The Phosphorus Rule - Impact on Lab Testing

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Phosphorus Rule Impacts

- **What's the issue?**
- **What have we done to date?**
  - Survey March 2011
  - Commission State Lab of Hygiene Study
  - Statistical Analysis
- **What problems do we face ?**
  - Contamination
  - Calibration concerns
  - Dealing with blanks
  - Determining an LOD correctly
- **What potential solutions exist?**
  - Major
  - Minor
- **Summary/recommendations**

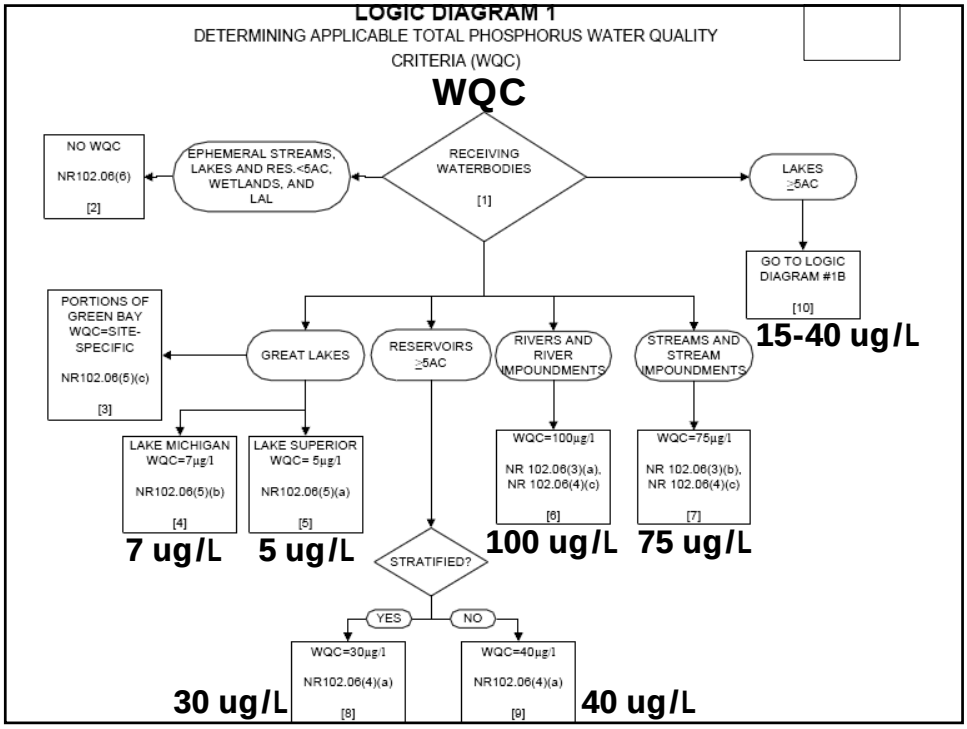
A turbulent year for Phosphorus…

Guidance for Implementing Wisconsin's Phosphorus Water Quality Standards for Point Source Discharges

August 25, 2011

DRAFT: FOR REVIEW ONLY

This guidance document was made available to key externals for comment. Comments should be submitted to the Department by September 30th at which point we will review and respond to comments, and edit the guidance document as appropriate.



Sampling and Testing Procedures

Sampling and laboratory testing procedures shall be performed in accordance with Chapters NR 218 and NR 219, Wis. Adm. Code and shall be performed by a laboratory certified or registered in accordance with the requirements of ch. NR 149, Wis. Adm. Code. The analytical methodologies used shall enable the laboratory to quantitate all substances for which monitoring is required at levels below the effluent limitation. Again, the Department recommends a level of detection at 30 ug/L and a level of quantitation at 90 ug/L. If the required level cannot be met by any of the methods available in NR 219, Wis. Adm. Code, then the method with the lowest limit of detection shall be selected. Additional test procedures may be specified in this permit.

LOD…Why Haven't We Cared?

Historically, permit limits for Total P have been 1.0 mg/L

∴ the highest an LOQ could be would be 1.0 mg/L

Generally, the LOQ is about 3.3 times the LOD

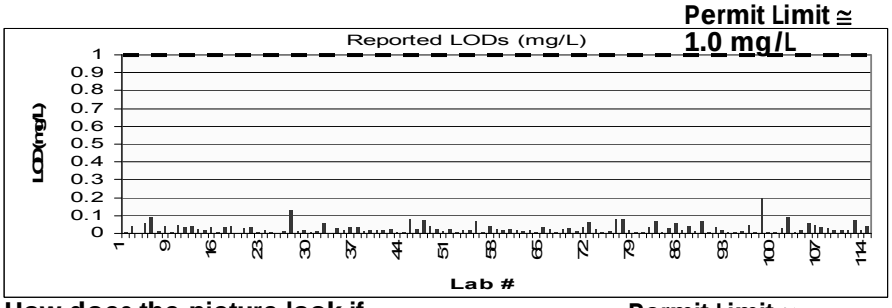
∴ the highest an LOD could be is about 0.3 mg/L

