.10	0.075	0.10	0.079	0.10	0.074	0.09	0.073	0.11	0.080	0.09			
MU	109	TNT (Test N' Tube)	0.02	0.2	0.17	0.052	0.15	0.048	0.16	0.051	0.16	0.050	0.17	0.053	0.16	0.050	0.17	0.052	0.16	0.049
MU	111	TNT (Test N' Tube)	0.02	0.7	0.71	0.403	0.72	0.407	0.71	0.404	0.71	0.402	0.72	0.409	0.71	0.402	0.70	0.397		
MU	13	TNT (Test N' Tube)	0.022	0.05	0.04	0.019	0.05	0.024	0.04	0.018	0.04	0.016	0.05	0.020	0.03	0.007	0.04	0.016		
MU	66	TNT (Test N' Tube)	0.022	0.2	0.20	0.058	0.20	0.060	0.20	0.058	0.21	0.062	0.19	0.056	0.19	0.056	0.20	0.058	0.20	0.060
MU	60	TNT (Test N' Tube)	0.025	0.1	0.08	0.078	0.08	0.077	0.09	0.082	0.07	0.072	0.09	0.079	0.09	0.082	0.07	0.072		
MU	58	TNT (Test N' Tube)	0.026	0.2	0.20	0.153	0.21	0.157	0.19	0.146	0.20	0.152	0.20	0.149	0.19	0.146	0.19	0.147		
MU	73	TNT (Test N' Tube)	0.027	0.1	0.11	0.112	0.10	0.107	0.10	0.108	0.11	0.112	0.11	0.116	0.12	0.120	0.12	0.121		
MU	15	TNT (Test N' Tube)	0.038	0.1	0.13	0.134	0.13	0.133	0.10	0.118	0.12	0.129	0.13	0.134	0.11	0.123	0.11	0.121		
MU	65	TNT (Test N' Tube)	0.0399	0.3	0.31	0.355	0.29	0.348	0.28	0.341	0.29	0.345	0.27	0.336	0.28	0.342	0.28	0.340		
MU	36	TNT (Test N' Tube)	0.040	0.2	0.20		0.18		0.20		0.21		0.20		0.19		0.18		0.17	
MU	87	TNT (Test N' Tube)	0.042	0.2	0.19	0.149	0.19	0.150	0.22	0.167	0.22	0.164	0.19	0.147	0.20	0.157	0.20	0.156		
MU	48	TNT (Test N' Tube)	0.043	0.2	0.22	0.136	0.23	0.144	0.22	0.134	0.24	0.149	0.23	0.141	0.21	0.132	0.21	0.128		
MU	18	TNT (Test N' Tube)	0.044	0.1	0.10	0.087	0.09	0.081	0.09	0.083	0.10	0.088	0.09	0.082	0.08	0.079	0.06	0.069	0.06	0.067
MU	114	TNT (Test N' Tube)	0.044	0.1	0.09	0.057	0.08	0.050	0.06	0.039	0.06	0.039	0.06	0.042	0.05	0.037	0.06	0.042		
MU	8	TNT (Test N' Tube)	0.046	0.2	0.23	0.131	0.20	0.119	0.22	0.127	0.19	0.110	0.22	0.128	0.20	0.117	0.22	0.126		
MU	106	TNT (Test N' Tube)	0.047	0.1	0.09	0.029	0.11	0.037	0.13	0.041	0.09	0.030	0.12	0.037	0.12	0.038	0.12	0.037		
MU	96	TNT (Test N' Tube)	0.048	0.1	0.13	0.105	0.12	0.103	0.11	0.095	0.13	0.108	0.10	0.089	0.14	0.110	0.11	0.093		
MU	72	TNT (Test N' Tube)	0.0612	0.2	0.20	0.106	0.17	0.090	0.18	0.097	0.22	0.116	0.21	0.114	0.18	0.095	0.18	0.099		
MU	55	TNT (Test N' Tube)	0.069	0.2	0.22	0.158	0.17	0.131	0.22	0.157	0.21	0.147	0.24	0.167	0.22	0.153	0.23	0.159		
MU	47	TNT (Test N' Tube)	0.073	0.2	0.20		0.23		0.20		0.26		0.23		0.25		0.21			
MU	6	TNT (Test N' Tube)	0.093	0.2	0.21		0.25		0.184		0.21		0.192		0.234		0.159			
PH	25	F/A	0.001	0.005	0.01	0.501	0.01	0.457	0.01	0.465	0.01	0.481	0.01	0.476	0.01	0.495	0.01	0.494		
PH	79	F/A	0.0088	0.025	0.03	0.096	0.03	0.079	0.03	0.106	0.03	0.097	0.03	0.102	0.03	0.085	0.03	0.087	0.02	0.077

