Who wants to be a Phosphorus and Ammonia Expert?!

WSLH WISCONSIN STATE LABORATORY OF HYGIENE

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Wisconsin Dept. of Natural Resources

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Disclaimer

- ★ Review Total Phosphorus procedures
  - ★ manual digestion/analysis procedure
  - ★ “Test ‘n Tube” procedure
- ★ Review ammonia procedure (ISE)
- ★ Troubleshoot: Common Problems
- ★ Demonstrate various aspects of testing
- ★ Review required QC samples
- ★ Discuss documentation required
- ★ Provide necessary tools to pass audits

Session Objectives

- Overview
- Calibration Basics
- Limit of Detection (LOD)
- **Phosphorus**
  - Calibration
  - Method Details
  - Troubleshooting
- **Ammonia**
  - Calibration
  - Method Details
  - Troubleshooting
- Quality Control
- Documentation

Course Outline

Ammonia

- Major excretory product of animals
- Oxidation of ammonia leads to nitrite and then nitrate
- Ammonia is toxic to organisms at high pH levels
- Total nitrogen reaching a municipal WWTP averages 15 - 50 mg/L
- 60% is ammonia

About 40% of small WWTP labs required to test for NH_3

You should be aware that the rules relating to ammonia are changing, maximum allowable levels are dropping, and more plants could be affected.

Ammonia Sources

Nitrification [NH_3 -> NO_2^- -> NO_3^-]

- *Nitrosomonas* : oxidation of ammonia to nitrite (NO_2^-)
- *Nitrobacter* : oxidation of nitrite to nitrate (NO_3^-)
- Oxidation of ammonia to nitrate requires 4.57 mg of oxygen for every mg of ammonia oxidized.
- Between 7.07 and 7.14 mg of alkalinity (as $CaCO_3$) consumed per mg of ammonia oxidized.
- Inhibited at 10°C or less.
- Optimum temperature is about 25° C.

Ammonia and Nitrification

Ammonia levels and effects

NH_3 level	Effects
0.06 mg/L	fish can suffer gill damage
0.1 mg/L	Usually indicative of polluted waters
0.2 mg/L	Sensitive fish like trout and salmon begin to die
2.0 mg/L	Ammonia-tolerant fish like carp begin to die

The danger ammonia poses for fish depends on the water's temperature and pH. The higher the pH and the warmer the temperature, the more toxic the ammonia.

Ammonia Critical Levels

Phosphorus

- Typically from fertilizers or phosphate-based detergents (*Alconox contains about 8.7%)*
- Natural sources include phosphate-containing rocks and solid or liquid wastes
- Key growth-limiting material for phytoplankton
- The human body releases about a pound of phosphorus per year
- Widely used in power plant boilers to prevent corrosion and scale formation
- As of January 1999, approximately (33%) of wastewater labs must test for phosphorus.
- …and it continues to be written into permits

Phosphorus sources

Phosphorus levels and effects	
mg/L as P	**Effects**
0.003-0.010	Amount of phosphate-phosphorus in most uncontaminated lakes
0.008	Accelerates the eutrophication process in lakes
0.033	Recommended maximum for rivers and streams

Phosphorus Critical Levels

Ammonia + Phosphorus =

Deleterious effects

…which leads to something like this

…or this

Deleterious Effects II

Calibration

Calibration Curve Frequency-Basics

- For best results, should be run daily.
- Alternatively, a “full” calibration can be analyzed initially and verified (with one or more standards) each day of analysis.
- Whenever any reagent is replaced.

- Ammonia: Curve should be re-prepared with each analysis
  - The method calls for a daily calibration
- Phosphorus:

  MUST prepare new calibration when check standards fail, GLP suggests, “whenever any reagent is replaced”.

  Bottom line: we’d like a new curve at least quarterly.

Calibration Frequency

Use an appropriate number of standards

MUST be constructed using at least 3 standards and a blank.

NR 149.13 (3) (b) … A calibration shall consist of at least 3 standards and a blank except as allowed in approved methods using ***ion selective electrodes*** *or inductively coupled plasma.*

There aren't any!

To include...or not to include (a blank)?

- *Rule of thumb*:
  if you can adjust your instrument to read zero with a blank, then include the blank in your calibration curve.
- Including a blank is generally appropriate for colorimetric procedures

Ammonia

DO analyze a blank
Do NOT include in calibration.

Phosphorus

DO analyze a blank
DO include in calibration.

Number of standards

Define your calibration range properly

- Range should be appropriate for the samples being analyzed
  (i.e. don't calibrate from 1- 5 mg/L when samples range 0.05 - 0.5 mg/L).
- Be aware of the linear range of the method used!
- Standards should also be evenly spaced.
  0.1, 0.2, and 10.0 are NOT good levels for a calibration
  NOTE: This is a general rule: electrodes are a bit different!
- Where possible…bracket samples with calibration standards.
  (but if your effluent NH_3 runs about 0.05ppm, DON'T use a standard at 0.02!)
- Low standard not more than 2 - 5X the LOD (best is = LOQ).
- Suggested range Ammonia: 0.2, 2, 20 mg/L

  Phosphorus: 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1 mg/L

NOTE: Calibration ranges for ammonia may change with the season

Calibration Range

Processing calibration data

- 🚫 pre-programmed calibrations
- 🚫 👎 👍 graph paper……
- 👍 linear regressions
- 👍 software

- Makes traceability virtually impossible
  - Significant variability in how the size of the graph constructed
  - Significant variability in how the scales of the graph are constructed
  - Significant variability in how any individual draws the "best fit" line
  - During QA/QC training, we showed that variability can be 1.0mg/L or more
- A standard procedure can eliminate sources of variability.

Regardless of technique used, it is critical to evaluate the calibration to ensure that is is valid

“Response to Concentration, over...”

Mandatory

Ongoing calibration checks

Optional

Correlation coefficient (if using linear regression)

Visual

Linearity check (response factors)

Residual analysis ("*back-calculation*")

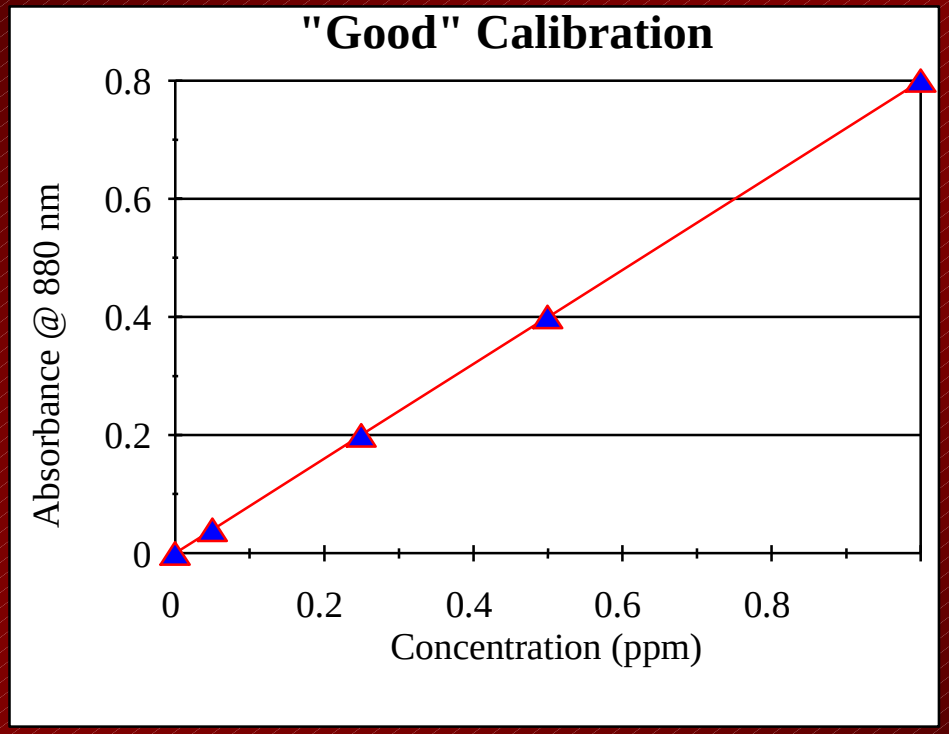
☆☆☆ **While these tools are not strictly required…** ☆☆☆

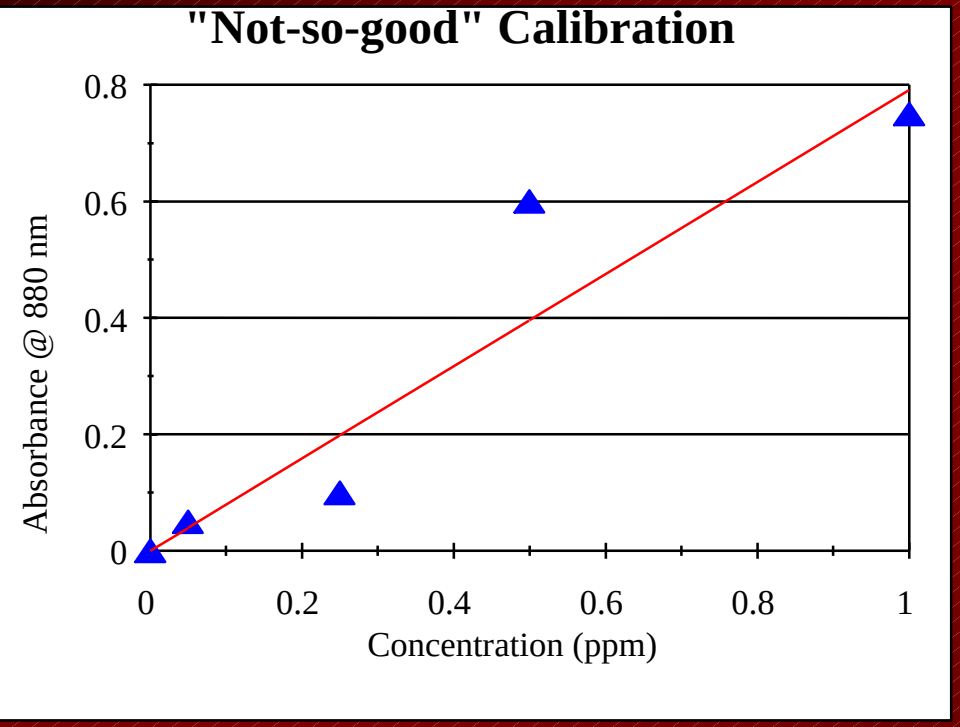
1) They are critical in obtaining a valid calibration
2) Often, calibration data will pass one or more, but not all of these checks

Calibration evaluation tools

Simple Visual Evaluation

Data points should very closely fit the resultant calibration line.





Unfortunately, rarely is it this easy.

Visual evaluation

Statistical Evaluation

If using a linear regression, the correlation coefficient "r" provides a measure of the acceptability of a particular calibration curve.

- " r " = complex mathematical equation
- Values between 0 (no correlation) and 1 (perfect correlation).
- Correlation coefficients can be obtained using:
  - any scientific calculator with 2-variable statistics capability
  - most spreadsheet programs, e.g., Excel, Lotus, QuattroPro

BOTTOM LINE: "r" *SHOULD* be 0.995 or greater

$r^2 \neq r$

---

if your instrument/software provides r^2, then:

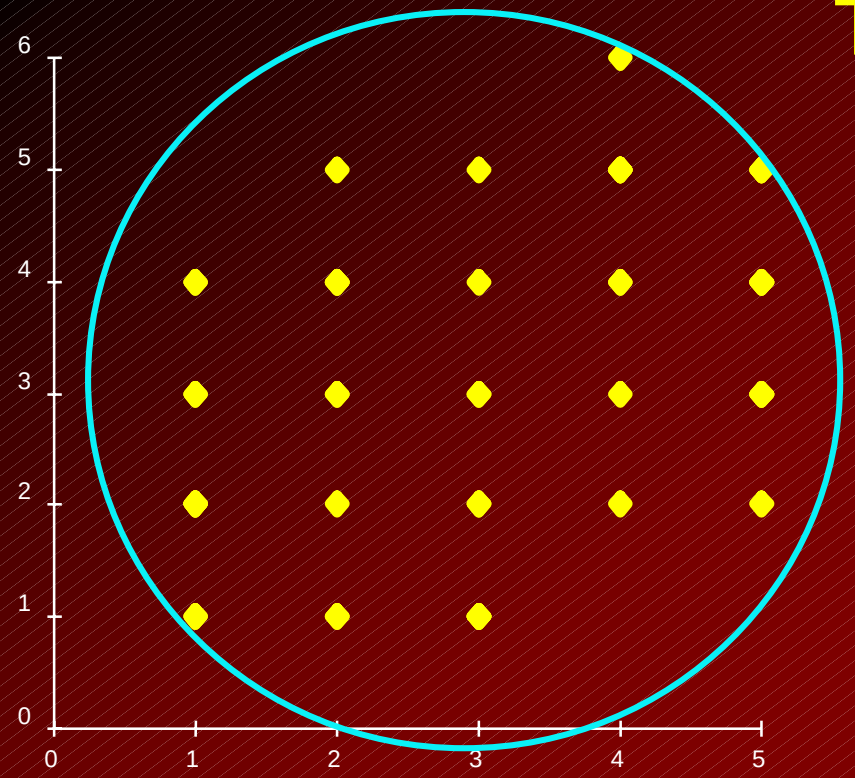
- take the square root of r^2 (which equals r) or
- an r^2 of 0.990 = r of 0.995

$$\sqrt{r} = r$$

Statistical evaluation

The “Rubber Band” test

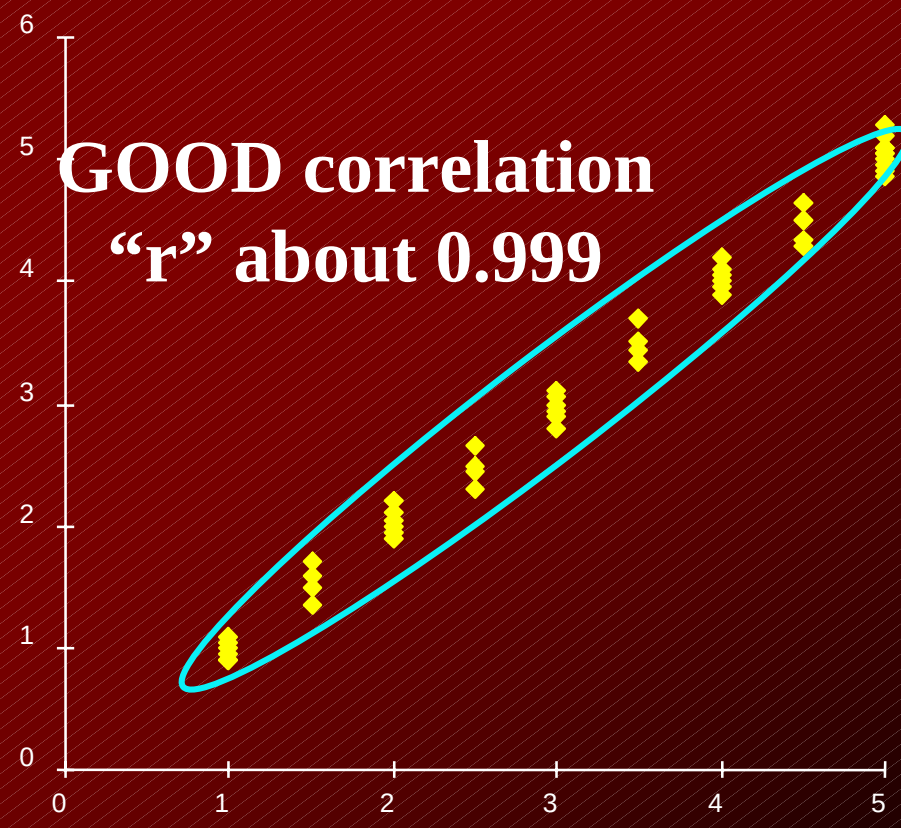
…simply visualize how a rubber band would “fit” the data...