…and no lab has had trouble obtaining an LOD < 0.3 mg/L

Lab LODs < 0.3 ppm < 1.0 mg/L Permit Limit

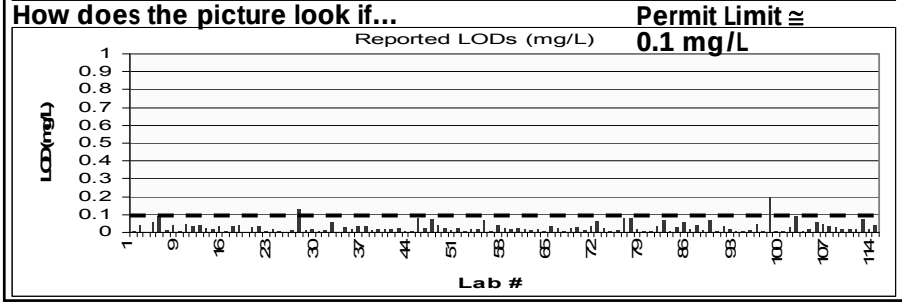
LODs Relative to Existing Permit Limits

Permit Limit ≅ 1.0 mg/L

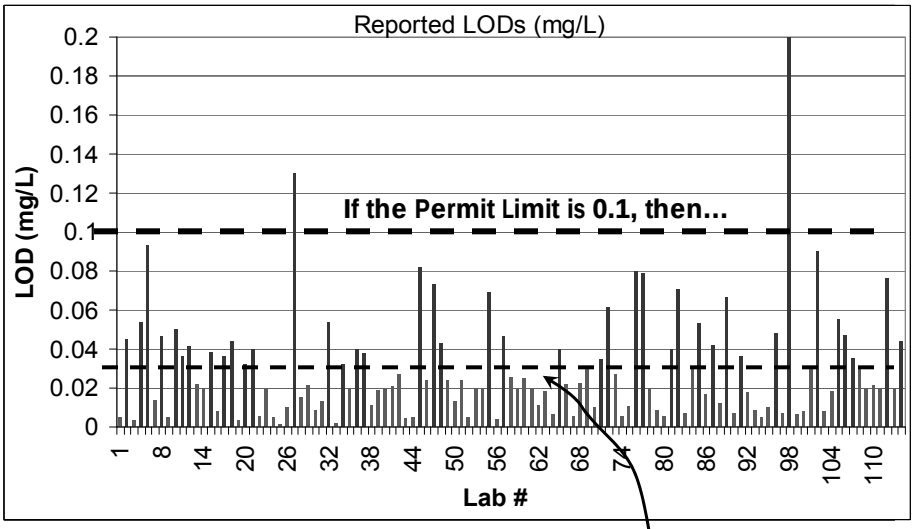


How does the picture look if…

Permit Limit ≅ 0.1 mg/L



Zooming in a little …



… and so we decided to conduct a little survey

Time to Revisit the LOD?

- Unless you've been hiding under a rock for the last year, you know the Department has enacted major revisions to Phosphorus rules.

- The new rules require LODs for Total P to be reported on DMRs and are a point of concern.
- During the winter of 2011 LabCert conducted a survey of Total P LODs.
- The results were disconcerting.

What did we learn?

Who's using what (all labs submitting results)?

	TNT	AC	HP	FIA	DA
Commercial	2	0	3	4	3
Public Health	-	-	-	2	-
Industrial	5	1	3	-	-
Large WWTP	1	5	2	2	1
small WWTP	26	27	23	-	-
	34	**33**	**31**	**8**	**4**

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

Ability to Meet A Specific Total Phosphorus LOD

Based on data as reported

	0.01 mg/L	0.02 mg/L	0.03 mg/L	
Commercial	1 of 11 labs	6 of 11	6 of 11	55%
Public Health	2 of 2	2 of 2	2 of 2	100%
Industrial	1 of 10	3 of 10	5 of 10	50%
Lg WWTP	2 of 11	7 of 11	7 of 11	64%
Sm WWTP	7 of 77	18 of 77	29 of 77	38%
Total	13 of 111	36 of 111	49 of 111	
	12%	**32%**	**44%**	

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 Meet Specific Total Phosphorus LOD By Technique

NOTE: These data are based on LODs reported on the survey

	# labs which could meet a specific LOD			
	0.01 mg/L	0.02 mg/L	0.03 mg/L	
Test N' Tube	3	13	15	(of 34)
Hot Plate	8	17	25	(of 31)
Autoclave	17	24	25	(of 33)
Flow Injection	5	6	6	(of 8)
Discrete Analyzer	2	2	2	(of 4)
Total	**35**	**62**	**73**	**(of 111)**

*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.*

Ability to Meet Specific Total Phosphorus LOD By Technique

NOTE: These data are based on adjusted "realistic" LODs

	0.01 mg/L	0.02 mg/L	0.03 mg/L	
Test N' Tube	0 labs	2 labs	2 labs	**(of 25)**
Hot Plate	1 lab	8 labs	17 labs	**(of 28)**
Autoclave	8 labs	16 labs	20 labs	**(of 26)**
Flow Injection	3 labs	6 labs	6 labs	**(of 8)**
Discrete Analyzer	0 labs	2 labs	2 labs	**(of 3)**
Total	12 labs	34 labs	47 labs	**(of 90)**

*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.*

Quality of Total Phosphorus LOD Data

Surveyed: 173 labs
Responses: 112
Return rate: 65%

	Valid LOD 47 (42%)	Questionable LOD 47 (42%)	Invalid LOD 18 (16%)
Commercial	6 of 12 labs	3 of 12 labs	3 of 12 labs
Public Health	2 of 2 labs	----------	----------
Industrial	3 of 10 labs	6 of 10 labs	1 of 10 labs
Lg WWTP	4 of 11 labs	6 of 11 labs	1 of 11 labs
Sm WWTP	32 of 77 labs	32 of 77 labs	13 of 77 labs

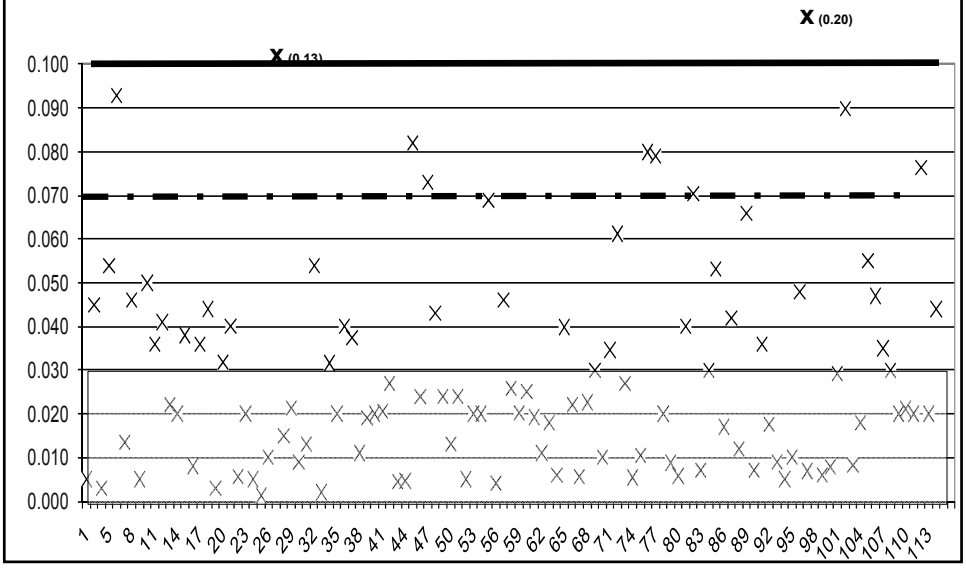
Valid LOD: *defined here as meeting the EPA's required acceptance criteria*

Invalid LOD: *defined here as unacceptable due to failure to meet one or more of the EPA's required acceptance criteria*

Questionable LOD: *defined here as one that meets the EPA's designated acceptance criteria, but that LOD reported cannot be substantiated when reviewed against blank data as being "significantly different" from a blank. It is possible for us to make a decision regarding what level of LOD can be supported.*

More Than 1 in 3 Can't Meet New LOD Requirements

Raw Data: 41 labs (37%) have LODs outside of 0.02 to 0.03 ppm



Tell Me Whyyyyyyy...