Appendix C: Survey Lab Recent Blank Data (1 of 3)

				Question 1		Blank AVG	Question 5 (blank data)												Blank AVG
			LOD				1		2		3		4		5		6		7		
Type	#	Technique	(mg/L)	mg/L	abs	mg/L	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	abs
CO	93	Discrete Analyzer	0.01	0.01	0.004	0.008	-0.002	0.004	0.011	0.001	0.012	0.001	0.012	0.001	0.015	0.001	0.012	0.001	0.013	0.001	-0.001
CO	102	Discrete Analyzer	0.09	-0.04	0.004	-0.060	-0.004	0.004	0.020	0.001	-0.050	0.005	-0.060	0.004	-0.060	0.005	0.030	0.002	-0.070	0.005	
CO	98	Discrete Analyzer	0.20	-0.07	0.007	-0.043	0.000	0.004	-0.102	0.004	-0.005	0.001	-0.070	0.004	-0.063	0.003	-0.110	0.005	-0.087	0.001	0.083
CO	19	FIA	0.003	0.002	0.002	0.000	0.071	0.005	0.123	0.002	0.091	0.001	0.081	0.003	0.101	0.001	0.064	0.000	0.053		
CO	43	FIA	0.005	-0.01	0.005	-0.037	0.095	0.023	0.136	0.025	0.119	0.025	0.080	0.034	0.105	0.005	0.078	0.028	0.104		
CO	94	FIA	0.01	0.003	0.003	0.002	0.070	0.001	0.071	0.001	0.033	0.003	0.094	0.004	0.070	0.004	0.065	0.004	0.069	0.027	
CO	76	FIA	0.08																		
CO	99	Hotplate	0.01				0.007		0.008		0.000		0.009		0.003		0.003		0.010	0.006	
CO	39	Hotplate	0.02	-0.01	0.009	-0.016	0.001	0.007	0.002	0.005	0.002	0.007	0.000	0.014	0.000	0.007	0.002	0.007	0.002	0.001	
CO	113	Hotplate	0.02	0.003	0.009			0.006		0.007		0.001		0.003		0.000		0.001			
CO	23	TNT (Test N' Tube)	0.02	-0.01	0.008	-0.008	0.022	0.004	0.024	0.021	0.015	0.030	0.010	0.038	0.006	0.002	0.025	0.007	0.030	0.019	
CO	78	TNT (Test N' Tube)	0.02	-0.003	0.003	-0.010	0.049	0.005	0.052	0.012	0.048	0.007	0.051	0.006	0.058	0.015	0.063	0.007	0.051	0.053	
IN	38	Autoclave	0.01	0.002	0.000	0.000	0.000	0.000	0.005	0.008	0.010	0.000	0.000	0.000	0.005	0.008	0.010	0.000	0.005	0.005	
IN	88	Hotplate	0.01	0.000	0.000	-0.001	0.004	0.004	0.002	0.001	0.002	0.002	0.003	0.005	0.002	0.005	0.002			0.003	
IN	84	Hotplate	0.03	0.005	0.005	0.006	0.004	0.002	0.004	0.007	0.004	0.004	0.002	0.004	0.003	0.005	0.003	0.005	0.003	0.003	
IN	20	Hotplate	0.03	0.002	0.002	0.001	0.011	0.001	0.010	0.002	0.012	0.001	0.011	0.004	0.016	0.004	0.016	0.001	0.011	0.012	
IN	41	TNT (Test N' Tube)	0.02	-0.01	-0.024	0.009	-0.005	0.019	-0.016	0.013	-0.003	0.020	-0.022	0.010	-0.007	0.018	-0.011	0.016	0.015		