POOR correlation

“r” = 0

...the more it is stretched taut in a “cigar” shape, the higher the correlation.



GOOD correlation

“r” about 0.999

Correlation coefficients

- Most analyses have a limited linear range
- Deviation from linearity usually related to concentration
- Can occur at both the lower and the upper end
- Unsure? Review “response factors” for each calibration level
- Look for the point at which deviation occurs

Response Factor (RF) = $\frac{\text{Response}}{\text{Concentration}}$ *(= Absorbance)*

*** If you identify **non-**linearity, reduce the calibration range ***

Linearity check

mg/L	Abs.	RF $\frac{Abs}{mg/L}$
0	0	
0.20	0.175	0.875
0.40	0.325	0.813
0.60	0.500	0.833
0.80	0.675	0.844
1.00	0.825	0.825
1.20	0.950	0.792
1.40	1.000	0.714
1.60	1.050	0.656
1.80	1.075	0.597
2.00	1.150	0.575

Low end variability

Midpoint: stability

Steady decline in RF characteristic of exceeding linear range

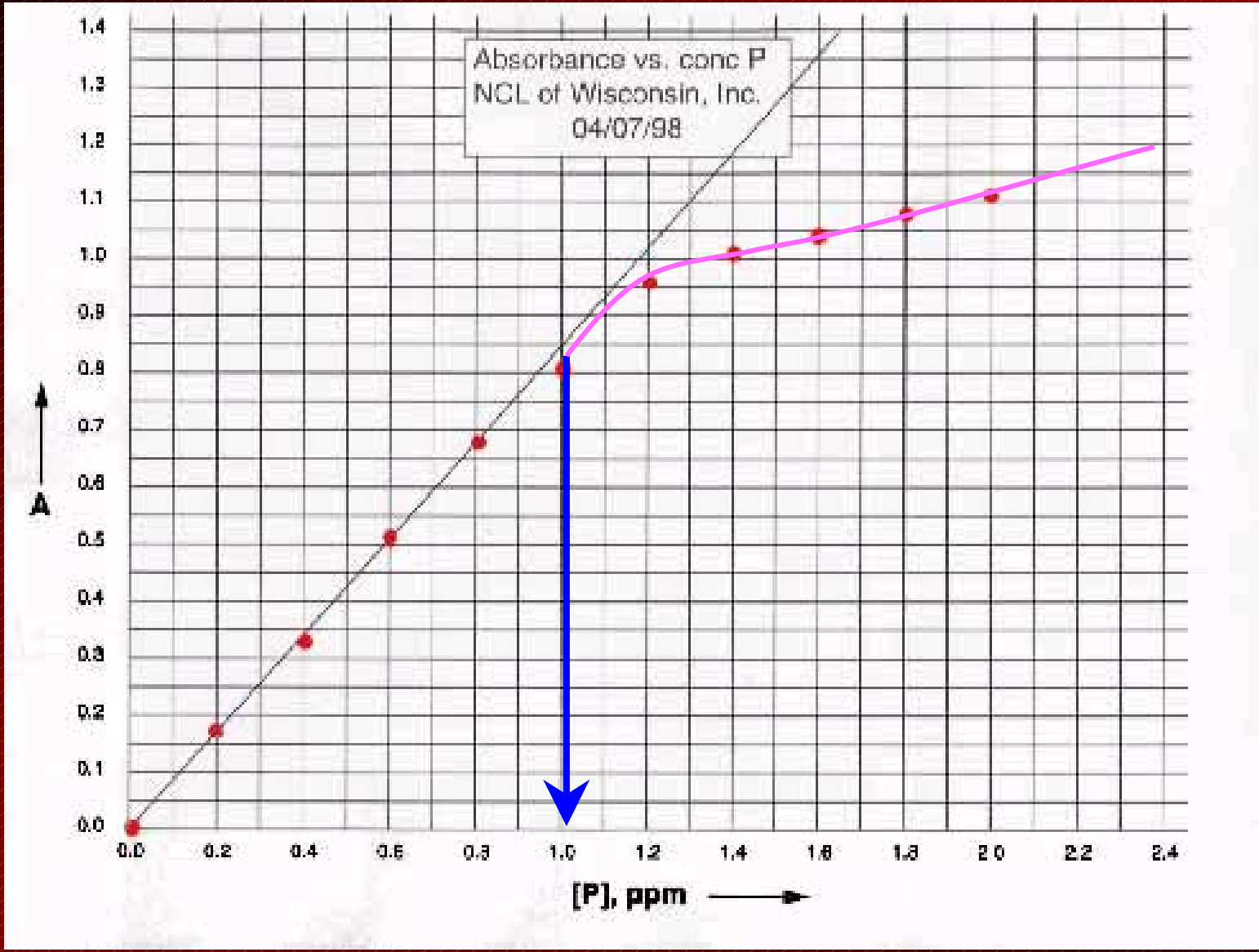
Rise in Abs. Per 0.2 mg/L

0.175
0.150
0.175
0.175
0.150
0.125
0.050
0.050
0.025
0.075

Data Source: North Central Labs at www.nclabs.com

Linearity check - response factors

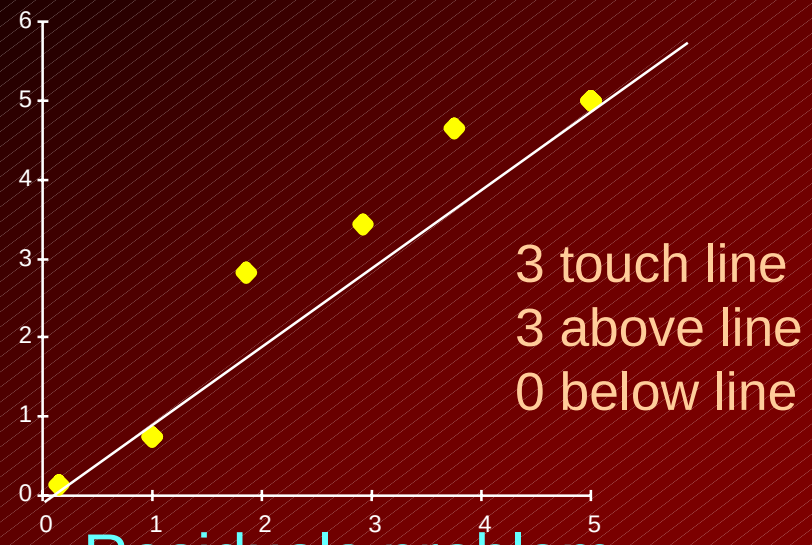
Phosphorus is non-linear above about 1.0 mg/L



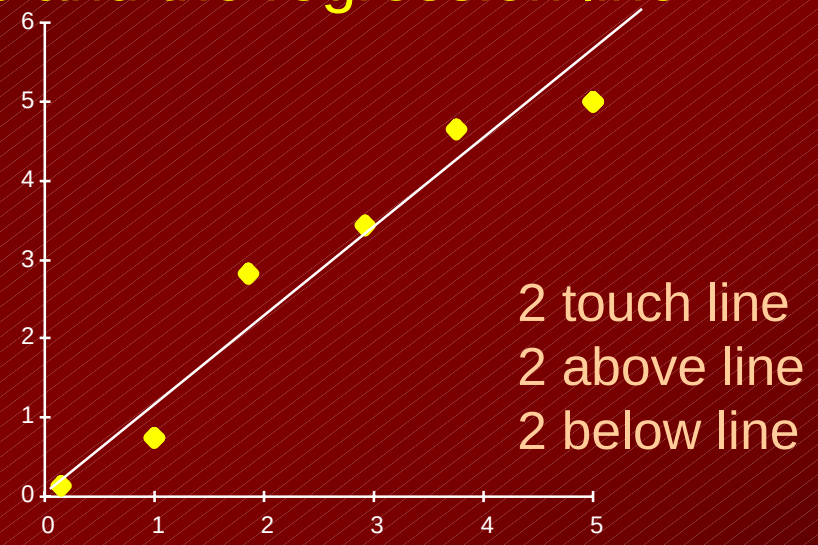
Source: North Central Labs at www.nclabs.com

TP calibration linearity

The purpose of a linear regression is to mathematically minimize the distance between all data points and the regression line



Residuals problem



Residuals minimized

"Residuals" represent the degree of agreement between True Value and back-calculated concentration

- ☆ "Plug" responses of standards into the regression equation
- ☆ Compare recovery: regression concentration vs. true value
- ☆ Recoveries should generally be within 90-110%
- ☆ It's more difficult to achieve ± 10% as you get close to the LOD
- ☆ The "apparent" concentration for the blank must not be > LOD

Back-calculation analysis

- Linear regression equations are in the form **Y = mX + b**
- m = slope; b = intercept
- Since X = concentration, and Y = absorbance…..
- This solves for absorbance…which we already KNOW
- Therefore we have to "re-arrange" the equation….

$$\frac{\text{Absorbance} - \textit{intercept}}{\textit{slope}} = \text{Concentration}$$

NOTE: Be aware that some calculators switch Concentration & Absorbance

First…a little math lesson

Calibration Data

mg/L P	Abs.
0	0
0.1	0.051
0.5	**0.25**
2	0.72
5	1.24

Slope (m) = 0.244713124

Intercept (b) = 0.080236051

	True Concentration (mg/L)	Regression Concentration (mg/L)	Percent Recovery
Absorbance = 0.000:	0	-0.328	???
Absorbance = 0.051:	0.1	-0.119	ND
Absorbance = 0.250:	0.5	0.69	138%
Absorbance = 0.720:	2	2.61	130.5%
Absorbance = 1.24:	5	4.74	94.8%

0.250 - 0.08024 = 0.16976 → 0.16976 / 0.2447 = 0.694

Back-calculation analysis

Analytical Evaluation - ongoing

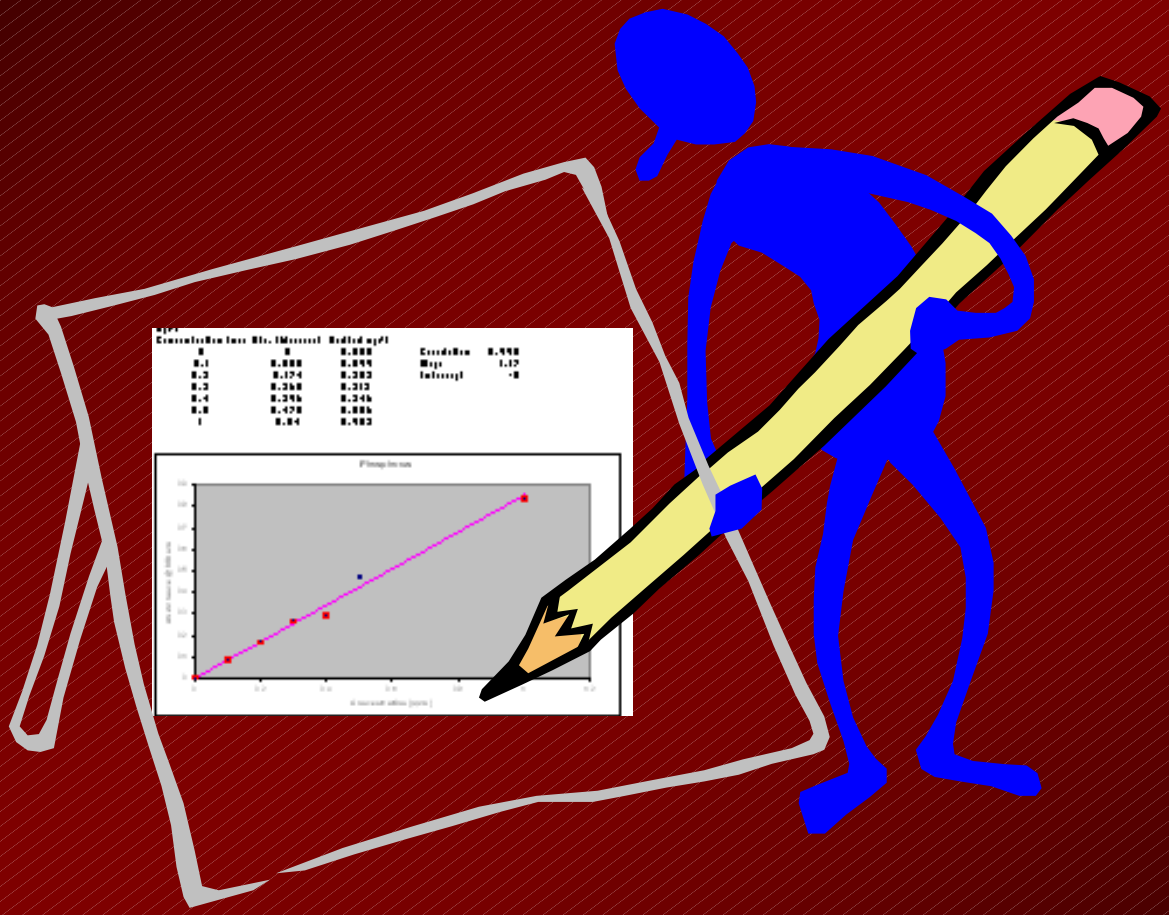
- Periodically confirm that response has not changed from initial.
- Use a midpoint standard (check standard) and blank.
- Calculate % recovery.
- Check at beginning, every 10 samples and end of each batch.

For WWTPs doing 1-2 samples/day, only need one

Recovery must be within 90-110% for phosphorus and ammonia.

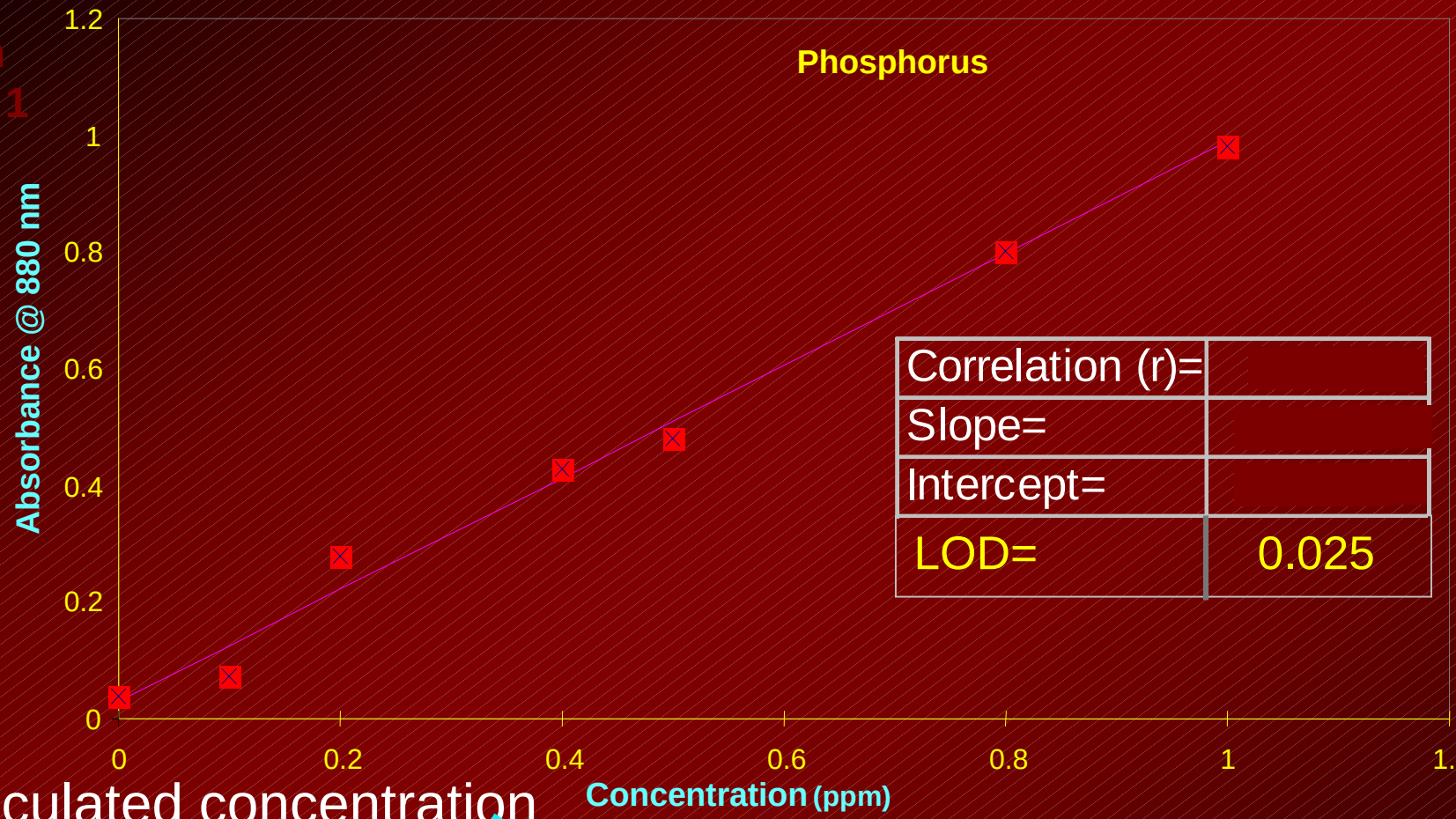
$$\% \text{ Recovery} = \frac{\text{measured Value}}{\text{True value}} \times 100$$

Calibration - daily checks



Calibration Exercise

Calibration Exercise # 1



Correlation (r)=	
Slope=	
Intercept=	
LOD=	0.025

Back-calculated concentration

Concentration (mg/L)	Abs. (Measured)	Residual mg/L
0	0.040	
0.1	0.075	
0.2	0.280	
0.4	0.430	
0.5	0.480	
0.8	0.800	
1	0.980	

YOU be the auditor!