- ...so many labs are having trouble with determining an LOD?
- ...so many labs cannot achieve the required LOD of 0.03 ppm?
- ...Test 'N Tube (TNT) seems to be the most challenging?

Problem # 1:

Test 'N Tube is the most popular method yet less than 10% of "TNT" labs can meet the required LODs. Really?.....

Are LODs obtained using Test 'N Tube really different from other methods?

Solving Problem # 1:

To answer this, we need to do a little statistics.

Statistics 101

"Statistics show:
every two minutes
another statistic is created."
-Anonymous

Some statistical terms to know and love

- **The "Student's" t-test:** one of the most commonly used techniques for testing a hypothesis on the basis of a difference between sample means.
- **Null hypothesis:** generally represents the default position in a t-test. "Null" mean "no", so the null hypothesis often is that there is no relationship (difference) between two measurements.
- **t-value**: the score obtained from a t-Test. It represents the difference between the means of two groups, while taking into account any variation in measurements.
- **p-value:** the probability of obtaining a test statistic at least as extreme as the one calculated, assuming that the null hypothesis is true. Usually one "rejects the null hypothesis" when the p-value is less than the significance level α which is often 0.05 or 0.01. Rejecting the null hypothesis means the result is statistically significant.

Probability in 30 seconds

- You can flip a coin 10 times and get "heads" each of the 10 times.
- But there is a probability associated with that.
- The probability in this case is 1 in 1024 "sets" of 10 coin flips.
- Or about 0.1% likelihood that any single set of 10 coin flips will produce heads each time.
- In our world, p= 0.001

t-values and statistical significance

t-Test Values Required to Reject the Null Hypthothesis (H_0).

H_0= There is no difference between LODs of two techniques.

(df)	α=.05	α=.01
20	2.09	2.85
25	2.06	2.79
30	2.04	2.75
35	2.03	2.72
40	2.02	2.71
45	2.01	2.70
50	2.01	2.68
55	2.00	2.67
60	2.00	2.66
65	2.00	2.66
70	2.00	2.65
75	1.99	2.64
100	1.98	2.63
∞	1.96	2.58

If t > 2.00 then we can "reject H_0" and declare the LODs to be significantly different with less than a 5% chance of making the wrong decision.

If t > 2.70 then we can "reject H_0" and declare the LODs to be significantly different with less than a 1% chance of making the wrong decision.

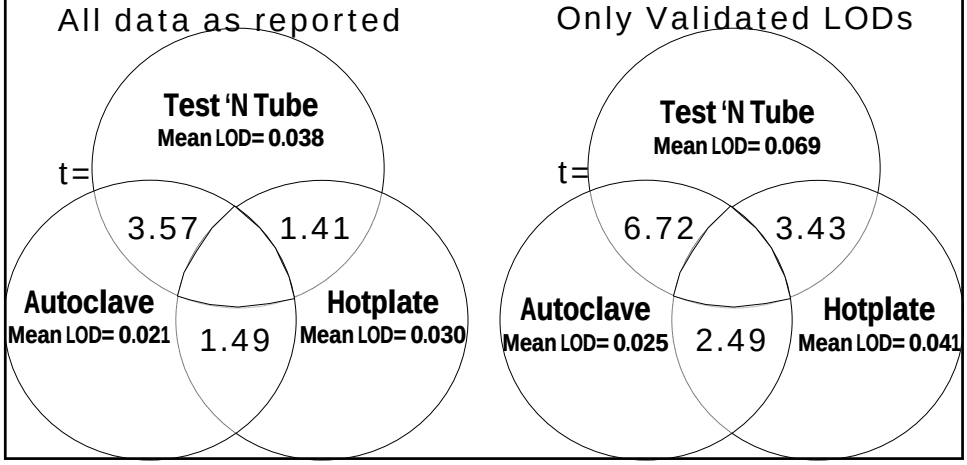
Minding your p's and t's

- You compare a t-value to a table to determine whether or not your difference is significant at a given level of confidence…usually 95% (0.05) or 99% (0.01).
- A p-value gives you the absolute level of confidence at which the difference is (statistically) significant.
- So…if t-value is 2.5 and the t-table criterion for the degrees of freedom is 2.0 at α=0.05, then the difference is significant at the 95% confidence level.
- A p-value of 0.0008 means there is a 0.08% chance that you could be making the wrong assessment.
- So….if p < 0.05 there is technically less than a 5% chance of making an incorrect assessment.

Student's t-values for t-test of means

Answers: Are these LODs significantly different?

A t-value greater than 2.00 means there is a significant difference between sample data at the 95% confidence level. "t" > 2.70 = 99% confidence



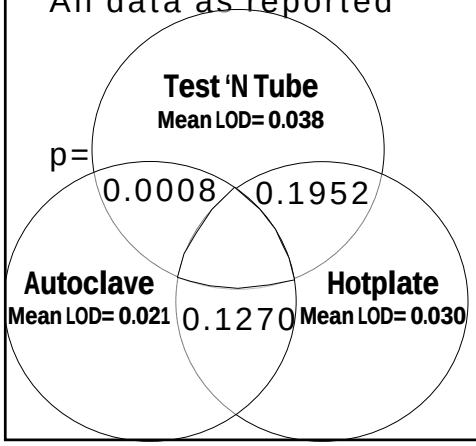
P-values: Are LODs significantly different?

"p" here represents the probability that the difference is coincidence

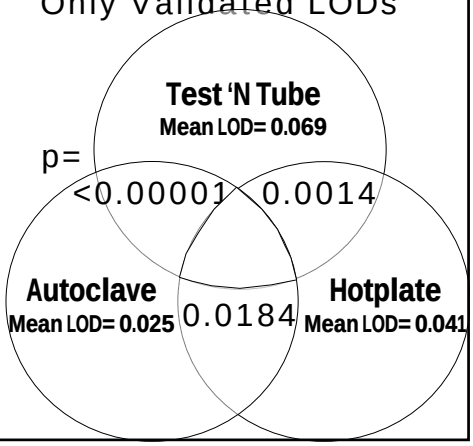
Random sampling of Test 'n Tube v. Autoclave would lead to a smaller difference in means in 0.08% of studies

Random sampling of Hotplate v. Autoclave would lead to a smaller difference in means in 1.84% of studies

All data as reported



Only Validated LODs



Bottom line on validated LODs

- LODs for the 3 main techniques are different
- You are most likely to meet new recommended LODs using Autoclave
- ...but there are reasons for that
- ...and our evaluation is based on the data received and our assessment of it

- Test N Tube **is** different
- **But we're not saying it's "no good"**
- We will be talking about ways you can improve the sensitivity of Test N Tube AND the other techniques