IN	21	TNT (Test N' Tube)	0.04	0.02	0.022	0.026	0.019	0.019	0.015	0.012	0.029	0.038	0.023	0.028	0.019	0.020	0.017	0.023			
IN	85	TNT (Test N' Tube)	0.05	0.03	0.026	0.027	0.052	0.048	0.019	0.029	0.030	0.038	0.013	0.026	0.038	0.040	0.045	0.044	0.036		
IN	89	TNT (Test N' Tube)	0.07			0.025		0.016		0.032		0.028		0.026		0.036			0.027		
IN	82	TNT (Test N' Tube)	0.07	0.03	0.019	0.012	0.046	0.020	0.002	0.007	0.046	0.020	0.014	0.002	0.029	0.015	0.069	0.027	0.015		
IN	103		0.01	-0.003	-0.007	-0.001	-0.005	0.000	-0.002	0.002	0.000	0.003	-0.005	0.000	0.000	0.003	-0.004	0.001	0.001		
lg MU	56	Autoclave	0.004	0.000	0.000	0.000	0.002	0.002	0.000	0.002	0.000	0.002	0.001	0.003	-0.002	0.001	-0.002	0.001	0.001	0.002	
lg MU	44	Autoclave	0.005	-0.003	0.000	0.003	-0.004	0.001	-0.004	0.001	-0.002	0.002	-0.004	0.001	-0.002	0.002	-0.004	0.001	0.002	0.002	
lg MU	59	Autoclave	0.02	-0.01	-0.005	0.012	-0.022	0.001	-0.022	0.001	-0.009	0.009	-0.006	0.011	-0.005	0.012			0.008		
lg MU	49	Autoclave	0.02	-0.06	-0.060	0.000	-0.060	0.001	-0.060	0.001	-0.060	0.001	-0.060	0.001	-0.060	0.001	-0.050	0.002	0.001		
lg MU	10	Autoclave	0.05	0.01	0.020	0.001	0.020	0.001	0.020	0.001	0.020	0.001	-0.001	-0.001	-0.001	-0.001	0.001	0.000			
lg MU	83	Discrete Analyzer	0.01	-0.01	-0.010	0.005	-0.017	0.003	-0.012	0.006	-0.015	0.006	-0.016	0.006	0.003	0.006	-0.009	0.002	0.005		
lg MU	53	FIA	0.02	0.01	0.014	0.001	0.004	0.000	-0.002	-0.001	0.007	0.000	0.032	0.002	0.004	0.001	0.010	0.001	0.001		
lg MU	37	FIA	0.04																		
lg MU	9	Hotplate	0.01	0.00	-0.008	0.001	-0.004	0.004	-0.009	0.000	-0.005	0.003	-0.009	0.000	0.008	-0.001	-0.005	0.003			
lg MU	27	Hotplate	0.13	0.000	0.000	0.002	0.000	0.003	0.000	-0.002	0.000	-0.001	0.000	-0.002	0.000	0.003			0.001		
lg MU	32	TNT (Test N' Tube)	0.05	-0.03	-0.050	0.027	-0.060	0.023	-0.030	0.040	-0.040	0.031	-0.030	0.038	0.020	0.061	-0.010	0.050	0.039		
MU	33	Autoclave	0.002	0.01	0.009	0.005	0.004	0.001	0.007	0.003	0.005	0.002	0.020	0.010	0.012	0.006	0.018	0.010	0.005		
MU	1	Autoclave	0.01	-0.001	0.006	0.005	0.000	0.001	-0.001	0.000	-0.001	0.000	0.000	-0.004	-0.006	-0.003	0.001	0.002	0.000		
MU	52	Autoclave	0.01	0.003	0.004	0.002	0.003	0.001	0.001	0.000	0.001	0.000	0.004	0.002	0.003	0.001	0.006	0.003			
MU	74	Autoclave	0.01		0.004	0.002	0.003	0.001	0.001	0.000	0.001	0.000	0.004	0.002	0.003	0.001	0.006	0.003			