Acceptable calibration?

CALIBRATION -

Entering Regression Data into Sharp EL-520L

Calibration Data	
mg/L P	Abs.
0	0.040
0.1	0.075
0.2	0.280
0.4	0.430
0.5	0.480
0.8	0.800
1.0	0.980

Set Mode to “2”:	MODE 2		
Clear the registers:	2nd F DEL		
Enter 1st data pair:	0 STO	• 0 4 M+	n=1
Enter 2nd data pair:	• 1 STO	• 0 7 5 M+	n=2
Enter 3rd data pair:	• 2 STO	• 2 8 M+	n=3
Enter 4th data pair:	• 4 STO	• 4 3 M+	n=4
Enter 5th data pair:	• 5 STO	• 4 8 M+	n=5
Enter 6th data pair:	• 8 STO	• 8 0 M+	n=6
Enter 7th data pair:	1 STO	• 9 8 M+	n=7

Obtain the calibration evaluation information

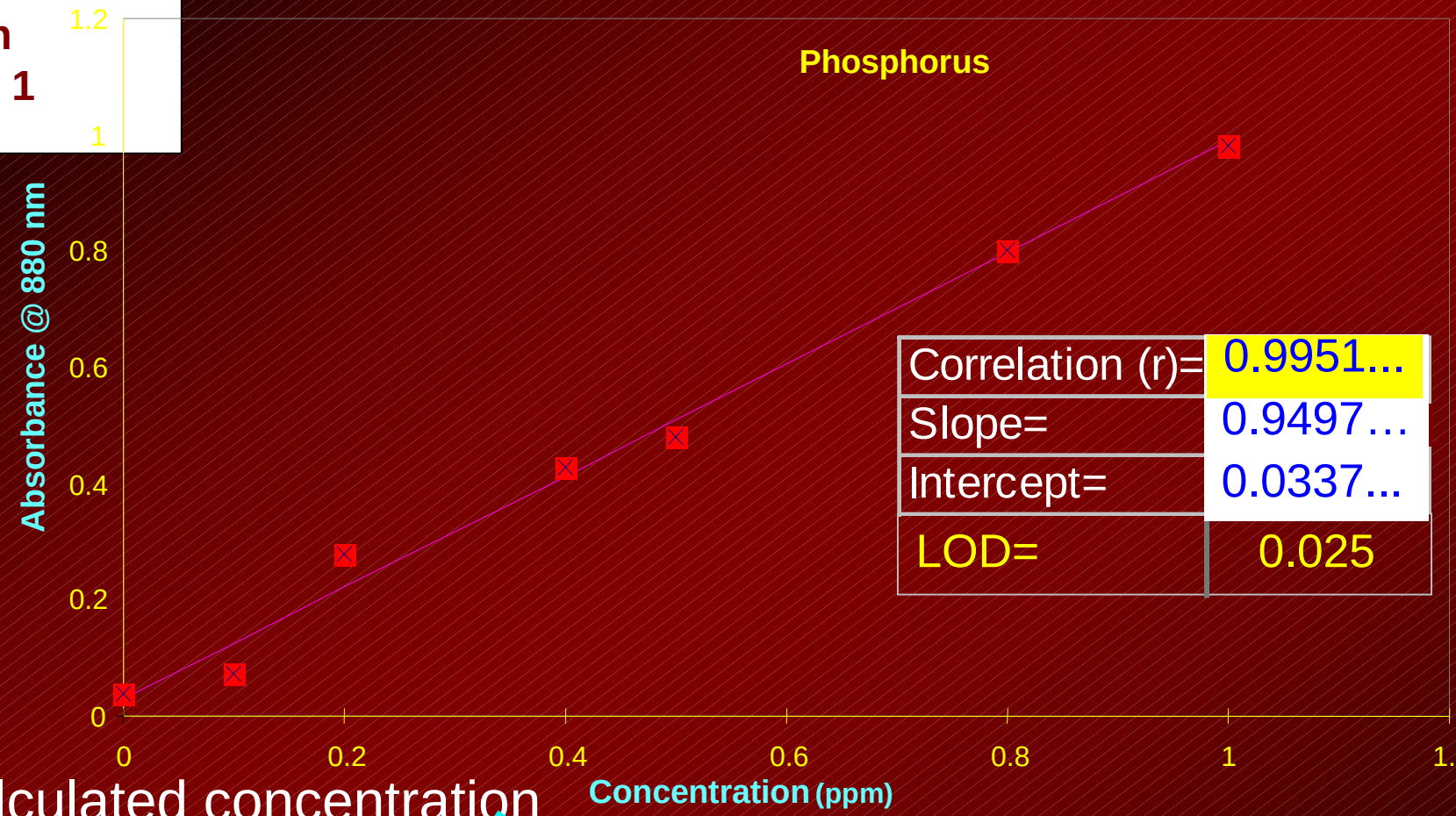
- ✓ Get the Correlation coefficient..: RCL ÷ r = 0.9951…..
- ✓ Get the Slope.....: RCL) b= 0.9497…..
- ✓ Get the Intercept: .....: RCL (a = 0.03368…..

Converting a sample absorbance to concentration

0.04:	• 0 4	2nd F (	**0.007**	**This value should be < LOD**										
0.075:	• 0 7 5	2nd F (	**0.044**	÷	• 1	×	1	0	0	=	**44 %**			
0.28:	• 2 8	2nd F (	**0.26**	÷	• 2	×	1	0	0	=	**130 %**			
0.43:	• 4 3	2nd F (	**0.42**	÷	• 4	×	1	0	0	=	**105 %**			
0.48:	• 4 8	2nd F (	**0.47**	÷	• 5	×	1	0	0	=	**94 %**			
0.8:	• 8	2nd F (	**0.80**	÷	• 8	×	1	0	0	=	**100 %**			
0.98:	• 9 8	2nd F (	**0.99**	÷	1	×	1	0	0	=	**99 %**			

"Back" Calculating Standards

Calibration Exercise # 1



Correlation (r)=	0.9951...
Slope=	0.9497…
Intercept=	0.0337...
LOD=	0.025

Back-calculated concentration

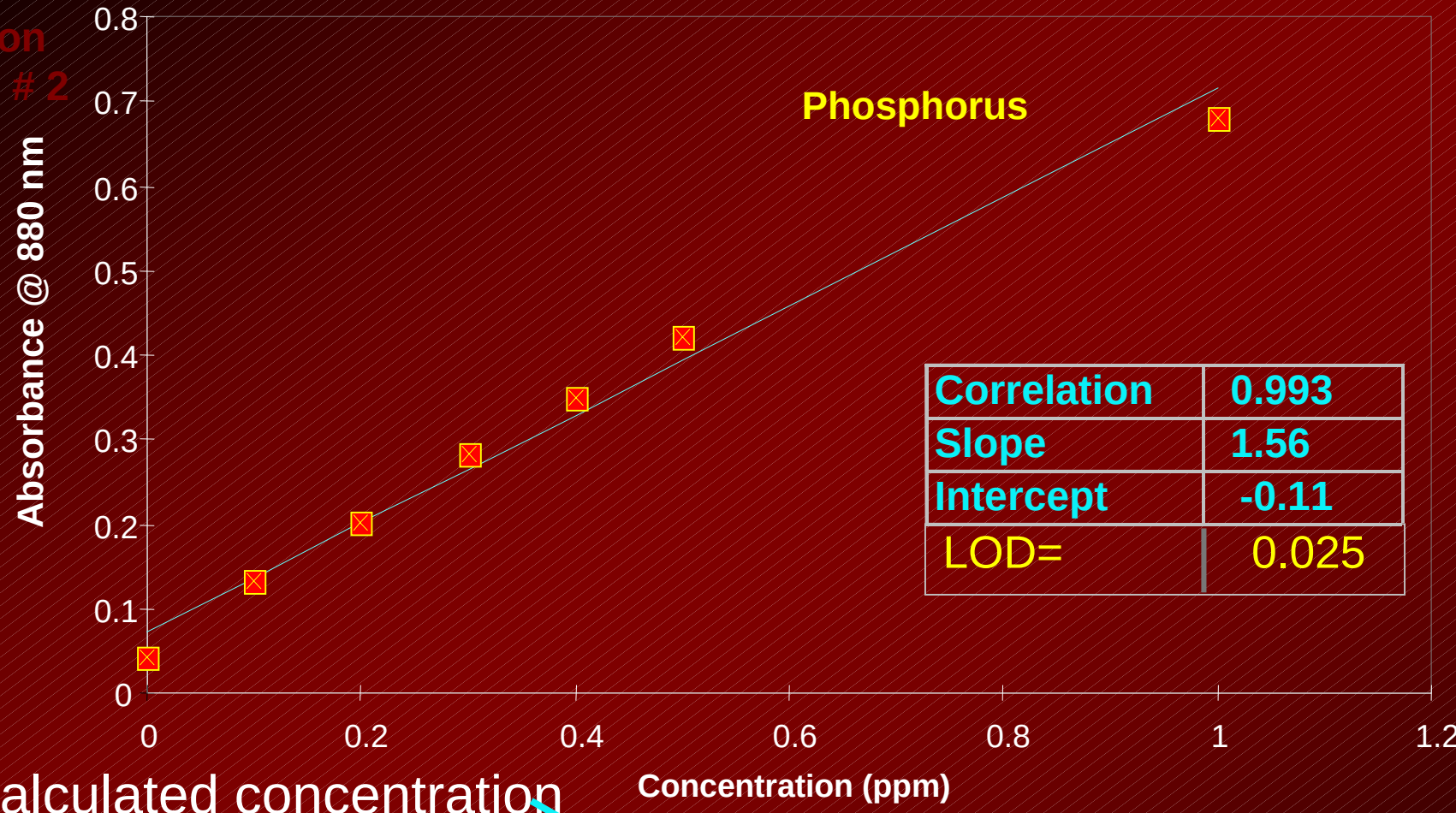
Concentration (mg/L)	Abs. (Measured)	Residual mg/L
0	0.040	0.0066…
0.1	0.075	0.0435 (43.5%)
0.2	0.280	0.259 (130%)
0.4	0.430	0.417 (104%)
0.5	0.480	0.470 (93.9%)
0.8	0.800	0.807 (101%)
1	0.980	0.996 (99.6%)

Visual: Good, not great

Correlation: OK

Residuals: Fail at low end

Calibration Exercise # 2



Correlation	0.993
Slope	1.56
Intercept	-0.11
LOD=	0.025

Back-calculated concentration

Concentration (mg/L)	Abs. (Measured)	Residual mg/L
0	0.041	-0.05
0.1	0.132	0.09
0.2	0.203	0.20
0.3	0.282	0.33
0.4	0.349	0.43
0.5	0.422	0.55
1.0	0.681	0.95

YOU be the auditor!

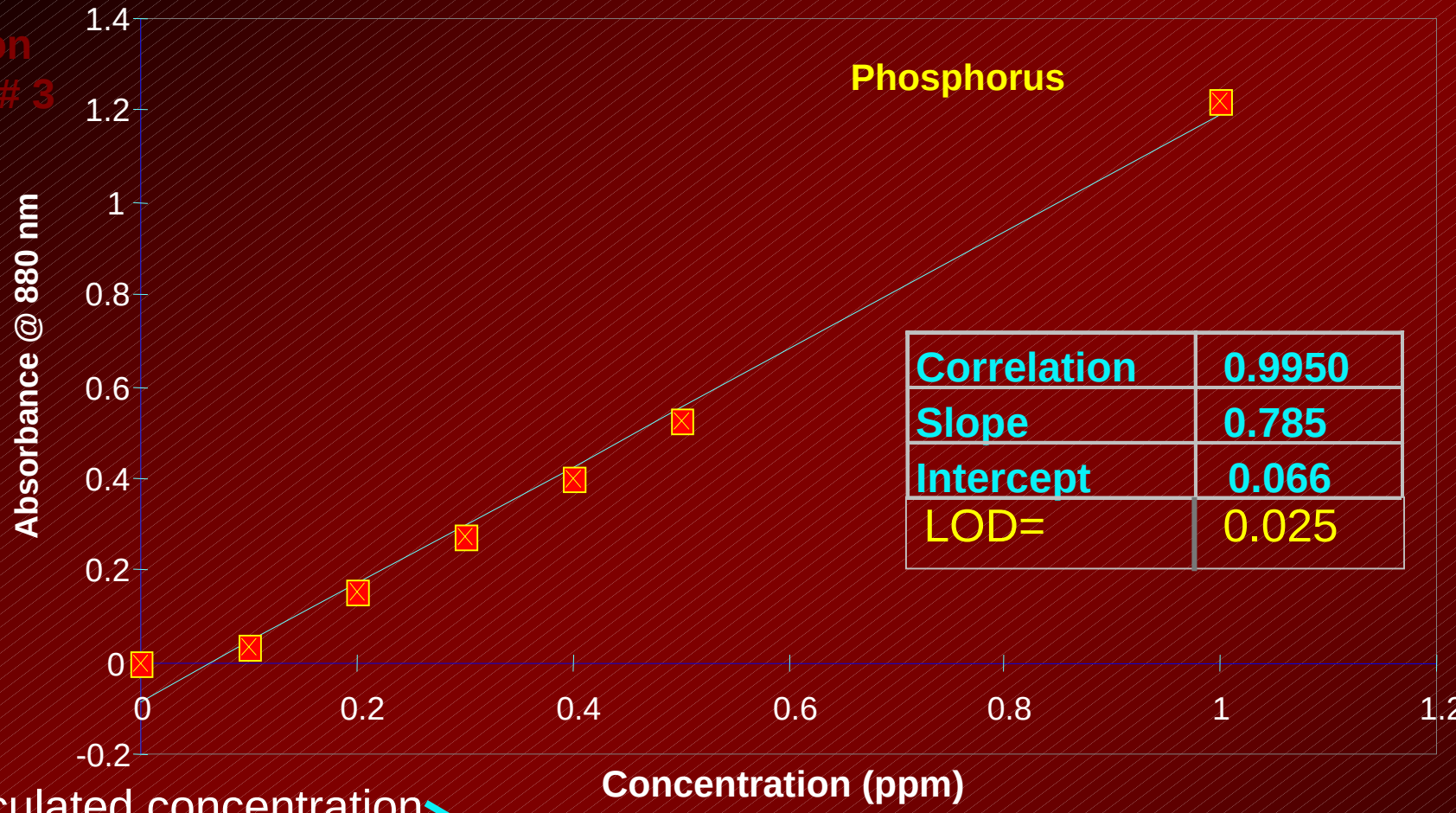
Acceptable calibration?