Problem # 2:
Labs are having trouble just determining an LOD correctly

Solving Problem # 2:
Back 2 Basics:

How to correctly determine an LOD

Revisiting Old Friends: LOD & LOQ

LOD

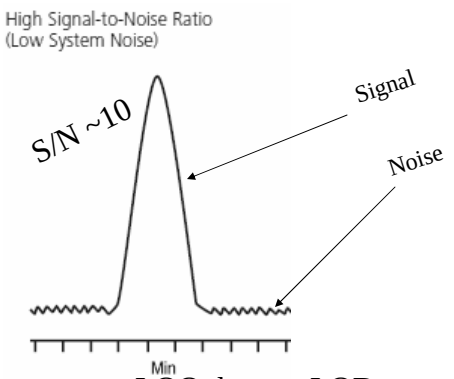
- ✓ "Limit of detection" or "LOD" means the lowest concentration or amount of analyte that can be identified, measured, and reported with confidence that the concentration is not a false positive value.
- ✓ For DNR purposes, the LOD approximates the MDL

LOQ

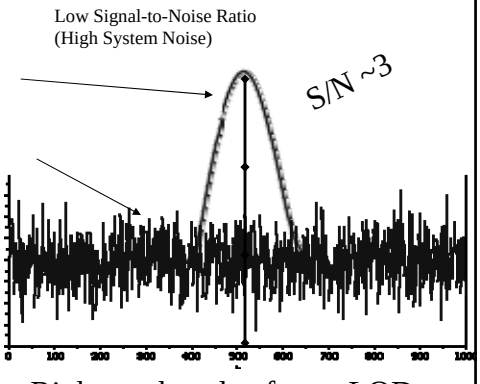
- ✓ "Limit of quantitation" of "LOQ" means the lowest concentration or amount of an analyte for which quantitative results can be obtained.
- ✓ Formerly defined in code as 10/3 times the LOD (i.e. 3.33 x LOD)
- ✓ NR 149 now requires labs to " *have procedures to relate the LOD to the LOQ* "

LOD is about Signal: Noise Ratio [S:N]

"Signal" =analyte response; "Noise" =blank response.
LOD typically viewed as 3:1 Signal: Noise (S:N)
LOQ is generally considered to be S:N of 10:1

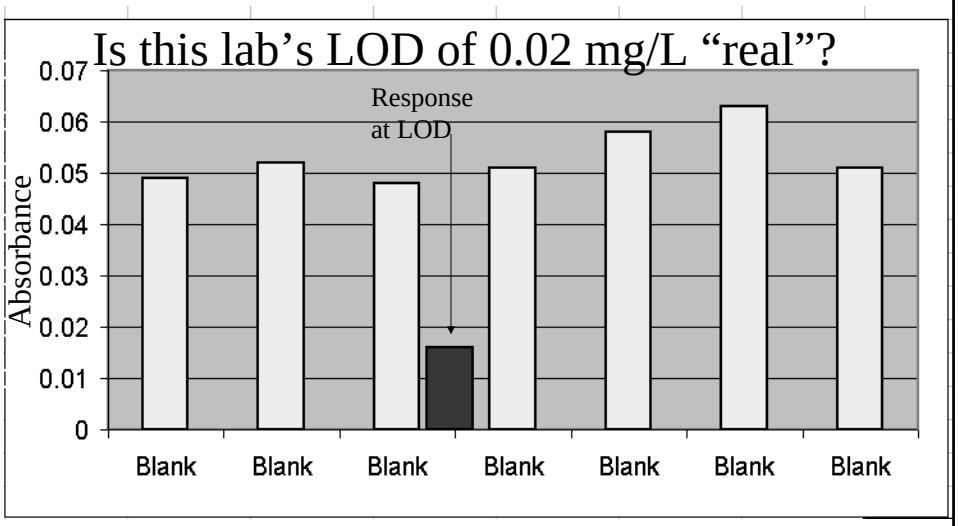


Closer to an LOQ than an LOD



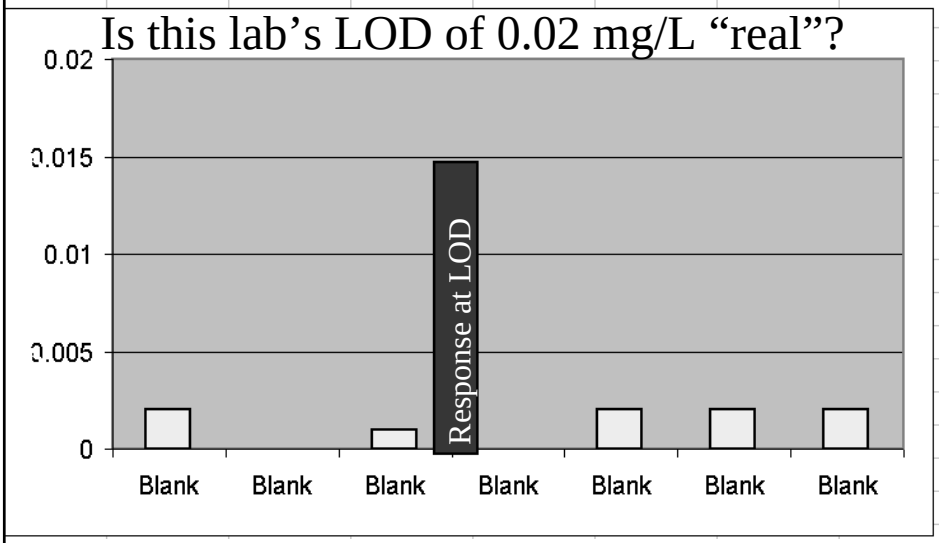
Right on the edge for an LOD

LOD-equivalent signal "in the weeds"



This lab submitted LOD results in which the theoretical response at the LOD level is well below that of blanks

LOD signal well above S/N ratio



What does a "good" LOD look like?

Which lab can substantiate an LOD of 0.02 ppm?

Lab# 29	**Lab# 78**
• Hotplate	• Test n' Tube
• Spike: 0.1 ppm	• Spike: 0.2 ppm
• Spike Abs: 0.073	• Spike Abs: 0.160
• Low std: 0.1 ppm	• Low std: 0.1 ppm
• Low std Abs: 0.076	• Low std Abs: 0.119
• LOD: 0.021 ppm	• LOD: 0.02 ppm
• LOD Equiv Abs: 0.016	• LOD Equiv Abs: 0.024
• Blank mg/L: -0.014 ppm	• Blank mg/L: -0.003 ppm
• Blank Abs: 0.0013	• Blank Abs : 0.0531

Equivalent absorbance calculated based on response factor from low standard or LOD spike. [0.076 Abs/0.1 ppm = 0.76 ABS/PPM x 0.021 ppm== 0.016

If your LOD is "in the weeds" it
really isn't realistic.
You can't really
"see" it

Let's review...