Appendix C: Survey Lab Recent Blank Data (2 of 3)

				Blank AVG	Question 1	Question 5 (blank data)														Blank AVG
						1		2		3		4		5		6		7		
Type	#	Technique	LOD (mg/L)	mg/L	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	abs
MU	80	Autoclave	0.01	-0.002	0.003	0.005	-0.009	-0.004	0.000	0.003	0.001	0.002	0.001	0.004	-0.001	0.002	-0.011	0.011	0.003
MU	97	Autoclave	0.01																
MU	90	Autoclave	0.01	0.004	0.010	0.003	0.006	0.000	0.009	0.002	0.004	0.004	-0.003	0.001	-0.001	0.002	0.002	0.004	0.002
MU	100	Autoclave	0.01	0.000	0.000	-0.002	0.000	0.001	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
MU	16	Autoclave	0.01	0.000	0.000	0.002	0.000	0.002	0.000	0.001	0.000	0.002	0.000	0.000	0.000	0.001	0.000	0.002	0.001
MU	26	Autoclave	0.01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MU	70	Autoclave	0.01	0.000	0.000	0.001	0.000	0.003	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.002	0.001
MU	62	Autoclave	0.01	0.01	0.005	0.001	0.005	0.001	0.005	0.001	0.008	0.002	0.008	0.002	0.008	0.002	0.010	0.003	0.002
MU	31	Autoclave	0.01	0.004	0.008	0.005	0.003	0.001	0.003	0.001	0.004	0.002	0.004	0.002	0.002	0.000	0.006	0.003	
MU	50	Autoclave	0.01	0.003	0.003		0.004		0.003		0.002		0.004		0.002		0.002		
MU	86	Autoclave	0.02			0.486		0.493		0.490		0.487		0.484		0.479		0.518	0.491
MU	63	Autoclave	0.02	-0.01	-0.014	0.005	-0.012	0.006	-0.012	0.006	-0.014	0.005	-0.011	0.007	-0.010	0.008	-0.017	0.003	
MU	61	Autoclave	0.02	0.008	0.012	0.004	0.007	0.002	0.007	0.002	0.007	0.002	0.004	0.001	0.015	0.005	0.009	0.003	0.003
MU	40	Autoclave	0.02	0.002	0.000	0.003	0.003	0.007	0.004	0.009	0.002	0.006	0.000	0.003	0.002	0.006	0.001	0.004	0.005
MU	68	Autoclave	0.02																
MU	42	Autoclave	0.03	-0.02	-0.023	0.001	-0.018	0.004	-0.021	0.002	-0.021	0.002	-0.019	0.003	-0.019	0.003	-0.014	0.005	0.003
MU	11	Autoclave	0.04	-0.003	-0.003	0.005	0.003	0.009	-0.001	0.006	-0.001	0.006	-0.007	0.002	-0.005	0.003	-0.008	0.001	0.005
MU	81	Autoclave	0.04	0.006	0.006														
MU	12	Autoclave	0.04	-0.004	0.000	0.004	-0.010	0.003	0.000	0.003	-0.010	0.002	-0.010	0.003	-0.010	0.002	0.010	0.005	0.003
MU	3	Autoclave	0.05	0.009	0.011		0.011		0.008		0.008		0.011		0.005		0.011		
MU	57	Autoclave	0.05	-0.083	-0.083	0.000	-0.083	0.000	-0.083	0.000	-0.083	0.000	-0.083	0.000	-0.083	0.000	-0.083	0.000	0.000
MU	5	Autoclave	0.05	0.007	0.001	0.012	0.009	0.028	0.002	0.014	0.017	0.042	0.004	0.018	0.008	0.029	0.005	0.021	0.023
MU	77	Autoclave	0.08																
MU	4	Hotplate	0.003																
MU	22	Hotplate	0.01	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.003	0.003	0.004	0.004	0.003	0.003	0.003	0.003	0.003
MU	67	Hotplate	0.01	0.000	0.000	0.007	0.000	0.003	0.000	0.004	0.000	0.003	0.000	0.003	0.000	0.005	0.000	0.003	0.004
MU	64	Hotplate	0.01	0.003	-0.002	0.000	-0.002	0.000	0.003	0.001	0.005	0.003	0.004	0.002	0.013	0.009	0.003	0.001	
MU	30	Hotplate	0.01			0.003		0.003		0.004		0.001		0.005		0.007		0.003	0.004
MU	92	Hotplate	0.02	0.01	0.000	0.007	0.010	0.015	0.030	0.054	0.010	0.018	0.000	0.004	0.000	0.000	0.010	0.010	0.015
MU	14	Hotplate	0.02	0.00	0.030	0.024	0.050	0.039	-0.040	0.001	-0.040	0.001	0.030	0.024	0.000	0.009	0.003	0.004	
MU	54	Hotplate	0.02	-0.004	-0.005	0.003	-0.003	0.004	-0.003	0.004	-0.011	0.005	-0.005	0.003	-0.001	0.002	-0.001	0.002	0.003
MU	110	Hotplate	0.02	-0.003	0.006	0.010	-0.009	-0.001	-0.005	0.002	0.000	0.005	-0.002	0.004	-0.006	0.001	-0.006	0.001	0.003
MU	29	Hotplate	0.02	-0.01	-0.016	0.002	-0.019	0.000	-0.018	0.001	-0.019	0.000	-0.016	0.002	-0.006	0.002	-0.006	0.002	0.001
MU	46	Hotplate	0.02	0.01	0.001	0.002	0.002	0.001	0.001	0.002	0.003	0.001	0.017	0.001	0.017	0.001	0.017	0.001	0.001
MU	51	Hotplate	0.02	-0.01	-0.012	-0.011	-0.008	-0.004	-0.003	0.004	-0.004	0.002	-0.005	0.001	-0.004	0.002	-0.001	0.007	0.000
MU	101	Hotplate	0.03	0.001	0.003	0.006	0.003	0.008	0.003	0.006	0.000	0.004	0.003	0.006	-0.001	0.003	-0.007	-0.001	0.005
MU	69	Hotplate	0.03	0.01	0.012	0.008	0.015	0.010	0.008	0.005	0.004	0.003	0.008	0.005	0.008	0.005	0.019	0.013	0.007