Visual: Good

correlation: Fails

Residuals: Good

Calibration Exercise # 3



Correlation	0.9950
Slope	0.785
Intercept	0.066
LOD=	0.025

Back-calculated concentration

Concentration (mg/L)	Abs. (Measured)	Residual mg/L
0	0	0.07
0.1	0.031	0.09
0.2	0.151	0.18
0.3	0.273	0.28
0.4	0.399	0.38
0.5	0.525	0.48
1	1.22	1.02

YOU be the auditor!

Acceptable calibration?

Visual: Good

correlation: OK

Residuals: OK except zero

Break!

What Graham's up to:

- TNT blank
- TNT check standard
- TNT high alkalinity
- TNT…skin residue
- TNT…ashes

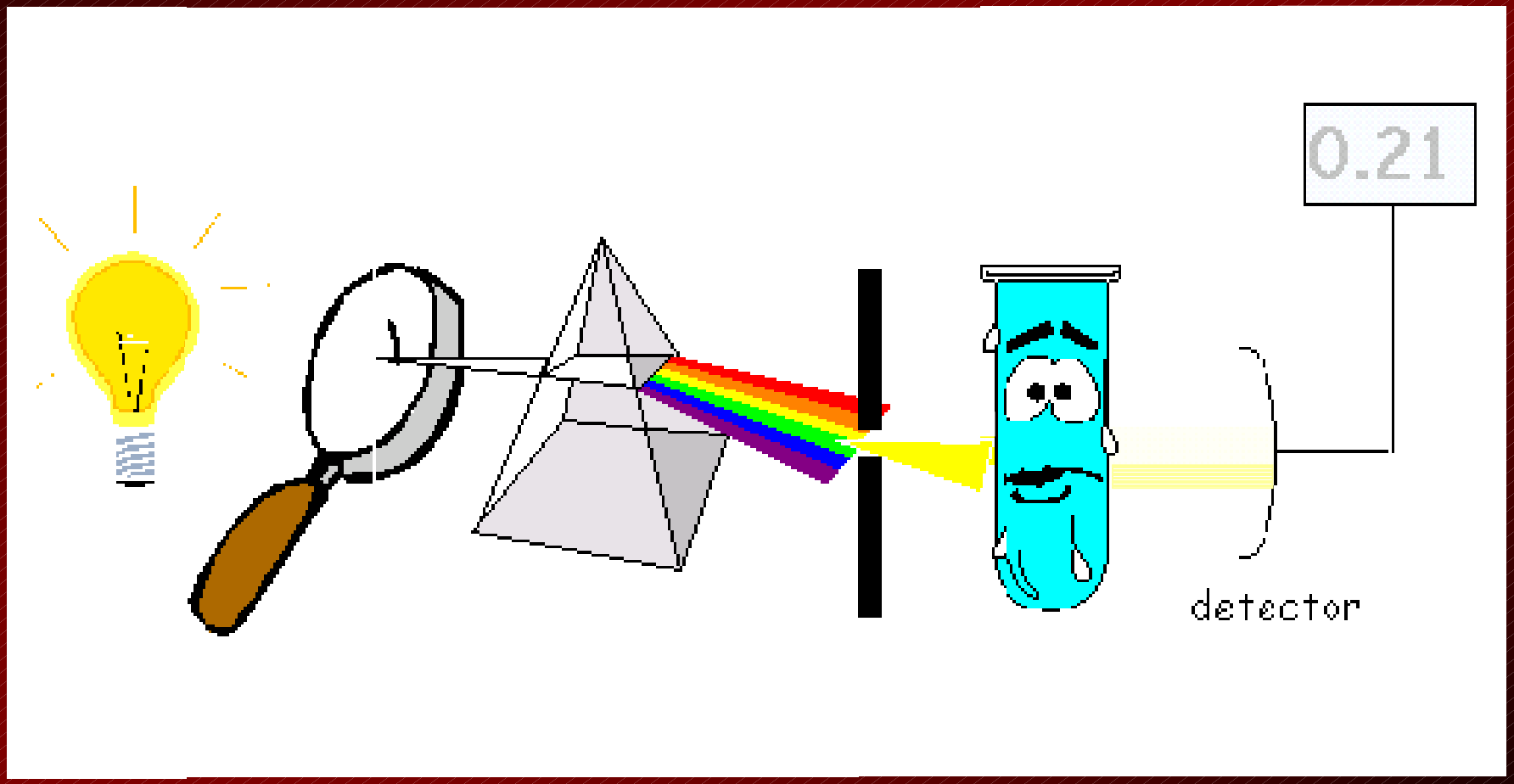
Method Details - Phosphorus

Courtesy of NCL:

Sequoia-Turner Model 390
Sequoia-Turner Model 690
Spectronics 20 Genesys

Courtesy of HACH:

HACH Model 2010
HACH COD Reactor



How the spectrophotometer works

1. **Digestion**
2. **Calibrate (or verify existing calibration)**
3. **Colorimetric analysis**
4. **Calculate sample concentration**
5. Calculate QC sample results
6. Evaluate QC data
7. Perform any necessary corrective action
8. Re-analyze any samples/QC as needed
9. Qualify data as needed

Key Steps

What are we trying to do here?

1. Convert all forms of phosphorus to phosphate (PO_4^{-3})

Organic + inorganic phosphorus $\xrightarrow[\text{acid}]{\text{Heat}}$ PO_4^{-3}

2. Produce a blue color equivalent to the amount of PO_4^{-3} present

$(NH_4)_2Mo_2O_7$ **+** $C_8H_4K_2O_{12}Sb_2$ **+** PO_4^{-3} $\longrightarrow$ $H_3PMo_{12}O_{40}$

$H_3PMo_{12}O_{40}$ **+** $\mathbf{C_6H_8O_6}$ $\longrightarrow$ Molybdenum blue

The Objective

Sample handling considerations

- ⇒ Refrigerate at 4°C; preserve w/ H_2SO_4 to pH < 2
- ⇒ Holding time = 28 days (not an issue for WWTPs)

*** Collect sufficient sample to allow re-testing if necessary ***

Critical reagent requirements

- ⇒ Dry potassium dihydrogen phosphate (KH_2PO_4) at 105°C for ≥ 1 hour before weighing (*better yet…purchase standards!*).
- ⇒ Prepare **ascorbic acid** (last addition to the combined color reagent) fresh weekly, store 4°C.
- ⇒ The combined solution should be mixed well after each solution addition.
- ⇒ **Combined color reagent** stable for only 4 hours. Warm all solutions; mix after each.
- ⇒ Wait ≥ 8 mins. After addition; read samples within 30 mins.

Sample and Reagent Handling

- 50 mL sample.
- +1 drop phenolphthalein; if pink, acidify with 30% H_2SO_4
- Add 1 ml of 11 N H_2SO_4
- Add 0.4 g ammonium *(or 0.5 g potassium)* persulfate.

Hotplate Digestion

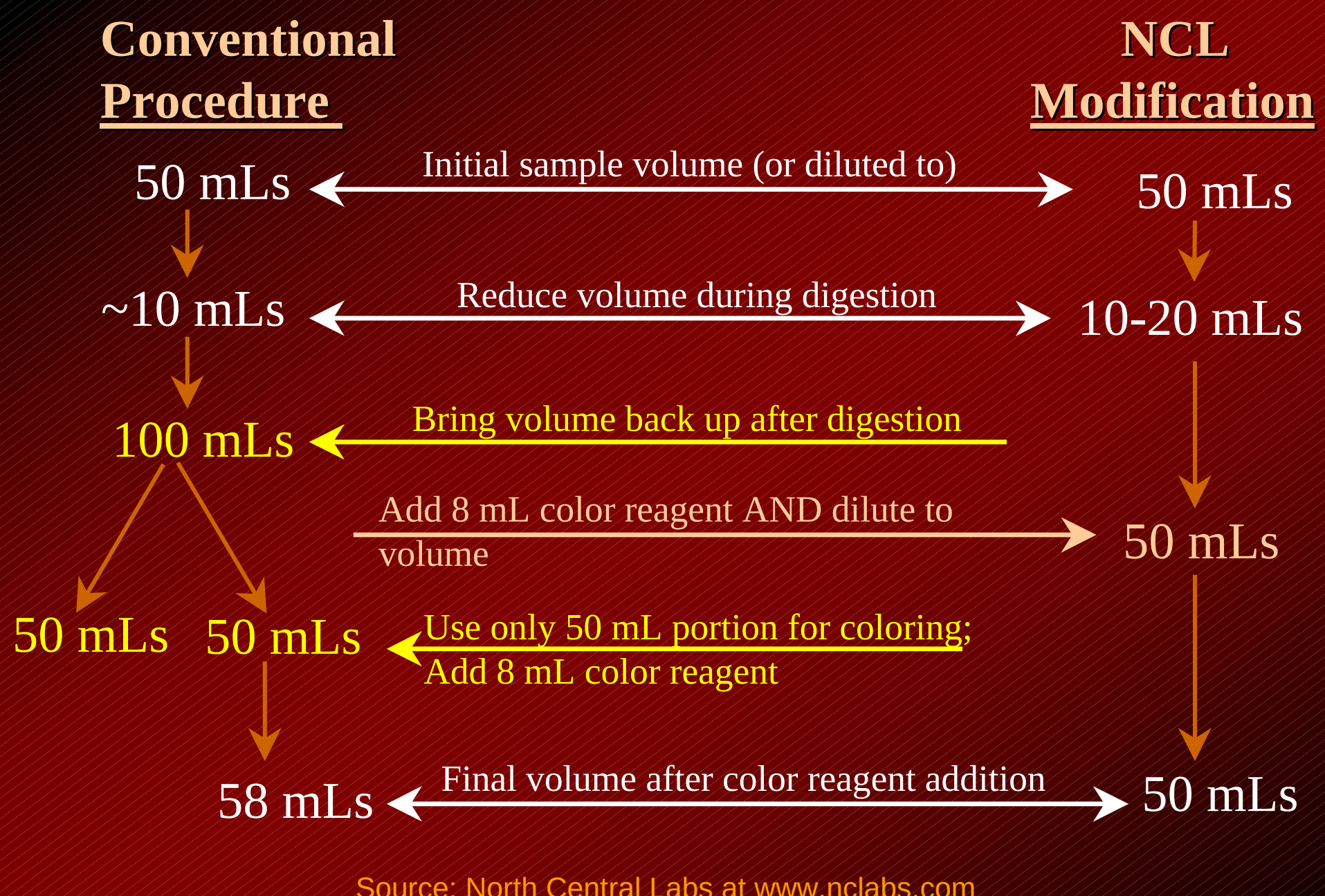
- Boil samples 30-40 mins. or until a final volume of 10 mL (whichever comes first)
- in no case should samples be boiled dry

Autoclave Digestion

- Autoclave for 30 minutes in an autoclave or pressure cooker
- Set the conditions for 15-20 psi. (98-137 kPa)
- Samples are not boiled dry

- Cool samples, standards, and blanks.
- +1 drop phenolphthalein. Neutralize w/ 1N NaOH ‘til faint pink.
- Dilute to 100 ml, but don’t filter.

Phosphorus Digestion



Source: North Central Labs at www.nclabs.com

An alternative approach

Three (3) techniques approved by NR 219:

- Single reagent, ascorbic acid (95 labs) *[650 or 880nm, BLUE]*
- Two reagent, ascorbic acid (1 lab) *[650 or 880nm, BLUE]*
- Automated, ascorbic acid (3 labs) *[650 or 880nm, BLUE]*

Since 95% of labs are using the ascorbic acid technique, we will limit our discussion to that method. QC will always be the same. Procedures very similar.

Other methods available (but not approved under NR 219)

- Vanadomolybdophosphoric acid *(400-490 nm, YELLOW color produced)*
- Stannous chloride *(690 nm, BLUE color, same principle as ascorbic acid)*

***Bottom line**: your lab needs to change procedures if….*
(1) you measure absorbance at <u>less than 650 nm</u>,
(2) the <u>color</u> of the solution you are measuring <u>is yellow</u>, or
(3) if you are <u>using stannous chloride</u> in the color-producing step.

Colorimetric Procedure

- Some plants develop seasonal color due to algae, etc.
- This color (or turbidity) may register background absorbance
- ...which amounts to high bias on phosphorus analysis
- Optimally, subtract “background” from true sample absorbance.
- Requires the measurement of absorbance from a "color blank".

PROBLEM:

How do we correct for bias
due to sample color/turbidity?

Dealing with natural sample color

- **Potassium antimonyl tartrate reagent**: Dissolve 1.3715 g $K(SbO)C_4H_{40} \cdot 1/2H_20$ in 400 ml reagent water in a 500 ml volumetric flask. Dilute to volume. Store in a glass-stoppered bottle.
- **Ammonium molybdate reagent:** Dissolve 20 g $(NH_4)_6Mo_70_{24} \cdot 4H_20$ in 500 ml reagent water. Store in a glass-stoppered bottle.
- **Sulfuric acid, 5N:** Dilute 70 ml conc. H_2S0_4 to 500 ml with reagent water.

***** *These first three reagents are typically purchased commercially* *****

- **Ascorbic acid, 0.01M:** Dissolve 1.76 g ascorbic acid in 100 ml reagent water. Store at 4°C. Discard after one week.

Combined Color Reagent (100 mLs)

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50 ml 5N sulfuric acid,
5 ml potassium antimonyl tartrate,
15 ml ammonium molybdate, and
30 ml ascorbic acid

Color Blank Reagent (100 mLs)

35 ml reagent water
50 ml 5N sulfuric acid, and

---

15 ml ammonium molybdate

Preparing color reagent

Absorbance of sample + color reagent

−

Absorbance of sample + color blank reagent

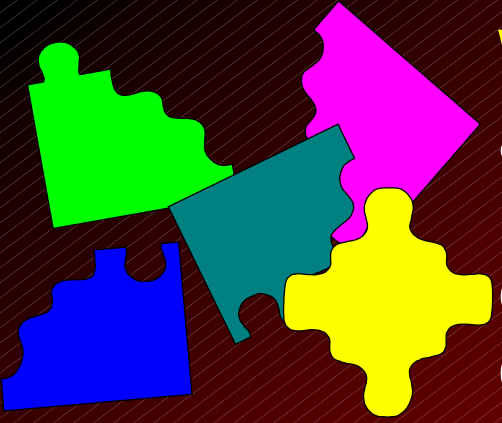
=

Absorbance due to phosphorus in sample

Convert absorbance to concentration using curve

NOTE: In order to do this, you will need to have 100 mL of sample

Correcting for sample color



What should we ZERO with:

a) undigested distilled water ONLY ?
b) undigested distilled water (+ color reagent) ?
c) DIGESTED distilled water (+ color reagent) ?
d) something else???

If you answered:

(a) Effect of digestion? Color Reagent?
(b) Effect of digestion?
(c) How to measure contamination?
(d) What else is there?

The methods don't help:

Standard Methods 4500-P E. [section c.]
...Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve

EPA method 365.2 [section 9.1.1.]
Process standards and blank exactly as the samples. Otherwise, silent on the issue.

Zeroing the spectrophotometer

The method blank tells us if there is any contamination related to the digestion/analysis procedure on that day.

Solution:

☑ No longer require calibration curve be digested.

☑ Zero the spec each day

☑ Zero with undigested reagent water + color reagent.

☑ With each "batch" of samples digested, there must be a Method Blank, and a Calibration Check

Can ONLY do this if you can demonstrate no significant difference between digested & undigested standards

Zeroing the spectrophotometer

mg/L Total P=

$$\frac{mg}{L} P \text{ (from curve)} \times V \times \frac{FV}{CV} \times \frac{1}{SV}$$

Where:

V = volume (mL) of sample + reagent water that was colored [**typically 50**]

FV = final volume (mL) after digestion [**typically 100**]

CV = volume (mL) of sample that was colored [**typically 50**]

SV = original volume of sample that was digested [**typically 50**]

Thus…using standard volumes for the manual test...

$$\frac{mg}{L} P \text{ (from curve)} \times \frac{2}{1}$$

$$= \frac{mg}{L} P \text{ (from curve)} \times 2$$

This ONLY works with the volumes listed. With any other volumes the formula changes.