EPA procedure for determining LOD

1. Determine a spike concentration (*close to the expected LOD*)
2. Prepare at least 7 spiked replicates of reagent water at this spike level
3. Calculate the mean (X) and standard deviation (SD)
4. Obtain the "t"-value associated with the number of replicates
5. Calculate the LOD: SD times t
6. Perform "5-point check" of the LOD

LOD Evaluation: The 5 + 1-point check

Mandatory checks (EPA)

1. **Is LOD greater than 10% of the spike level?**
   If you spike LOD replicates at 0.1, LOD must be no less than 0.01
   Otherwise, re-peat at lower spike level
2. **Is the spike level greater than the LOD?**
   Common sense: if LOD > spike level, couldn't detect it
3. **Is the LOD below any relevant permit limit?**
   TP Permit limits eventually likely to be = 0.075 to 0.10 mg/L

Additional *(strongly encouraged)* checks

4. **Is the signal-to-noise ratio (S/N) between 2.5 and 10?**
   S/N est = mean/std dev.
5. **Is mean recovery within reasonably expected limits?**
   Mean recovery= mean/spike level x 100 *Expect 80 to 120%*
6. **Is average response of blank < 3 times response at LOD**
   If not, your LOD is probably not "real"

LOD DOs and DON'Ts

- DO use reagent water
- DO follow the 5-point check
- DO repeat at a lower/higher level if needed
- DO compare LOD signal to blank signal
- DO ask your auditor about a "realistic" LOD

- DON'T use absorbance (response) to calculate.
- DON'T use less than 7 replicates
- DON'T ignore blank response relative to LOD

What does <u>YOUR</u> LOD "look" like?

- There is value in preparing a "standard" at a concentration equal to (or very close to) your calculated LOD ***<u>and</u>*** one at the LOQ (use 3x LOD).
- Compare the signal at your LOD (and LOQ) to a typical method blank.
- Is your LOD "in the weeds"? Your LOQ?
- If so you have two options:
  1. "Trim" the weeds (i.e., reduce background "noise")
  2. Raise your LOD until it rises clearly above the weeds.
  3. Re-set your LOQ

Problem # 3:
Labs will have trouble meeting the new LODs.

What else can we do to improve LODs?

Solving Problem # 3

Option 1: Purchase a fancy instrument

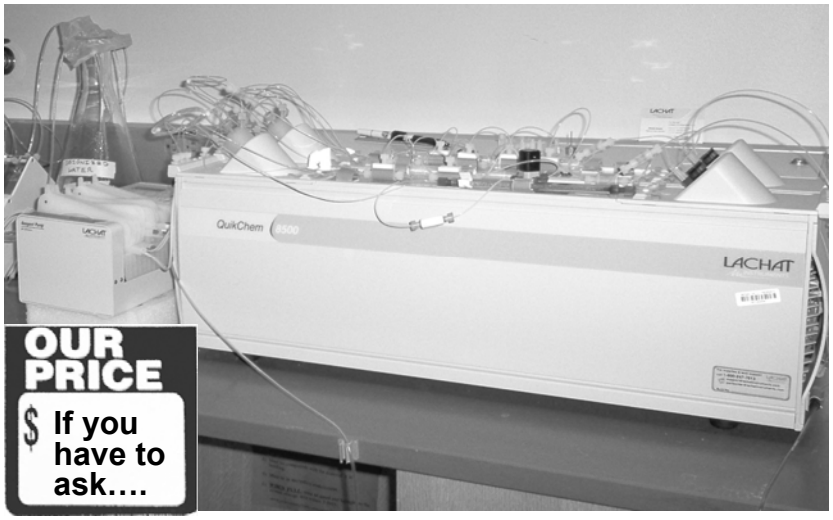
Option 2: Bench level changes

- **calibration errors WILL affect LOD**
- **know thine opponent**
- **proper care and feeding of cuvettes**
- **you're not re-using TNT vials…right?**

Option 3: Effecting real change

- **The State Lab helps prove a theory**
- **using the right cuvette**

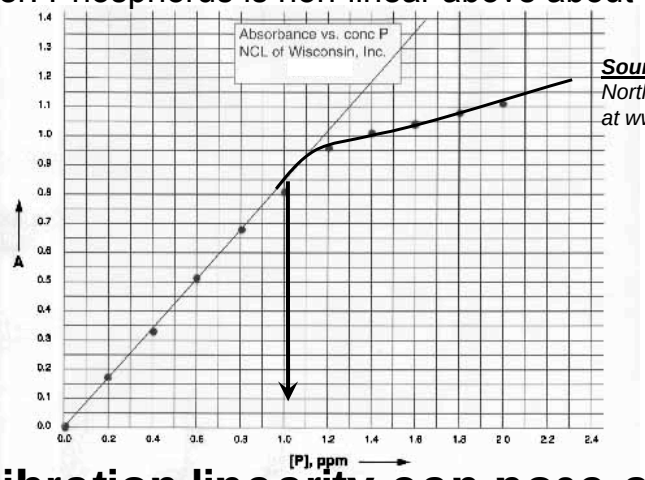
Option 1 : Purchase an FIA (flow injection analyzer) system



Downside: Cost may be a little too prohibitive for small labs

Option 2 : Look at what you're doing and make some adjustments

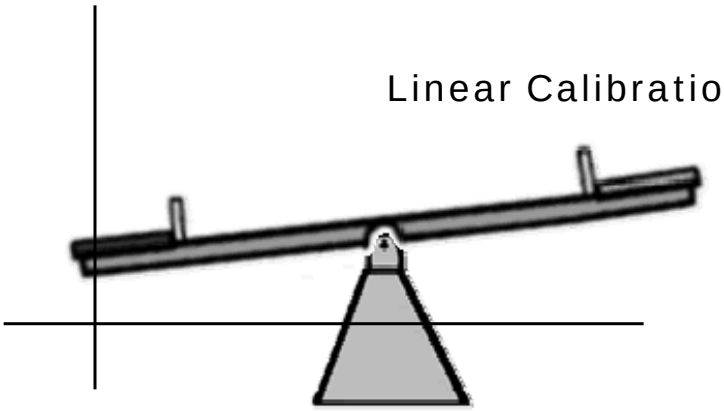
Remember: Phosphorus is non-linear above about 1.0 mg/L



Source:
North Central Labs at www.nclabs.com

Calibration linearity can pose a problem when determining LODs

How does calibration affect the LOD?