Appendix C: Survey Lab Recent Blank Data (3 of 3)

Type	#	Technique	(mg/L)	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	mg/L	abs	abs
MU	108	Hotplate	0.03	0.01	0.010	0.001	0.000	0.001	0.010	0.002	0.010	0.001	0.000	0.001	0.010	0.002						
MU	34	Hotplate	0.03	0.000	0.000	0.002	0.000	-0.003	0.000	0.001	0.000	0.001	0.000	-0.001	0.000	0.002						0.000
MU	71	Hotplate	0.03	0.01	0.007	0.002	0.007	0.002	0.010	0.004	0.000	0.000	0.007	0.002	0.007	0.002						0.002
MU	107	Hotplate	0.04	0.02	0.025	0.006	0.028	0.008	0.026	0.007	0.025	0.006	0.022	0.004	0.020	0.003						0.006
MU	17	Hotplate	0.04	0.01	0.020	0.018	0.024	0.021	0.016	0.015	0.010	0.011	0.005	0.007	0.002	0.002						0.012
MU	91	Hotplate	0.04	0.01	-0.001	0.024	-0.006	0.016	0.005	0.033	0.017	0.051	0.022	0.059	0.018	0.053						0.043
MU	105	Hotplate	0.06	-0.05	-0.049	0.009	-0.073	0.007	-0.073	0.007	-0.037	0.010	-0.049	0.009	-0.037	0.010						0.009
MU	112	Hotplate	0.08	0.01	0.013	0.009																0.009
MU	45	Hotplate	0.08	0.01	0.024	0.009	0.010	0.005	0.010	0.005	0.010	0.005	0.010	0.005	0.010	0.014	0.006					0.006
MU	104	Oven (250C)	0.02	0.01	0.005		0.000		0.010		0.008		0.010		0.010							
MU	95	TNT (Test N' Tube)	0.01	0.002	0.006	0.010	-0.003	0.005	0.006	0.010	-0.005	0.004	0.003	0.008	0.004	0.009	0.003	0.008				0.008
MU	75	TNT (Test N' Tube)	0.01	-0.004	-0.001	0.014	-0.001	0.014	-0.008	0.010	-0.004	0.012	0.000	0.015	-0.009	0.010	-0.003	0.013				0.013
MU	7	TNT (Test N' Tube)	0.01	-0.01	-0.011	0.018	-0.009	0.019	-0.007	0.020	-0.015	0.016	-0.017	0.015	-0.017	0.015	0.008	0.028				0.019
MU	28	TNT (Test N' Tube)	0.02	-0.002	-0.002		-0.002		-0.006		-0.002		-0.001		-0.002		0.003					
MU	35	TNT (Test N' Tube)	0.02	0.02	0.028	0.036	0.024	0.034	0.044	0.044	0.021	0.032	0.007	0.025	-0.007	0.027	-0.003	0.029				0.032
MU	109	TNT (Test N' Tube)	0.02	0.02	0.020	0.007	0.020	0.006	0.000	0.000	0.010	0.004	0.020	0.006	0.030	0.008	0.020	0.006				0.005
MU	111	TNT (Test N' Tube)	0.02	-0.002	-0.006	0.011	-0.004	0.012	0.000	0.014	0.004	0.016	-0.006	0.011	0.004	0.016	-0.004	0.012				0.013
MU	13	TNT (Test N' Tube)	0.02	0.01	0.000	0.000	0.010	-0.007	0.000	0.000	0.010	0.005	0.000	0.000	0.040	0.015	0.000	0.000				0.002
MU	66	TNT (Test N' Tube)	0.02	0.02	0.025	0.005	0.003	0.011	0.021	0.006	0.025	0.005	0.018	0.007	0.028	0.004	0.036	0.002				0.006