Calculations

Problem	Possible Cause	Suggested Corrective Action
Standards / samples don't turn blue	Missing or bad ascorbic acid	Prepare fresh ascorbic acid & new combined color reagent
Samples yellow after digestion; but not standards	Excessive organic matter; oxidation of iron in sample	Use smaller sample volume; if iron: treat samples and standards with sodium bisulfite (EPA 365.3-3)
Mixed color reagent is dark yellow	Ascorbic acid may be bad	Prepare fresh ascorbic acid & new combined color reagent
All samples, standards and blanks turn blue after color reagent is added	Phosphorus contamination	Clean all glassware with dilute HCl; prepare all new reagents

Troubleshooting

Contamination!

- Wash glassware well, using a non-phosphate detergent
- THROW OUT your Alconox (~8.7% phosphorus)
- Rinse with dilute (1%) hydrochloric acid
- Even new glassware needs to be washed
- DO NOT touch <u>inside</u> glassware with bare hands!
- DO NOT smoke anywhere near testing or glassware storage
- Cover samples if you use autoclave for digestion
- Segregate glassware for TP (*but still rotate glassware*)

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.

Common Problems

Method Details - Test N’ Tube

- ☑ Turn on COD Reactor; set at 150º C (early versions say 105ºC)
- ☑ Add 5.0 mL sample to a "Total/Acid Hydrolyzable" Test Vial
- ❑ *(early version says) Add 2 mLs of 1.00 N sulfuric acid*

  newer vials already contain this
- ☑ Add one Potassium Persulfate Powder Pillow
- ☑ Cap; shake; set in COD Reactor for 30 minutes
- ☑ Cool
- ☑ Add 2.0 mLs of 1.54 N sodium hydroxide; cap & mix (early versions say 1.0 N sodium hydroxide)
- ☑ Add one PhosVer 3 Powder Pillow (does NOT fully dissolve)
- ☑ Cap; shake 10-15 secs.
- ☑ Time for 2 minutes
- ☑ Put vial into instrument and read*

** Read samples between 2 and 8 mins. after PhosVer 3 addition*

Test N' Tube instructions

Metals (a number of them) - But at levels you would not routinely encounter in wastewater

Arsenates - At any level. But these should be quite rare

Color/ High Turbidity - Inconsistent results.
Acid in powder pillow may dissolve some of the suspended particles.
Also, variable desorption of orthophosphate from the particles

Highly buffered samples or pH extremes - May exceed buffering capacity of the reagents and require pretreatment.

Test N' Tube interferences

Keep in mind:

A. The upper range of calibration (~ 1.0ppm)

B. The background concentration of your effluent (~0.5)

C. Spike concentration must be 1-5x background (0.5 to 2.5ppm)

Maneuvering room is A - B = 0.5 ppm

…or else less than 5 mLs of sample must be used

Suggestions:

- Spike with 0.5 mLs of a 5.0 mg/L Phosphorus solution (=0.5ppm)
- Use 4.5 mLs of sample (or less if background is > 0.5 ppm)
- Use same volume of sample in spiked and unspiked analysis
- Use reagent water as necessary to make total volume 5.0 mLs

Test N' Tube spiking

ADVANTAGES

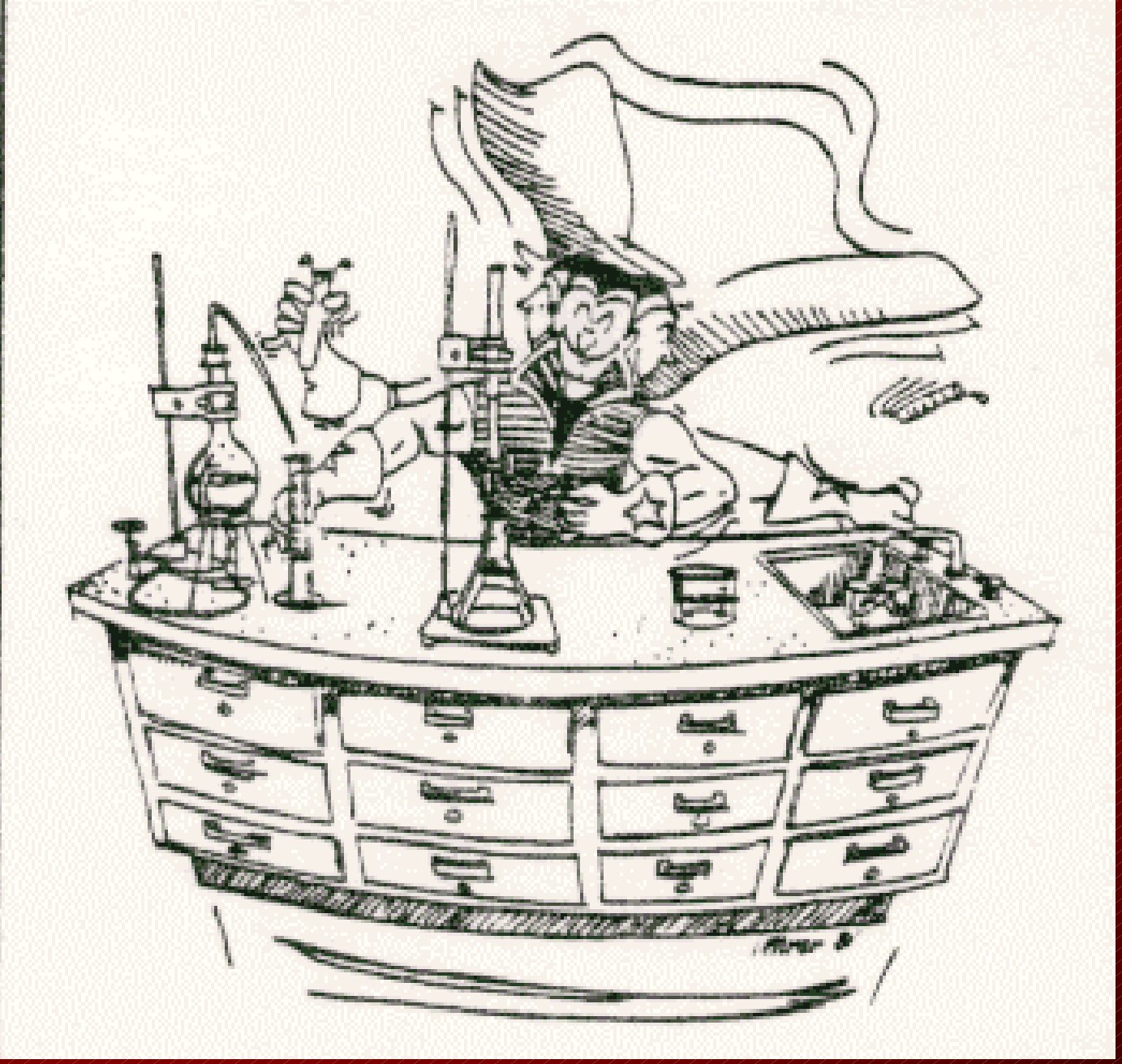
- ☑ Simple
- ☑ Quick
- ☑ Don’t have to deal with reagents
- ☑ No babysitting (won’t boil dry)

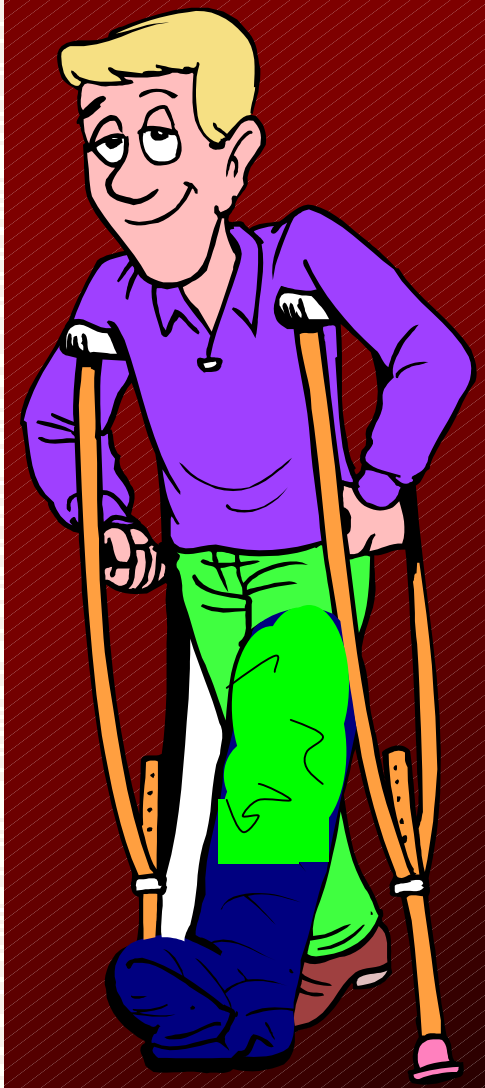
DISADVANTAGES

- ☑ Problematic for colored or turbid samples
- ☑ Not suited for highly buffered samples (high alkalinity)
- ☑ Preparing matrix spikes is difficult (limited sample volume)

Consider purchasing an autopipettor…it will simplify life

Test N’ Tube summary





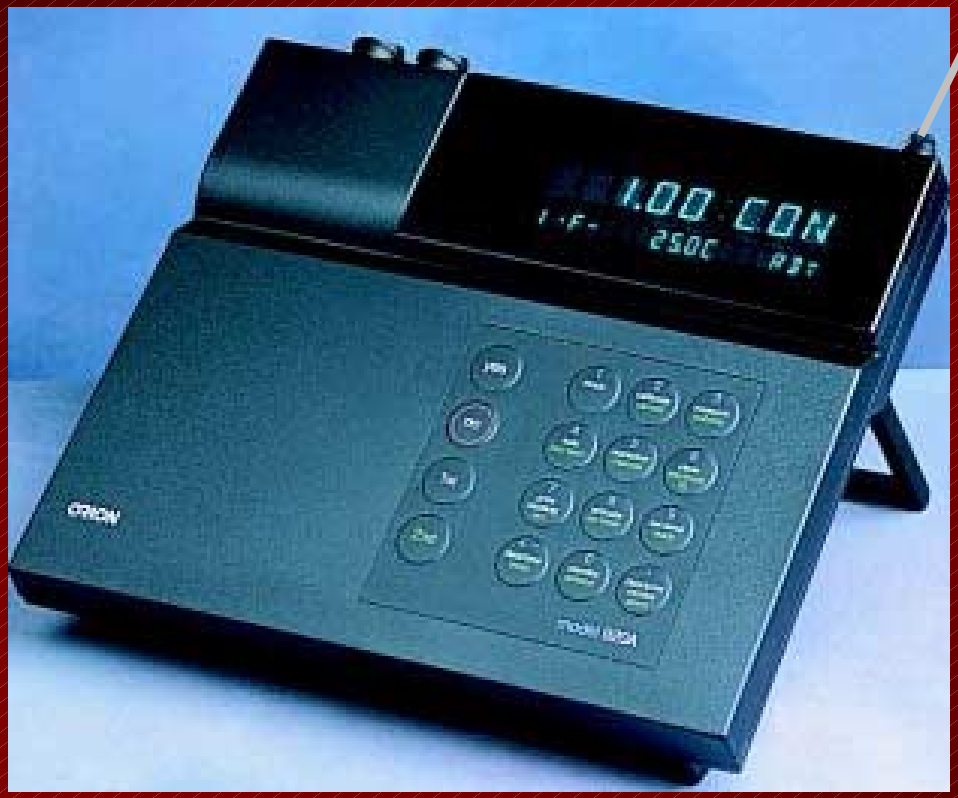
The amazing Graham!

Lunch!

Method Details - Ammonia

Courtesy of Orion:

Orion Model 720A
Orion Model 920A
ammonia ISE



Ammonia Equipment on display

1. Distillation??????????
2. **Calibrate**
3. **Determine sample concentration and millivolts**
4. Convert millivolts to **concentration**
5. Calculate QC sample results
6. Evaluate QC data
7. Perform any necessary corrective action
8. Re-analyze any samples/QC as needed
9. Qualify data as needed

Key steps

NR 219: distill samples…,or prove that [it] is not needed.

State Laboratory of Hygiene & DNR conducted a study:

- Municipal wastewater effluent
- Tested ammonia by ion selective electrode
- Comparison of values with and without distillation
- Conclusion: distillation not required
- Result: SLH granted a variance which extends to WWTPs

Variance limited to: domestic municipal effluent

Distillation, or a similar study, required if:

- receiving industrial or pre-treatment wastes,
- Paper mill effluents (a unique analytical matrix)
- if dealing with anything other than domestic municipal WW effluent,

Keep a copy of the SLH study on file

Obtain copies from:

- Laboratory Certification Program
- the State Lab of Hygiene

"Evaluation of Preliminary Distillation Prior to Ion Selective Electrode Determination of Ammonia in Municipal Wastewater Effluent"

To Distill or NOT to distill...

About 40% of small WWTP labs required to test for NH3:

Electrode (88% of the labs)

Nesslerization (5 labs)

Titration (4 labs)

Phenate (automated) (3 labs)

Not sure (*wrong or unclear method listed*) (15 labs)

We will be limiting our discussion to this, the predominant technique

Nessler reagent contains 100 g mercuric iodide per liter

2 mL Nessler reagent to 50 mL sample in Nessler tube

Each 2 mL of nessler reagent contains 88 mg of pure mercury

***** Mercury causes severe neurological disorders *****

88 mg in 52 mLs = 1.69 mg/L as mercury

Drinking water limit for mercury is 0.0002 mg/L [~ 1/10,000]

Contents of ONE tube would have to be diluted with ~ 130 gallons of water

Determinative Method Options

Make sure meter is warmed up!

1. Pour out 50 or 100 mL of sample.
2. Place the electrode in the beaker.
3. Turn on the magnetic stirrer.
4. Set the meter to begin reading
5. Add 1 ml of buffer solution (typically 10 M NaOH).

   (SM Buffer solution is based on 1 mL per 100 mLs of sample)

   (Orion buffer is based on 2 mLs per 100 mLs of sample)

   (Orion buffer stays blue if the sample pH is > 11)
6. Read millivolts and concentration (if using onboard software).

- Keep the electrode at an angle to minimize air bubbles
- Stir at the same speed for standards and samples.
- Prevent heating the solution; insulate between beaker & stirrer
- Do not add NaOH before immersing electrode

  --ammonia is in gaseous form at this pH and will be lost!