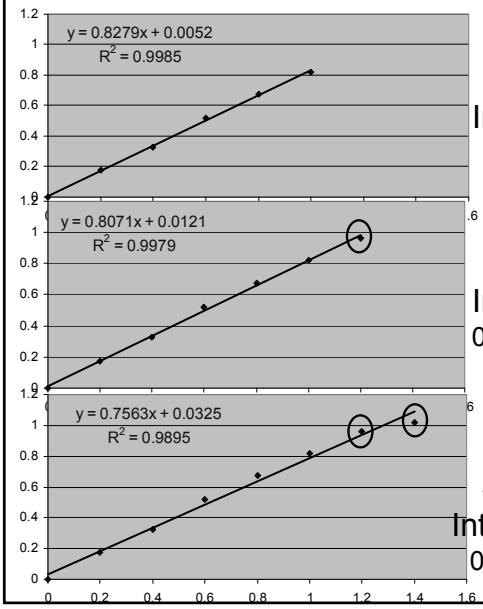


Is your Y-intercept creeping up?
Getting "negative" blanks?

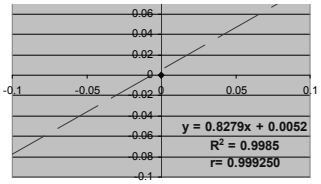
Calibrations, Non-linearity, & Negative Blanks

- As the upper end of the calibration "drops" the linear regression line "teeters" and the low end rises
- The Y-intercept increases…
- Translating to increasing negative values for a 0 response (i.e. blanks)
- This can be caused by exceeding the linear range
- …poorly prepared standards…
- …or a diminished spectrophotometer bulb
- …optics getting coated with HCl

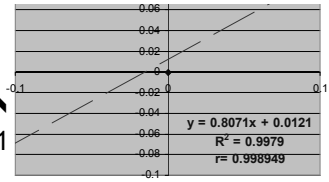
Calibrations, Non-linearity, & Negative Blanks



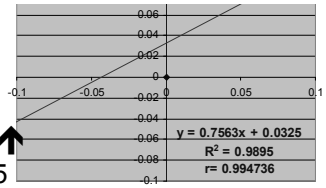
Linear
Intercept=0



Still linear
Intercept ↑
0 abs ≅ - 0.01



Still linear
Intercept ↑ ↑
0 abs ≅ - 0.05



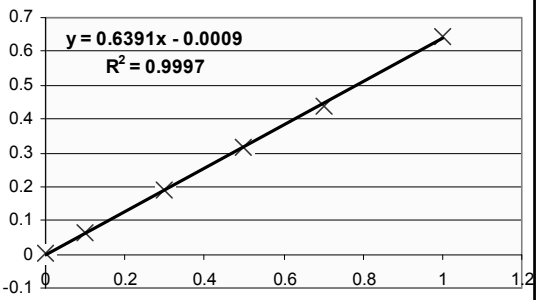
Not buying it? Here's some real data

WWTP "X" 1/13/10

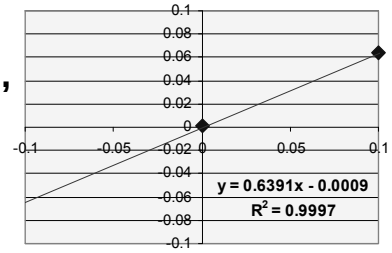
Hotplate abs read in disposable cuvette

mg/L	Abs @ 880 nm	RF
0	0.001	
0.1	0.064	0.640
0.3	0.191	0.637
0.5	0.317	0.634
0.7	0.439	0.627
1	0.644	0.644

slope= 0.639112
intercept= -0.000949
r= 0.999834



- **Response factors (RF) look good,**
- **Y-intercept is effectively zero**
- **Correlation is "3 Nines" plus**
- **Life is good**



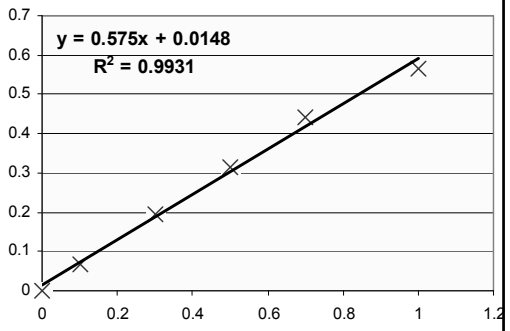
What a difference a year makes!
Would you notice this? DO anything?

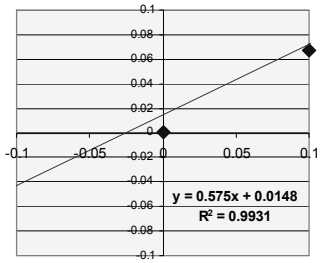
WWTP "X" 1/20/11

Hotplate abs read in disposable cuvette

mg/L	Abs @ 880 nm	RF
0	0.001	
0.1	0.067	0.670
0.3	0.194	0.647
0.5	0.316	0.632
0.7	0.441	0.630
1	0.565	0.565

slope= 0.575047
intercept= 0.014813
r= 0.996567





- **Response factor for 1 ppm drops**
- **Y-intercept now approaching 0.020**
- **Correlation is OK but "2 Nines"& 6**
- **0 response now = negative mg/L**
- **Something happened**
- **How would this affect LOD?**

To Follow NR149 or Follow the Method

<u>That</u> is the question

Spectrophotometer zeroed with:

Zero Blank (no Color reagent) | **Method blank**

True	Sample ID	mg/L	Abs		Conc.	Abs	
0	Zero Blk	**0.00**	0.000		**0.00**	- 0.032	
0	Method Blk	**0.03**	0.032		**0.00**	0.000	
0.5 ppm	LCS/CCV	**0.56**	0.326	112%	**0.50**	0.294	100%
0.5 ppm	LCS/CCV	**0.53**	0.308	106%	**0.48**	0.279	96%
0.05 ppm	LOD 1	**0.08**	0.058	160%	**0.02**	0.020	40%
0.05 ppm	LOD 2	**0.07**	0.054	140%	**0.02**	0.022	40%
0.05 ppm	LOD 3	**0.09**	0.064	180%	**0.03**	0.032	60%
0.05 ppm	LOD 4	**0.10**	0.068	200%	**0.04**	0.034	80%
0.05 ppm	LOD 5	**0.08**	0.060	160%	**0.02**	0.027	40%

Results obtained using Test 'N Tube

Know Thine Opponent

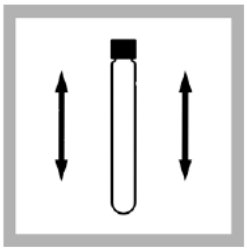
The Facts of [Test 'N Tube] Life

- Test 'N Tube is convenient…but it IS different
- Instead of using a single, optical quality cuvette for measurements, EACH TUBE is its own cuvette
- Are they lined up properly?
- The same optical quality?
- Smudge-free?
- Micro-scratch free?
- and what about that powder?