MU	60	TNT (Test N' Tube)	0.03	-0.03	-0.036	0.017	0.011	0.040	-0.026	0.020	-0.028	0.019	-0.047	0.009	-0.036	0.015	-0.042	0.012				0.019
MU	58	TNT (Test N' Tube)	0.03	-0.01	-0.040	0.029	-0.050	0.024	0.000	0.049	0.030	0.067	-0.010	0.047	0.010	0.043	0.010	0.047				0.044
MU	73	TNT (Test N' Tube)	0.03	-0.02	-0.027	0.021	-0.037	0.016	-0.039	0.015	-0.040	0.014	-0.018	0.026	-0.004	0.033	0.009	0.040				0.024
MU	15	TNT (Test N' Tube)	0.04	0.24	0.191	0.269	0.265	0.368	0.230	0.299	0.200	0.239	0.281	0.400	0.246	0.331	0.245	0.329				0.319
MU	65	TNT (Test N' Tube)	0.04																			
MU	36	TNT (Test N' Tube)	0.04	0.07	0.063	0.040	0.182	0.133	0.090	0.053	0.061	0.039	0.042	0.061	0.004	0.037	0.046	0.062				0.061
MU	87	TNT (Test N' Tube)	0.04	0.03	0.042		0.023		0.013		0.019		0.038		0.045		0.040					
MU	48	TNT (Test N' Tube)	0.04	0.03	0.036	0.020	0.040	0.022	0.036	0.020	0.027	0.015	0.030	0.017	0.030	0.017	0.023	0.013				0.018
MU	18	TNT (Test N' Tube)	0.04			0.079		0.090		0.093		0.092		0.078		0.084		0.090				0.087
MU	114	TNT (Test N' Tube)	0.04	0.000	0.000	-0.032	0.000	-0.051	0.000	-0.025	0.000	-0.025	0.000	-0.041	0.000	-0.028	0.000	-0.038				-0.034
MU	8	TNT (Test N' Tube)	0.05	0.01	0.000	0.008	0.010	0.023	0.010	0.024	0.000	0.016	0.000	0.004	0.010	0.015	0.010	0.014				0.015
MU	106	TNT (Test N' Tube)	0.05	0.01	0.028	0.011	0.018	0.008	0.028	0.011	0.003	0.004	0.011	0.006	0.007	0.005	0.000	0.003				0.007
MU	96	TNT (Test N' Tube)	0.05	-0.02	-0.009	0.033	-0.025	0.025	-0.021	0.027	-0.009	0.033	-0.011	0.033	-0.022	0.027	-0.019	0.029				0.030
MU	72	TNT (Test N' Tube)	0.06	0.02	0.022	0.017	0.016	0.014	0.008	0.010	0.012	0.012	0.018	0.015	0.018	0.015	0.020	0.016				0.014
MU	55	TNT (Test N' Tube)	0.07	-0.01	0.005	0.044	-0.036	0.023	-0.039	0.022	-0.020	0.031	0.035	0.040	-0.027	0.008	0.004	0.024				0.027
MU	47	TNT (Test N' Tube)	0.07	0.01	0.009		0.012		0.009		0.013		0.007		0.008		0.010					
MU	6	TNT (Test N' Tube)	0.09	-0.02	-0.017	0.067																0.067
PH	25	FIA	0.001	0.001	0.000	0.129	0.000	0.134	0.000	0.141	0.000	0.141	0.001	0.160	0.001	0.196	0.001	0.177				0.154
PH	79	FIA	0.01	-0.02	0.006	0.080	-0.030	-0.053	-0.036	-0.109	-0.039	-0.117	-0.017	-0.058	-0.015	-0.054	-0.021	-0.070				-0.054