Ammonia ISE Procedure

- Electrodes work <u>slightly</u> differently than other analyses
- Response follows a logarithmic pattern (*Nernst equation*)
- Nernst's law: slope MUST be -54 to -60 mV (target = -58)

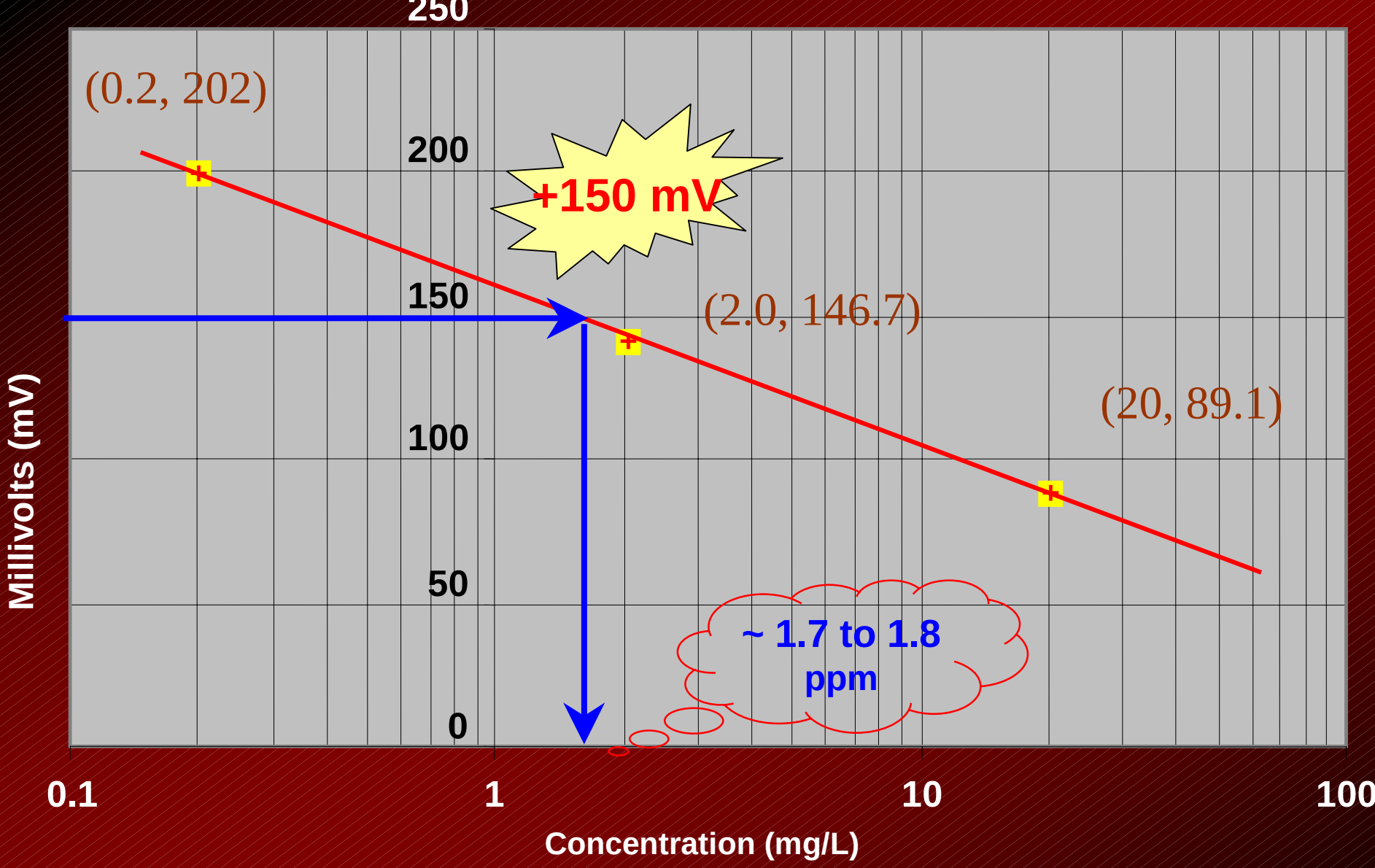
NOTE: if your full calibration range is TWO "decades, (e.g., 0.2 to 20),the difference in mV between the upper and lower standard should be 2 x 58.3 = 117mV

- Procedure identical to regular linear regression <u>*except*</u>:

….MUST first convert concentration to logarithm

…. Then, a second conversion is required to convert to mg/L

Log conversions



Unknown sample having response of +150 absolute mV

Calibration by Semi-Log Graphing

Calculator steps

Calibration Data

mg/L	mV
0.2	202
2.0	146.7
20	89.1

Set Mode to “2”: MODE 2

Clear the registers: 2nd F DEL

Enter 1st data pair: log 0 • 2 STO 2 0 2 M+

Enter 2nd data pair: log 2 STO 1 4 6 • 7 M+

Enter 3rd data pair: log 2 0 STO 8 9 • 1 M+

Obtain the calibration evaluation information

- ✓ Get the Correlation coefficient..: RCL ÷ r = - **0.99993**
- ✓ Get the Slope.....: RCL) b= -56.45
- ✓ Get the Intercept: .....: RCL (a = 162.9264

CALIBRATION - Entering Regression Data into Calculator

Converting millivolts (mV) to concentration

Perform a “back-calculation” check of the calibration

mV = 202
0.2 mg/L

2 0 2 2nd F (**-0.692**

2nd F log +/- • 6 9 2 = **0.203** **(101.5%)**

mV = 146.7
2.0 mg/L

1 4 6 • 7 2nd F (**0.287**

2nd F log • 2 8 7 = **1.94** **(97%)**

mV = 89.1
20 mg/L

8 9 • 1 2nd F (**1.308**

2nd F log 1 • 3 0 8 = **20.3** **(101.6%)**

Slope from 0.2 to 2.0 = **202 - 146.7= 55.3** should be 54 to 60

Slope from 2 to 20 = **146.7 - 89.1= 57.6** should be 54 to 60

Millivolt difference 0.2 to 20 = **202 - 89.1 = 112.8** (should be 108-120

CALIBRATION - Regression using the Sharp EL-520L

Probe, Probe, Probe!

Probes do NOT last forever!

- ✓ Average life expectancy is 2 years or less.
- ✓ If your probe is > 2 yrs old, consider getting a new one

DO NOT store probes in lab reagent water!

- ✓ Your probe will be deader than the proverbial doornail.
- ✓ DO store the probe in 1000 ppm NH_4Cl solution

AVOID calibrating below about 0.2 mg/L!

- ✓ It takes longer to stabilize than meter pre-set timer.
- ✓ Result will often be a poor slope or bias at the low end

Common problems - NH_3

Probe, Probe, Probe!

If you get negative mVs for any standards (≤ 20 ppm)…

…it's a sure sign that the probe/membrane is deteriorating

- ✓ Change membrane monthly or as needed
- ✓ You will ONLY see this if you calibrate "manually"

Be wary of temperature changes!

- ✓1-2% error per degree C change.
- ✓Samples & standards must be at the same temperature

Conditioning required?

- ✓If consistently have problems achieving valid calibration
- ✓try "shocking" the probe with a high (~ 10 ppm) standard

Common problems - NH_3

1. After receiving a new Ammonia probe, condition it in accordance with the manufacturers instructions.

2. With a new probe, you get new membrane filling solution. Throw old membranes and filling solution away...
   - membranes develop pinholes;
   - filling solution suffers from bacterial buildup.

3. Change the membrane and filling solution every 2-4 weeks.

4. Allow AT LEAST 2 hours stabilization time after changing the membrane and/or filling solution. *Overnight is better.*

5. For Orion probes: After filling the outer body, hold it at an angle and tap gently to get rid of any air bubbles.
   - After assembling the probe, GENTLY pull on the cable at the top of the probe to get rid of trapped air bubbles.
   - One major cause of unstable readings is trapped air bubbles at the bottom of the probe!

Source: North Central Labs at www.nclabs.com

Important Ammonia Probe hints

6. For short term storage (≤ 1 week):

Store probe in 1000 ppm Ammonia Standard, WITHOUT NaOH or ISA buffer.

For longer periods of storage:

- Disassemble probe, drain filling solution,
- Rinse inner and outer body with DI water,
- Air-dry, and reassemble dry.

7. Calibrate from lowest to highest concentration
8. Put probe into solution at an appropriate angle
9. Allow sufficient time for stabilization

NCL suggests waiting at least 4 minutes after adding NaOH.

Keep in mind:

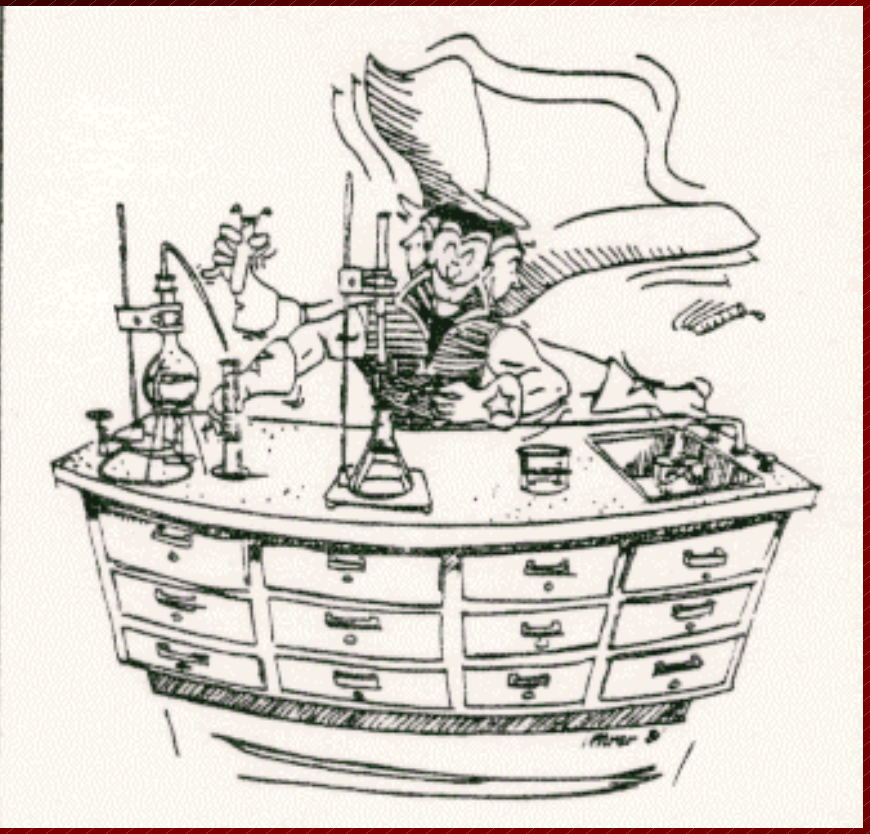
Standards made with Ammonium Chloride(NH_4Cl); probe measures ammonia (NH_3).
High pH buffer converts NH_4Cl to NH_3, but the reaction IS NOT instantaneous.
Many report low initial slope; if recalibrate w/ same standards, slope is in range.
Calibration must be completed within 20 minutes, as the ammonia gas volatilizes.

10. Consider performing an "inner body" check (see handouts)

Source: North Central Labs at www.nclabs.com

Important Ammonia Probe hints

back for an encore performance...



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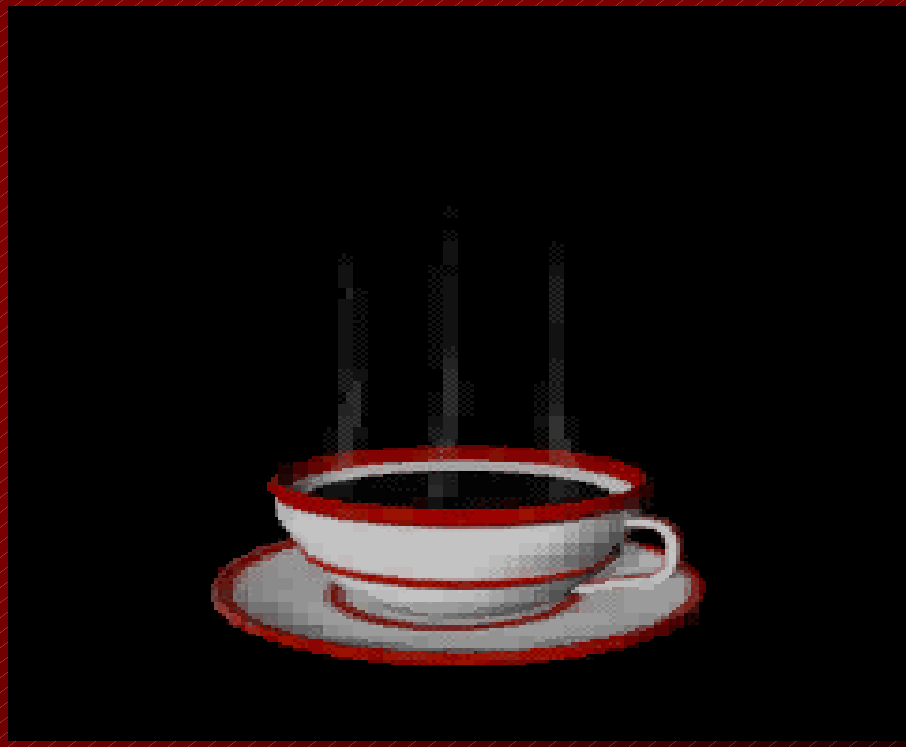


The amazing Graham!

Quality Control

1. Spike concentration (should be close to the expected LOD)
   Ammonia: Try 0.5 to 1.0 mg/L
   Phosphorus: Try 0.1 to 0.2 mg/L
2. 7 replicates at this concentration
3. Use EPA formula (**see handout**)
4. Perform the mandatory first 3 of the "5-point check"
5. **Repeat annually, or with any "major" changes**

***** If you need assistance, talk to us at a break or call us *****

LOD determinations (*see handouts*)

Why: To evaluate the accuracy of method as influenced by specific matrices (sample types).

How: Add known amount of analyte to randomly selected routine samples

When: 5% of samples (1 per 20 samples)

NOTE: Raw + effluent = 2 samples

What:

1. Calculate % recovery
2. Evaluate performance against control limits

Matrix Spikes

1. Consider the upper calibration range!

If you calibrate to 1 ppm...
your effluent is about 0.25 ppm....
and you spike at 1.0 ppm.....will exceed the calibration range.

2. Spike at 1 to 5 times the level in the sample

If effluent averages 1 ppm, spike between 1 and 5 ppm

- If you spike at less than 1x, risk the ability to recover it.
- As spike increases beyond 5X, TOO easy to recover.

3. Limit additional volume to <10% of sample

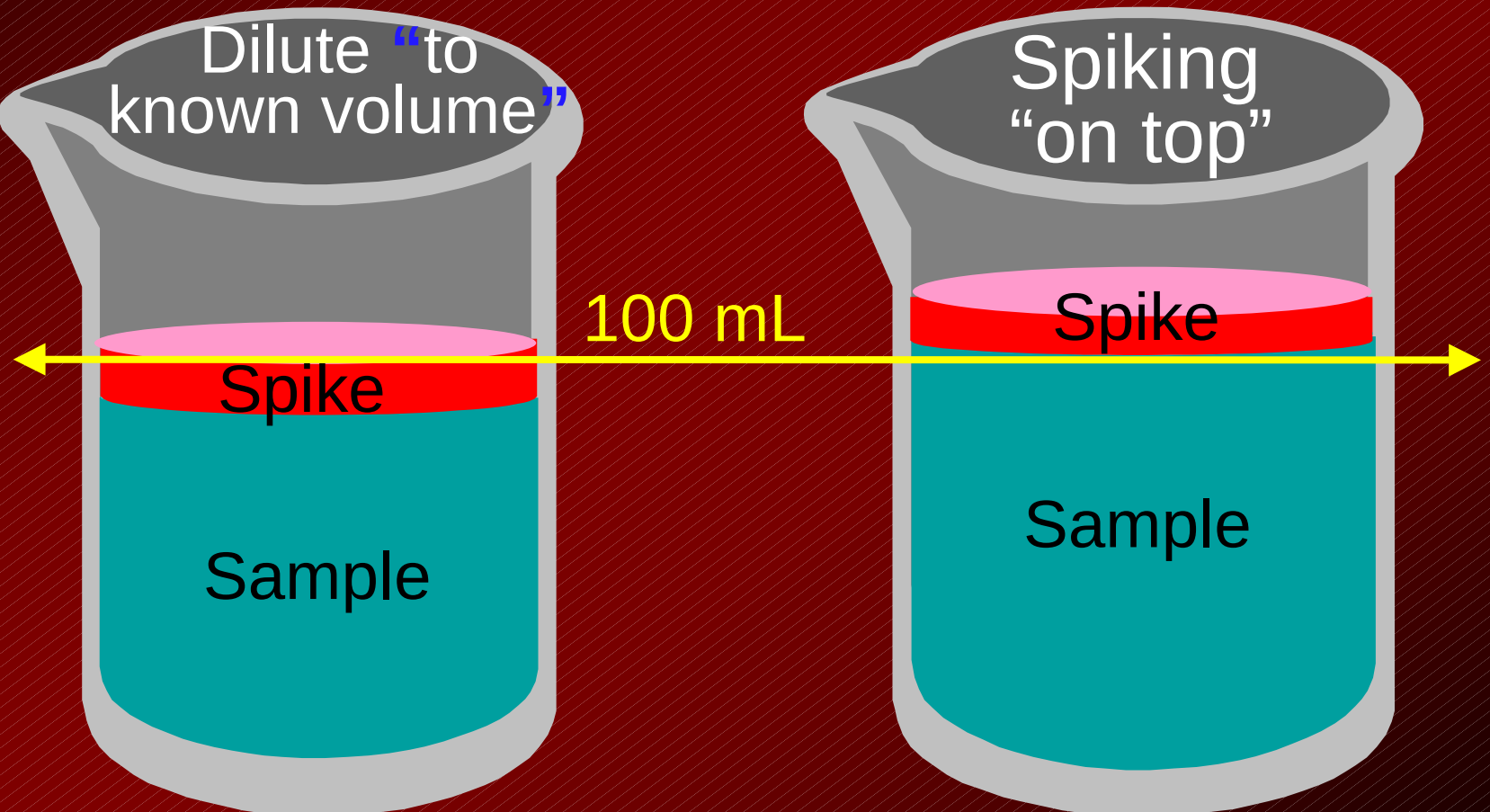
(i.e., 0.5 mL standard to 5 mL with sample)

- The spike faces less matrix than in the unspiked sample

Matrix Spikes: How <u>much</u> should you spike?