Know thine opponent

Know thine self

13. Use a funnel to add the contents of one PhosVer 3 Powder Pillow to the vial.



14. Immediately cap tightly and shake to mix for 20–30 seconds.

The powder will not dissolve completely.

So it is said that if you know your opponent and know yourself, you can win a hundred battles without a single loss.

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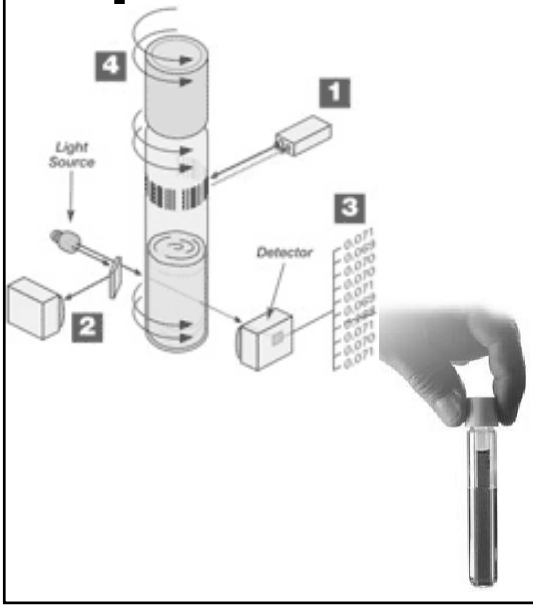
-- Sun Tzu, "The Art of War"

Source: HACH Method 8190

Newer instruments help mitigate the problems



1. **Barcode Recognition**: Simply drop in the vial and get results immediately with automatic method detection.
2. **Reference Detector**: Monitors and compensates for optical fluctuations.
3. **10X Measurement and Outlier Elimination**: Dirty, scratched, or flawed glassware, including fingerprints, is no longer an issue - instrument averages 10 readings and rejects outliers.
4. **Self-Contained Packaging - Reagents Inside Sealed Cap**: Reduces exposure to chemicals - no need to open pillows or clean glassware.

Reviewing Common TP Issues

Contamination!

- Wash glassware well, using a non-phosphate detergent
- Rinse with dilute (1-10%) hydrochloric acid
- Never re-use HCl solution to wash glassware
  - *Used acid soon becomes contaminated ➔ contaminates all of your glassware.*
- Even new glassware needs to be washed
- DO NOT touch inside glassware with bare hands!
- DO NOT smoke or use air fresheners in the laboratory.
- Cover samples if you use autoclave for digestion
- Segregate glassware for TP

Combined (color) reagent

- Make your Combined Reagent fresh daily
- It should be a light straw or light yellow color.

Sample pH

Not at proper pH prior to adding the Combined Reagent.

Other considerations –

Optimizing Spectrophotometer Performance

- Clean up spills
- Periodically clean cell compartment-wipe out with soft damp cloth
- Avoid exposing instrument to corrosive environment
  - acid vapors, dust and moisture can coat optics and degrade performance
- Consider changing lamp/bulb annually (and before doing new calibration curve)
- Recalibrate anytime major maintenance is performed.
- Track absorbance of CCV to ensure sensitivity does not degrade over time
- May wish to have wavelength accuracy and performance checked by outside vendor every few years

It's Hip to be Square

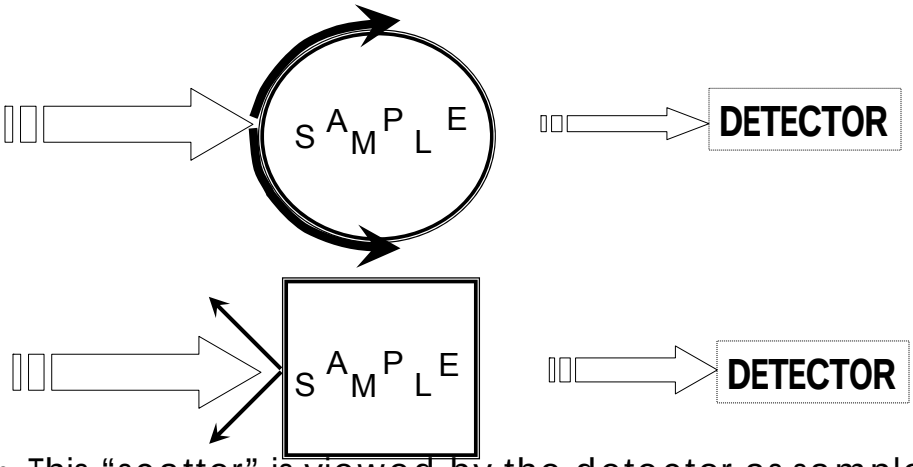
Cuvette shape matters

- **Square cuvettes, or cells will be found to be most precise** since the parameters of pathlength and parallelism are easier to maintain during manufacture. Round cuvettes have the advantage of being less expensive.
  --Spectrometry Principles
- **Square cuvettes gave the most accurate results**, compared to round glass test tubes and UVettes. The square geometry also made it easier to calculate the effect of refraction.
  --UCSD 2010 "Dynamic Light Scattering"
- **Inexpensive cuvettes are round** and look similar to test tubes. Disposable plastic cuvettes are often used in fast spectroscopic assays, where speed is more important than high accuracy.
  --Swarthmore College Chemistry Dept.

Light diffraction and cuvette shape

Detectors see the difference between light going in and coming out as absorption

- There is some diffraction of light by round cuvettes



- This "scatter" is viewed by the detector as sample absorbance, resulting in slight high bias