Appendix D: Single Cuvette Study Data*

Note: "TNT" = Test 'N Tube ™

Calibration Data

Abs read in TNT tube (13 mm)

mg/L	Abs @ 880 nm	RF
0	0.009	
0.1	0.067	*0.670*
0.2	0.125	*0.625*
0.4	0.234	*0.585*
0.6	0.348	*0.580*
0.8	0.448	*0.560*
1	0.560	*0.560*

slope=	0.5487
intercept=	0.013
correlation	0.99985

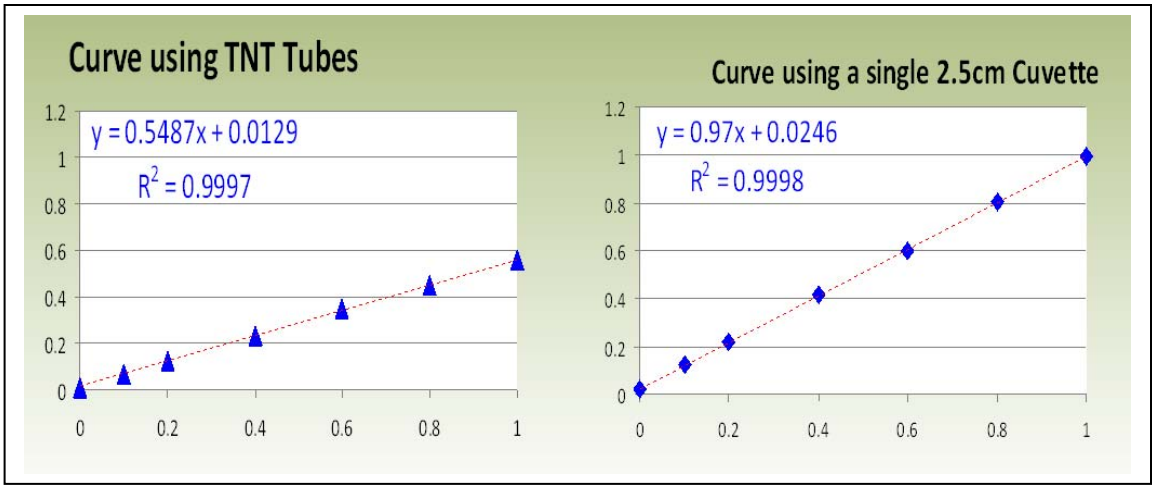
NOTE: "RF" = "Response Factor" = Response ÷ Concentration

Abs read in 25 mm Cuvette

mg/L	Abs @ 880 nm	RF
0	0.022	
0.1	0.127	*1.270*
0.2	0.217	*1.085*
0.4	0.416	*1.040*
0.6	0.598	*0.997*
0.8	0.802	*1.003*
1	0.997	*0.997*

slope=	0.9700
intercept=	0.025
correlation	0.99992

NOTE: "RF" = "Response Factor" = Response ÷ Concentration



LOD Data

Abs read in TNT tube (13 mm)

LOD replicates (0.1 mg/L)

0.099	0.115
0.121	0.095
0.123	0.115
0.100	0.125
0.102	0.120
0.111	

Mean= **0.111**
Range: **0.095 to 0.125** (0.030)
Std Deviation= **0.01074**
RSD= **9.7%**
LOD= 0.030 mg/L

Abs read in 25 mm Cuvette

LOD replicates (0.1 mg/L)

0.090	0.095
0.090	0.097
0.092	0.088
0.085	0.095
0.089	0.096
0.099	

Mean= **0.092**
Range= **0.085 to 0.099** (0.014)
Std Deviation= **0.00439**
RSD= **4.8%**
LOD= 0.012 mg/L

* Data provided by the Wisconsin State Laboratory of Hygiene.

Science Services

Center for Excellence –
providing expertise for science-based decision-making

We develop and deliver science-based information, technologies, and applications to help people make well-informed decisions about natural resource management, conservation, and environmental protection.

Our Mission: The Bureau of Science Services works across agency divisions to support all Wisconsin Department of Natural Resources programs and their partners by:

- conducting applied research and acquiring original knowledge.
- analyzing new information and emerging technologies.
- synthesizing information for policy and management decisions.
- applying the scientific method to the solution of environmental and natural resources problems.
- providing science-based support services for management programs department-wide.
- collaborating with local, state, regional, and federal agencies and academic institutions in Wisconsin and around the world.