- If you dilute spike (with sample) to a known volume….
  (amount of sample used in spike is LESS than that in the unspiked)
  sample concentration must be adjusted.

- If the spike is added "on top of" the sample
  (amount of sample used in spike is SAME as in the unspiked)
  the *spike concentration* must be adjusted as well.



The goal of a matrix spike is to provide us with information regarding how accurate our sample analysis results are.

If spike recovery is only 50%, then the potential exists that the true concentration of the target parameter is as much as twice the measured concentration.

Dilution - How much is too much?

- ✔ The bottom line is that you want to use as much sample for the matrix spike as you did for the sample itself.
- ✔ References vary on how much dilution is TOO much.
- ✔ Our guidance has been to limit dilution to 10% or LESS.
- ✔ If dilution from the spike is kept to 1% or less… direct subtraction of unspiked sample is allowed.

Labs frequently prepare spikes by diluting a sample 50:50 with the spike solution. The next slide provides a more graphic explanation for why this is inappropriate.

Matrix Spikes

Calculation of % Recovery

$$\% \text{ Recovery} = \frac{\text{Spiked Sample} - \text{Unspiked sample}}{\text{Amount of spike added}} \times 100$$

seems simple enough.....BUT….

it really works *only* if dilution from the spike is kept to 1% or less

Results will vary depending on whether you

- simply add the spike on top of the sample, or you
- add the spike first & dilute to a fixed volume with sample, and
- is further compounded by whether or not you are digesting
- …and even depends on the type of digestion involved

Alternative: Employ a "mass-based" calculation approach

Matrix Spikes-Recovery

	Phosphorus			Ammonia	
	Hot-Plate	Autoclave	Test N' Tube	Pre-Distill	As is
Spike "To a Known Volume"	DV	DV	DV	DV	DV
Spike "On Top"	---	DV, SC	DV	---	DV, SC

DV= Correct for difference in sample volume used in sample vs. matrix spike
If I use 50 mLs for the sample, but only 45 mLs in the spiked sample
I cannot just subtract the concentration of the sample
Instead, the subtraction is (45/50) x the sample concentration

SC= Correct for spike concentration changes due to additional volume
If I spike 10 mLs of a 100 ppm standard on top of 100 mLs of sample,
The spike concentration is NOT 10 ppm (I.e. 10% dilution of 100 ppm)
The spike concentration is (10/110) x 100 ppm

Spike calculation considerations

Calculation of % Recovery

$$\% \text{ Recovery} = \frac{\text{Spiked Sample} - \text{Unspiked sample}}{\text{Amount of spike added}} \times 100$$

Wastewater Lab operator/analyst "Joe" measures out 50 mLs of sample, and places the beaker on a stir plate. He then adds 1 mL of buffer solution. After stabilizing, the meter reads 2.0 mg/L ammonia.

Unspiked sample 2.0 ug/mL
Unspiked Sample Volume 50 mL

Joe" then measures out another 50 mLs of sample to prepare a mnatrix spike. To the 50 mLs of sample he adds 5 mL of a 25 mg/L ammonia standard. This beaker is then placed on the stir plate. He then adds 1 mL of buffer solution. After stabilizing, the meter reads 4.25 mg/L ammonia.

Spike volume	**5 mL**	**Spiked sample**	**4.25 ug/mL**
Spike Conc.	**25 ug/mL**	**Total volume**	**55 mL**

What's the % recovery?

Spike Recovery Exercise

A. Determine the contribution (ug) from the sample in the spike

1. Subtract the mLs of the spike from the total mLs of sample + spike

2. Multiply the answer from A.1 (above) by the sample concentration

B. Determine the # of ug (of analyte) **spiked**

Multiply the concentration of the solution used to spike by the # mLs spiked

C. Determine the # of ug (of analyte) **in the spiked sample**

Multiply the spiked sample concentration by the # mLs of this sample

D. Determine the # of ug (of analyte) **<u>recovered</u>**

Subtract "A" from "C"

% Recovery = *Divide "D" by "B" and multiply by 100*

key relationship: mg/L = ppm =ug/mL

Calculating %Recovery by mass

Unspiked sample **2.0 ug/mL** **Spiked sample** **4.25 ug/mL**
Unspiked Sample Volume 50 mL **Total volume 55 mL**

Spike volume 5 mL
Spike Conc. 25 ug/mL

A. Contribution (ug) from the sample in the spike = **100**

2.0 ug/mL X (55 mL - 5 mL) = 2.0 X 50

B. The # of ug (of analyte) **<u>spiked</u> =** **125**

25 ug/mL X 5 mL

C. The # of ug (of analyte) **in the spiked sample =** **233.75**

4.25 ug/mL X 55 mL

D. The # of ug (of analyte) **<u>recovered</u> =** **133.75**

= C - A = *233.75 - 100*

% Recovery = **107.0%**

= D / B =*(133.75 / 125) X 100*

Example: Ammonia- adding "on top"

Matrix Spikes: Ammonia example- adding "on top"

Unspiked sample 2.0 ug/mL **Spiked sample 4.25 ug/mL**
Unspiked Sample Volume 50 mL **Total volume 55 mL**

Spike volume 5 mL
Spike Conc. 25 ug/mL

A. Correct the concentration in the unspiked sample = 1.82

2.0 ug/mL X (50/55) mL = 2.0 x 0.91

B. Correct the spike concentration = 2.27

25 ug/mL X (5/55) mL = .091

C. Calculate recovered concentration = 2.43

=(4.25 ug/mL - 1.82 ug/mL

% Recovery = 107.0%

= (C / B) x 100 *=(2.43 / 2.27) X 100*

Conventional calculation- "adding on top"

Unspiked sample 3.263 ug/mL **Spiked sample 5.625 ug/mL**
Unspiked Sample Volume 100 mL **Spiked sample volume 100 mL**

Spike volume 20 mL
Spike Conc. 20 ug/mL

Contribution (ug) from the sample in the spike = _____
_____ *ug/mL X (_____ mL - _____ mL) = _____ X ____*

B. The # of ug (of analyte) **spiked =** _____
_____ *ug/mL X _____ mL*

C. The # of ug (of analyte) **in the spiked sample =** _____
_____ *ug/mL X _____ mL*

D. The # of ug (of analyte) **recovered =** _____
_____ - _____

% Recovery = ____%
(_____ / _____) X 100

Example: Ammonia- dilute to known volume

Unspiked sample .246 X 25 = 6.15ug/mL
Unspiked sample Volume 2mL=>50 mL

Spiked sample .346x 25= 8.65 ug/mL
Total volume 2 mL + 1 mL =>50 mL

Spike volume 1 mL
Spike Conc. 5 ug/mL

A. Contribution (ug) from the sample in the spike = _____

_____ *ug/mL X* ____ *mL* ***or*** _____ *ug/mL x* ____ *mL*

B. The # of ug (of analyte) **spiked** = _____

_____ *ug/mL X* _____ *mL*

C. The # of ug (of analyte) **in the spiked sample** = _____

_____ *ug/mL X* _____ *mL* ***or*** _____ *ug/mL x* _____ *mL*

D. The # of ug (of analyte) **recovered** = _____

= C - A = _____ - _____

% Recovery = ____%

= D / B =(_____ / _____) *X 100*

Example: Total Phosphorus (Influent)

Unspiked effluent = 0.40 ug/mL
Unspiked sample Volume 4.5mL

Spiked effluent = 0.80 ug/mL
Total volume= 5 mL

Spike volume 0.5 mL
Spike Conc. 5 ug/mL

A. Contribution (ug) from the sample in the spike = _____

_____ ug/mL X [____ mL - ____ mL] = _____ ug/mL X ____ mL

B. The # of ug (of analyte) **<u>spiked</u> =** _____

_____ ug/mL X _____ mL

C. The # of ug (of analyte) **in the spiked sample =** _____

_____ ug/mL X _____ mL

D. The # of ug (of analyte) **<u>recovered</u> =** _____

= C - A = _____ - _____

% Recovery = ____%

= D / B =(_____ / _____) *X 100*

Example: Test N' Tube

Why: Used to evaluate repeatability (reproducibility)

How: Analyze randomly selected routine samples in duplicate (including digestion steps)

When: 5% of samples (1 per 20 samples)

NOTE: Raw + effluent = 2 samples

What: 1. Calculate Range (or RPD)
2. Evaluate performance against control limits

NOTES:
1. Replicates are frequently termed “Duplicates”. The terms are interchangeable.
2. Precision is concentration dependent

Replicates

Evaluating Replicates

Based on absolute difference (Range) or Relative percent difference (RPD) between duplicates

Example

Sample = 2.2

Replicate =1.8

Range

expressed in same units as values

= Absolute Difference

= Larger value – smaller value

Range = 2.2 - 1.8 = 0.4

RPD

expressed as %

$$\text{RPD} = \frac{\text{Range}}{\text{Mean of the replicates}} \times 100$$

RPD = Range / Mean

Range = 0.4

Mean = (2.2 + 1.8)/2

= 2.0

RPD = (0.4/2.0) x 100

= 20%

Replicates - measuring precision

“Precision is concentration dependent”

Which is a brief way of saying that precision is difficult to evaluate without knowing the concentration levels involved.

Consider the following Phosphorus results

- The range of replicates is 0.5 mg/L
- First thought: “Gee...that’s terrible!”
- But.....what if the two values were 10 and 10.5 mg/L?
- Now 0.5 doesn’t look so bad.
- But....your opinion changes if the values are 0.6 and 1.1
- At this level, the range could cause a permit exceedance

Concentration dependency

- Consider using RPD
- Separate control limits based on concentration

  Ex. effluent NH_3 about 0.01 to 1 ppm; influent 0.1 to 50 ppm
- Start at 10 x the LOD or LOQ…or even the permit limit?
- MAY need <u>more</u> than two levels

  But you don't want so many different levels that you will never generate enough data to create your own limits either

Concentration dependency

Sample	Replicate	Range	RPD
152	161	9	5.8%
161	168	7	4.3%
143	151	8	5.4%
136	142	6	4.3%
155	160	5	3.2%
172	177	5	2.9%
164	155	9	5.6%
150	158	8	5.2%
145	137	8	5.7%
140	147	7	4.9%
125	119	6	4.9%
170	162	8	4.8%
143	149	6	4.1%
132	136	4	3.0%
152	146	6	4.0%
144	138	6	4.3%
189	180	9	4.9%
167	175	8	4.7%
130	140	10	7.4%
153	146	7	4.7%
Mean	**151.75**	**7.1**	**4.7%**
Stdev			1.1%
UWL		17.8	6.8%
UCL		**25.3**	**7.9%**

Sample	Replicate	Range	RPD
200	230	30	14.0%
300	330	30	9.5%
400	**430**	**30**	**7.2%**

As concentration increases, a point is reached where range fails but RPD passes.

Sample	Replicate	Range	RPD
100	107	7	6.8%
50	57	7	13.1%
25	32	7	24.6%
10	17	7	51.9%
5	12	7	82.4%

As concentration decreases, a point is reached where range passes but RPD fails.

RPD or Range?

Sample	Replicate	Range	RPD
200	216	16	7.7%
250	270	20	7.7%
300	324	24	7.7%
350	378	28	7.7%
400	**432**	**32**	**7.7%**

As concentration increases, the absolute range can increase accordingly with NO CHANGE in RPD

For a given range, as concentration increases, RPD decreases

For a given range, as concentration decreases, RPD increases

When might this be of use?

If your system is susceptible to high I & I

If your system exhibits high variability in influent loading

Is RPD for you?

There are many statistical tests available for identifying outliers. One that is relatively easy to use is the Grubbs test.

$$Z = \frac{|\text{mean} - \text{questionable data point}|}{SD}$$

- Ignore the sign of the "Z" value….is always " **+** "
- For replicates, test <u>only</u> the highest value
- For spikes, test both the lowest & highest values
- <u>Include</u> suspect outlier when calculating mean, SD
- If the calculated Z-value **>** Critical Z value
  for that number of data points,
  then the value is an outlier

N	Critical Z
18	2.65
19	2.68
20	**2.71**
21	2.73
22	2.76
23	2.78
24	2.80
25	2.82
26	2.84
27	2.86
28	2.88
29	2.89
30	2.91
35	2.98
40	3.04
50	3.13
60	3.20

Dealing with outliers

DATE	Range
11/01	0.4
11/04	1.1
11/07	1.0
11/10	1.1
11/13	1.0
11/16	1.2
11/19	5.8
11/22	0.2
11/25	0.5
11/28	0.4
12/01	0.1
12/04	0.4
12/07	0.1
12/10	0.7
12/13	0.1
12/16	1.0
12/19	0.9
12/22	0.8
12/25	0.8
12/28	0.2

1. Calculate the mean and SD

Mean = 0.890 SD = 1.216 **Control Limit = 2.91**

2. Test the high value (**5.8**)

$$Z = \frac{5.8 - 0.89}{1.216} = 4.0378$$

N	Critical Z
18	2.65
19	2.68
20	2.71

3. Discard outliers; re-calculate mean and SD

Since $Z_{5.8}$ > criterion, 5.8 is an outlier

new Mean = 0.6316 new Control Limit = **2.06**

NOTE: Step 3 may also require a re-check for additional outliers!

For spikes, test both outlying high AND low values

For replicates, test only outlying HIGH values

Testing for outliers

Matrix spike & RPD Control limits

1. Test the data for and eliminate outliers before proceeding.
2. Calculate the mean and standard deviation of the data.
3. Warning limits = Mean $\pm$ 2 standard deviations
4. Control limits = Mean $\pm$ 3 standard deviations

NOTE: RPD is a 1-tailed test, so only Mean +

Range Control limits

1. Test the data for and eliminate outliers before proceeding.
2. Calculate the mean of the data.
3. Warning limits = 2.51 x Mean
4. Control limits = 3.27 x Mean

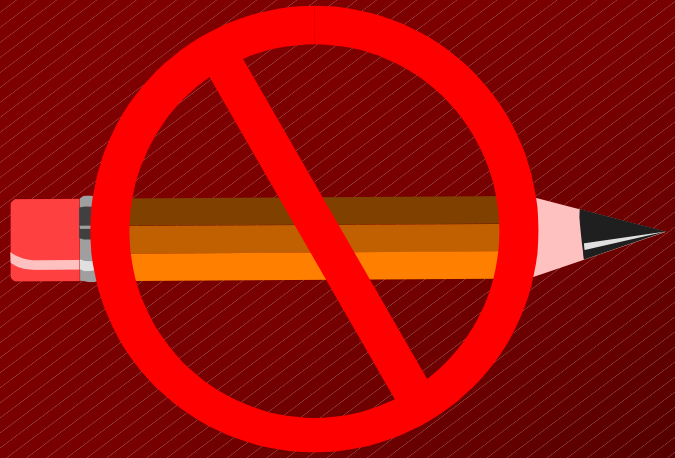
Calculating control limits

Documentation

A laboratory is required to:

maintain records: **NR 149.06 [esp. (5)]**

- which are <u>un-alterable</u>, *[what does THAT mean?]*
- which enable complete traceability *[by whose definition?]*
- for a given three-year compliance period



<u>Operating Principles</u>

☑ If you didn't document it, you didn't do it

☑ You did the work.....take credit for it!