Cuvette Care and Feeding

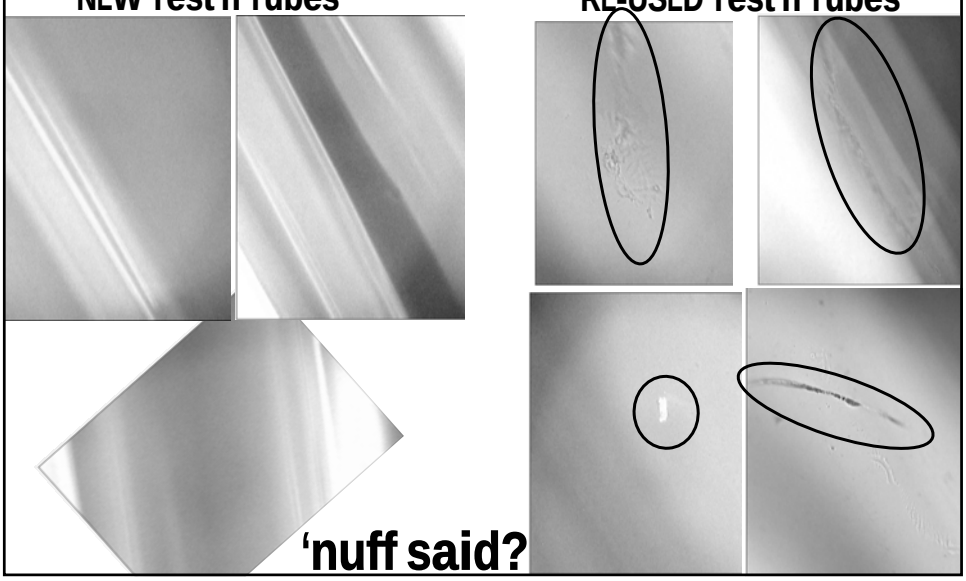
Would you treat your eyeglasses like you do cuvettes?

- Anything that hinders light passage through the cuvette will produce abnormally high absorbance readings. For example, scratches on the cuvette are a major problem.
- 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 soap solution.
- After the cuvette is scrubbed inside and out, the soap should be removed by rinsing with tap water and then distilled water.

Don’t Re-use Test ‘n Tubes!

NEW Test n Tubes

RE-USED Test n Tubes



‘nuff said?

So…Clearly we have some problems

…and so we worked with the State Lab of Hygiene to come up with some options

SLH Study – A Tale of Two Techniques

Perform TNT analysis of TP as directed

measurements taken in individual ~13 mm TNT vials

Perform TNT analysis of TP <u>with a twist</u>

all measurements taken using a single, <u>high</u> <u>quality</u> 2.5 cm cuvette

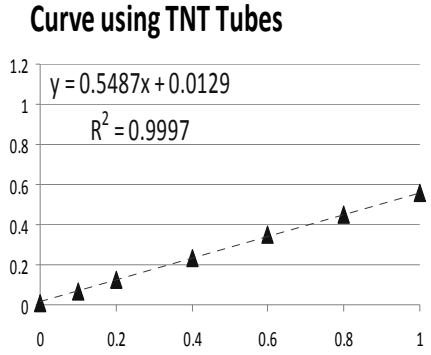
- Prepare a calibration curve
- On each of 4 separate days, prep/analyze:
  - A QCS (CCV)
  - A method blank
  - 2-3 LOD spikes

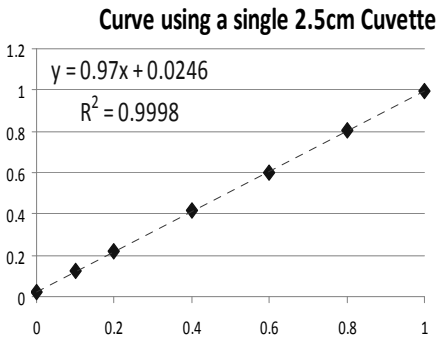
Calculate LOD ⬅ **COMPARE** ➡ Calculate LOD

SLH Study Notes

- Hach method 8190 followed.
- Single wave length user program at 880 nm.
- Cuvette used Hach cat # 249502 2.5 cm round glass.
- Vortexer used for mixing.
- Potassium persulfate did not completely dissolve until cooked. Likely not an issue but duly noted.
- PhosVer 3 color reagent did not completely dissolve as stated.
- Incompletely dissolved color reagent could have effect on LOD determination. Did not observe any significant sticking of it on cuvette walls. It seemed to settle to bottom in both TNT and cuvette.

Calibration differences:
TNT vs. single cuvette





1. **Longer path length = greater absorbance response**
2. **Slope doubles….but so does Y-intercept**

Calibration differences:
TNT vs. single cuvette

Abs read in tube		
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	0.56	0.560
slope=	0.548703	
intercept=	0.01286	
correlation	0.999849	

Abs read in 2.5 cm 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=	0.970034	
intercept=	0.02456	
correlation	0.999916	

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

Compare: LOD data and final LODs

Test ‘n Tube (read in tubes)
LOD spike=0.1 mg/L

- 0.099
- 0.121
- 0.123
- 0.100
- 0.102
- 0.111
- 0.115
- 0.095
- 0.115
- 0.125
- 0.120

Mean= 0.111
Range: 0.095 to 0.125 (0.030)
Std Deviation= 0.01074
LOD= 0.0297 mg/L

t-value = 5.3454
P-value = < .00001

Test ‘n Tube (2.5 cm cell)
LOD spike=0.1 mg/L

- 0.090
- 0.090
- 0.092
- 0.085
- 0.089
- 0.099
- 0.095
- 0.097
- 0.088
- 0.095
- 0.096

Mean= 0.092
Range= 0.085 to 0.099 (0.014)
Std Deviation= 0.00439
LOD= 0.01215 mg/L

Data Observations – the 411

- Data obtained using a single cell were much “tighter” than those obtained from individual TNT tubes. *Single tube vs. many = lower stdev*
- “Tighter” (higher precision) values yield a lower LOD
- Concentrations obtained from the analyses performed using a single 2.5 cm cuvette are about 20% less than those obtained using standard TNT tubes. *Hmmmmm*
- Yet, responses are about twice as high due to the longer path length

Some cuvette options

Product #: 122800
USD Price: $6.45

1" Round Glass, 10mL

Overview | Details | Parameter/Reager

Zeroing vial for AccuVac Ampules.

Product #: 2427606
USD Price: $24.75

Product #: 2401906
USD Price: $36.75

pkg of 6

Product #: 2665908
USD Price: $470.00

Sample Cell: 1" Square

Overview | Details | Accessories

set of 8; 4 matched pairs

What have we learned?

- Labs in general need to revisit LOD protocols.
- Don't just do the minimum to meet EPA requirements for LOD…obtain a valid LOD.
- You will need to obtain a valid LOD of at least 0.03 mg/L
- That will be very difficult using Test 'N Tube (*without making adjustments*)
- We **strongly** *recommend* TNT users adopt the single quality cuvette approach.

NOTE: *We have not evaluated the LOD using the TNT-Plus method and the "rotational measurement" technology". Early indications suggest these to be important enhancements*

Less Talking & More Doing

Talk doesn't cook rice.
~Chinese Proverb

Thanks for having us!

Acknowledgements

Thanks go to:

•Pat Gorski and Graham Anderson of the State Lab for analytical support.

•Jim Burke of Hach Co. for providing an instrument and supplies for the Test 'N Tube.

•Labs that responded to our survey.

Any questions?