Documentation basics

- Tables are better than lots of text!
  - ✓the old “a picture is worth 1000 words” concept
  - ✓Tables FORCE you to be brief

3 rules for building a QA Plan by tables

What am I evaluating? (parameter)

How do I evaluate it (criteria)

What if it doesn’t meet specifications? (Corrective Action)

Setting up an effective QA Plan

Evaluating?	Criteria	Corrective Action
Method Blank	< LOD	1) Identify contamination source 2) Correct Problem 3) Qualify data
Calibration slope (ammonia)	-54 to -60 mV target = **-58** mV (at 20° C)	1) Enough time to stabilize? 2) Low standard too low? 3) Change membrane 4) Perform inner body check
Matrix Spike	Within Control Limit(s)	1) Correct calculation? 2) Spike 1-5x sample? 3) Spike another sample 4) Qualify data
Replicates	Within Control Limit(s)	1) Homogeneous sample? 2) Analyze a third time 3) Review control limits 4) Qualify data

Your QA Plan

	Ammonia	Phosphorus
Calibration	Each analysis day At least 3 stds Slope 54-60 mV if LSR, r $\geq$ 0.995	When check fails at least quarterly At least 3 stds + blank if LSR, r $\geq$ 0.995
Calibration Check	90-110% of true value 1/10 (if needed)	each day of analysis 90-110% of true value
Blank	each day of analysis less than the LOD	
LOD	determine initially repeat annually	

QC Requirement summary

Ammonia & Phosphorus

Matrix Spikes	1/20 samples/matrix Calculate control limits Optional: 80-120% Check for outliers
Replicates	1/20 samples/matrix Calculate control limits if RPD: < 20% Check for outliers
Reference Samples	Pass at least 1 per yr
Blind Standards	Analyze & pass 3/yr 3-4 months apart if fail, analyze another

QC Requirement summary II

Have available for any inspection

- Digestion (or distillation information) *copy of SLH study!!*
- Calibration data (be able to relate to analyses)

If asked to see data from August 1997…can you show the calibration data?

- ALL sample-related information and raw data
- Clearly show any initial dilutions
- Matrix spike preparation and calculations
- Calculations and data associated with control limits
- Control limits in use over time (most recent 3 years)
- Any Corrective Action (including maintenance)
- Blind Standard performance (3 yrs) *+ any corrective action*

Documentation

Facility Name: Tree City, WI 111222333

X Ammonia X Total Phosphorus Benchsheet

Sample Location (specific)		Sample Type (grab, __ hr Comp, etc.)
Raw	see schematic	24 hr. composite (flow weighted)
Final	see schematic	24 hr. composite (flow weighted)
Other		

Sample Date: 5/2/00 Distillation Date: N/A Analyst: _____

Collected by: RGM Digestion Date : 5/2/00 Analyst: RGM

Analysis Date: 5/2/00 Analyst: RGM

Calibration Date: Phosphorus: 4/3/00 Ammonia 5/2/00

General Benchsheet Info

For all calibrations

Slope = 0.8553 (monitor for consistency or significant changes)

Intercept = -0.0018 (*should* be less than the LOD)

For linear regressions

correlation coefficient (r)= 0.999972 (*should* be ≥ 0.995)

Concentration mg/L = [sample absorbance - intercept]

slope

For ALL calibrations ----------→ For linear regressions

Standard Concentration (mg? or mg/L?)	Absorbance @ 880 nm	Regression concentration[1] (mg? or mg/L?)	
Blank 0	0	0.002	
1. 0.1	0.081	0.096	96%
2. 0.2	0.170	0.201	100%
3. 0.4	0.339	0.398	99.6%
0.5	0.428	0.502	100%
0.8	0.682	0.799	100%

LOD= 0.025

[1] obtained by solving for concentration using the absorbance of the standards

Phosphorus Benchsheet: Calibration

True=	[1]Known Standard 0.6 mg/L	Influent (Raw)	Effluent (Final)	Replicate (of Final)	Matrix Spike # (of Final)
Sample Volume mLs	50	0.5@	25	25	0.5@
Absorbance (*after coloring*)	0.531	0.037	0.486	0.418	0.234
Absorbance (*before coloring*)	-----	-----	-----	-----	-----
Net Absorbance	0.531	0.037	0.486	0.418	0.234
Dilution Factor (DF)	1	$\frac{50}{5} \times \frac{50}{5}$=100	50/25=2	50/25=2	$\frac{50}{5} \times \frac{50}{5}$=100
* mg/L (*from calibration*)	0.622	0.045	0.570	0.490	0.276
** Final mg/L as P	103.8%	4.50	1.14	0.98 Range = 0.16 RPD = 15.1%	% Recovery = 115.5%

@= Raw sample: Diluted 5 mLs to 50 mLs initially, then took 5 mLs of that dilution and made up to 50 mLs for digestion

#= Matrix spike = 2 mLs of a 5ppm standard (Lot #0112437) added to the 50 mLs of sample prepared as with the unspiked raw sample

** Final concentration = $\frac{\text{mg}}{\text{L}}$ *from calibration* X DF

0.276 x 50 = 13.8 ug
-
0.045 x 50 = 2.25 ug
11.55 ug

DF = $\frac{\text{Initial dilution final mLs}}{\text{Initial dilution sample mLs}}$ X $\frac{\text{mLs digested for standards}}{\text{mLs digested for sample}}$ X $\frac{\text{mLs colored for standards}}{\text{mLs colored for sample}}$

$\frac{11.55 \text{ ug}}{2 \times 5 = 10 \text{ ug}}$ X100= 115.5%

Phosphorus Benchsheet: Data

Calibration by: Internal (Direct Read):_____ Linear regression: __X__
Relative millivolts: _____ Semi-logarithmic paper: _____

All calibrations - Slope (per decade*) = __-54.5__ (must be -54 to -60 mV)

** "decade" refers to a 10-fold change in concentration, e.g. 1 to 10 ppm*

Linear regressions - Intercept = __90.34__ (should not be > LOD)
correlation (r)= __-0.99866__ (*should* be ≥ 0.995)

$\Delta_{0.2\text{-}20}$ = **109 mV** expect 108-120
$\Delta_{0.2\text{-}2.0}$ = **49.6 mV** expect 54-60
$\Delta_{2\text{-}20}$ = **59.4 mV** expect 54-60

Standard Concentration (mg/L)	Millivolts (mV)	Log_{10} of concentration[1] (mg/L)	Regression concentration[2] (mg/L)	
Blank				
0.2	126.8	- 0.669	$10^{-0.669}$ = 0.214	107 %
2.0	77.2	0.241	$10^{0.241}$ = 1.742	87.1 %
20	17.8	1.33	$10^{1.33}$ = 21.38	106.9%

$$\text{Concentration} = \text{Inverse/antilog of } \frac{[\text{sample mV} - \text{intercept}]}{\text{slope}}$$

Ammonia Benchsheet: Calibration

	Influent (Raw)	Effluent (Final)	Replicate (of Raw@)	Matrix Spike (of Final#)
Distilled? (Y/N)	N	N	N	N
Sample Volume mLs	10	50	10	45→50 with spike
Dilution Factor (DF)	50/10=5	1	50/10=5	1
Millivolts (mV)	94.9	114	99.7	85.4
* mg/L from calibration	$10^{-0.084}$=0.824	$10^{-0.434}$= 0.368	$10^{-0.172}$=0.673	$10^{0.091}$= 1.23
** Final mg/L as NH_3	4.12	0.37	3.36 Range = 0.76 RPD = 20.3 %	%Recovery=89.7%

@= Raw sample: Diluted 10 mLs of raw to 50 mLs with deionized water for analysis

#= Matrix spike = 5 mLs of a 10ppm standard (Lot #Xb7A28) diluted to a final volume of 50 mLs with sample.

1.23 x 50 = 61.5 ug
-
0.37 x 45 = 16.65 ug

44.85 ug

** Final concentration = mg/L from calibration X DF

Dilution Factor (DF) = $\frac{\text{mLs used for standards}}{\text{mLs used for sample}}$

$\frac{44.85 \text{ ug}}{10 \times 5 = 50 \text{ ug}}$ X100= 89.7%

Ammonia Benchsheet: Data

- ☑ Reviewed background information
  - Ammonia
  - Phosphorus
- ☑ Discussed calibration concepts
- ☑ Reviewed the methods in detail
- ☑ Highlighted QA/QC requirements
- ☑ Provided resolutions to common problems
- ☑ Discussed what documentation is required
- ☑ Put it all together [your QA manual]

Summary

We **DO** realize you are frequently “wearing many hats”

Ever feel like this?

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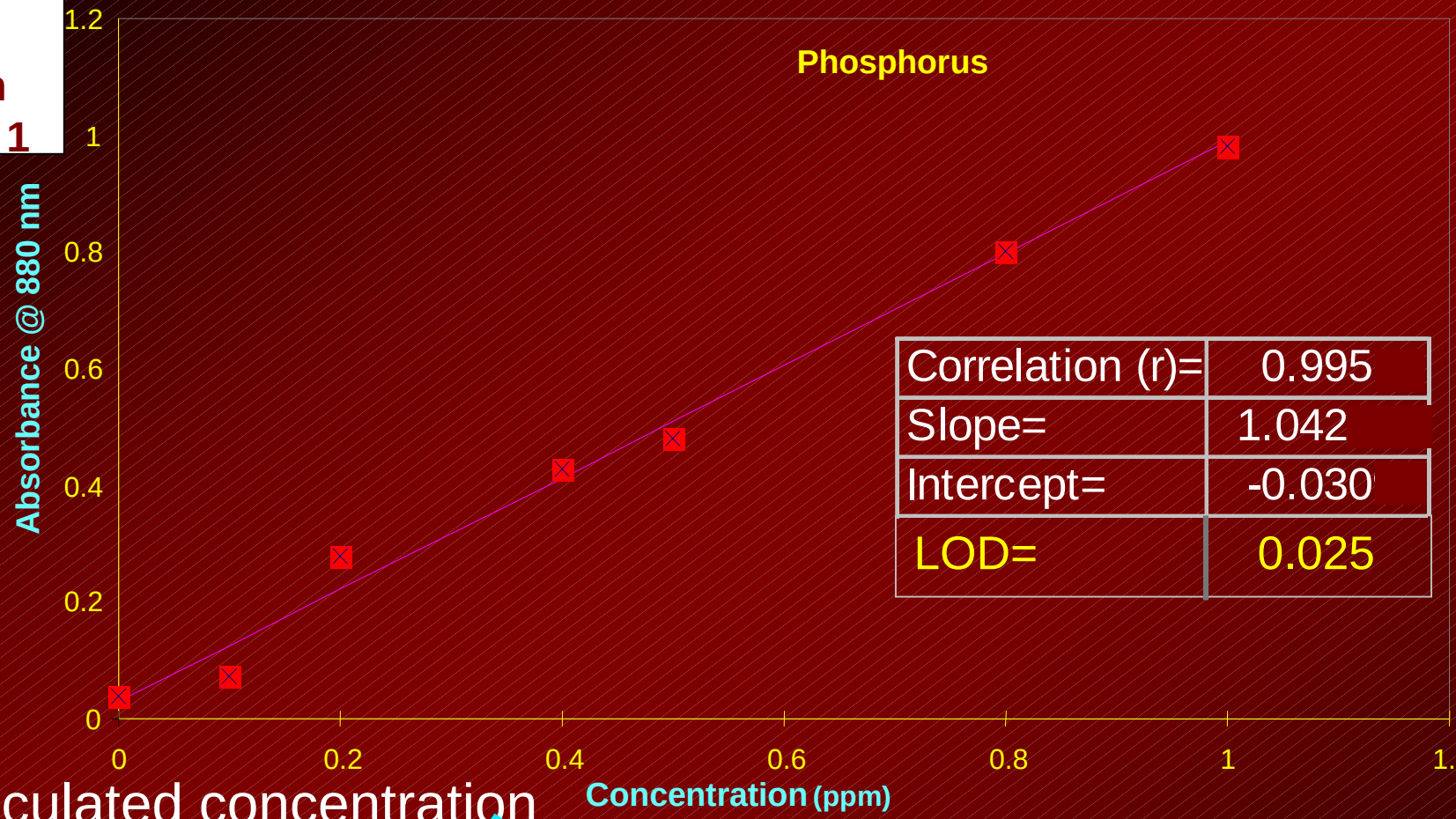
http://www.dnr.state.wi.us/org/es/science/lc/

LabCert "toolbox":
http://www.dnr.state.wi.us/org/es/science/lc/toolbox/

For more information

Answer to Calibration Exercise # 1



Correlation (r)=	0.995
Slope=	1.042
Intercept=	-0.030
LOD=	0.025

Back-calculated concentration

Concentration (mg/L)	Abs. (Measured)	Residual mg/L
0	0.04	0.01
0.1	0.075	0.05
0.2	0.28	0.26
0.4	0.43	0.42
0.5	0.48	0.47
0.8	0.8	0.80
1	0.98	0.99

YOU be the auditor!

Acceptable calibration?

Visual: Good, not great

correlation: OK

Residuals: Fail at low end

Unspiked sample 3.263 ug/mL **Spiked sample 5.625 ug/mL**
Unspiked Sample Volume 100 mL **Spiked sample volume 100 mL**

Spike volume 20 mL
Spike Conc. 20 ug/mL

A. Contribution (ug) from the sample in the spike = 261.0

3.263 ug/mL X (100 mL - 20 mL) = 3.263 X 80

B. The # of ug (of analyte) **spiked = 400**

20 ug/mL X 20 mL

C. The # of ug (of analyte) **in the spiked sample = 562.5**

5.625 ug/mL X 100 mL

D. The # of ug (of analyte) **recovered = 301.5**

562.5 - 261.0

% Recovery = 75.4%

(301.5 / 400) X 100

Solution: Ammonia- dilute to known volume

Unspiked sample .246 X 25 = 6.15ug/mL
Unspiked sample Volume 2mL=>50 mL

Spiked sample .346x 25= 8.65 ug/mL
Total volume 2 mL + 1 mL =>50 mL

Spike volume 1 mL
Spike Conc. 5 ug/mL

A. Contribution (ug) from the sample in the spike = **12.3**

0.246 ug/mL X 50 mL ***or*** *6.15 ug/mL x 2 mL*

B. The # of ug (of analyte) **<u>spiked</u> =** **5**

5 ug/mL X 1 mL

C. The # of ug (of analyte) **in the spiked sample =** **17.3**

0.346 ug/mL X 50 mL ***or*** *8.65 ug/mL x 2 mL*

D. The # of ug (of analyte) **<u>recovered</u> =** **5**

= C - A = *17.3 - 12.3*

% Recovery = **100.0%**

= D / B =*(5 / 5) X 100*

Solution: Total Phosphorus (Influent)

Unspiked effluent = 0.40 ug/mL
Unspiked sample Volume 4.5mL

Spiked effluent = 0.80 ug/mL
Total volume= 5 mL

Spike volume 0.5 mL
Spike Conc. 5 ug/mL

A. Contribution (ug) from the sample in the spike = 1.8

0.4 ug/mL X 5 mL- 0.5 mL = 0.4 ug/mL X 4.5 mL

B. The # of ug (of analyte) **<u>spiked</u> = 2.5**

5 ug/mL X 0.5 mL

C. The # of ug (of analyte) **in the spiked sample = 4.0**

0.80 ug/mL X 5 mL

D. The # of ug (of analyte) **<u>recovered</u> = 2.2**

= C - A = *4.0-1.8*

% Recovery = 88.0%

= D / B =*(2.2 / 2.5) X 100*

Solution: Test N' Tube