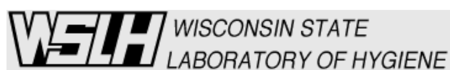
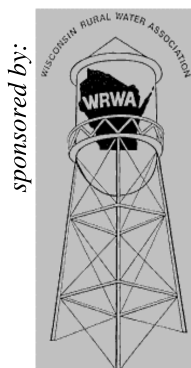
Phosphorus and Ammonia: Testing, QC, and Troubleshooting





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Disclaimer

Session Objectives

- ✶ 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 critical QC requirements
- ✶ Discuss documentation required
- ✶ Provide necessary tools to pass audits

Course Outline

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

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

In WI 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

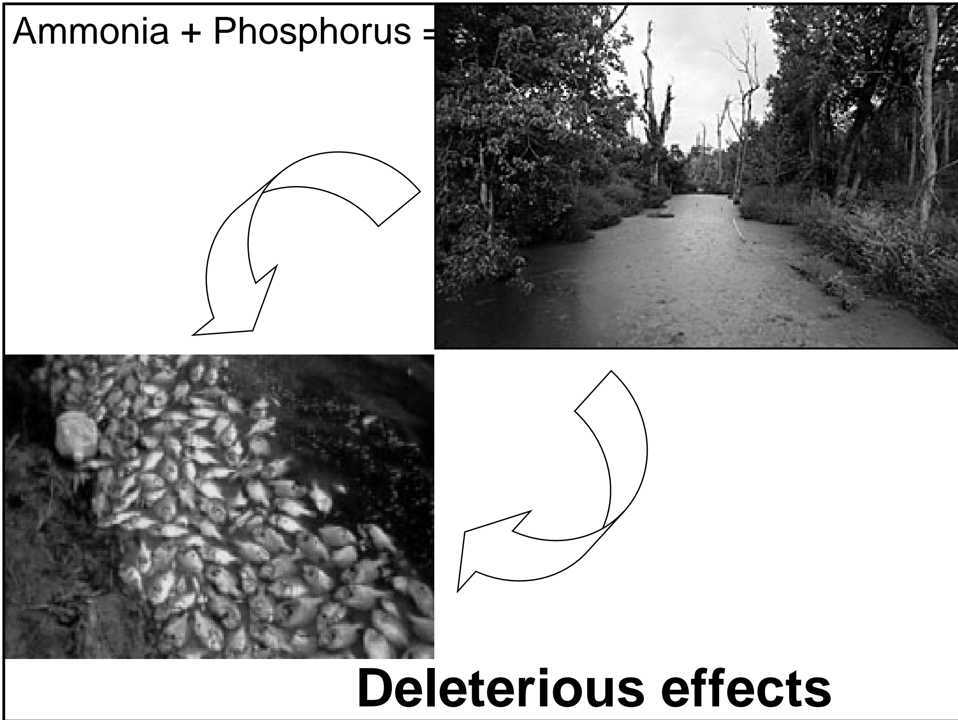
Phosphorus

- 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 WI wastewater labs must test for phosphorus.
- …and it continues to be written into permits

Phosphorus sources

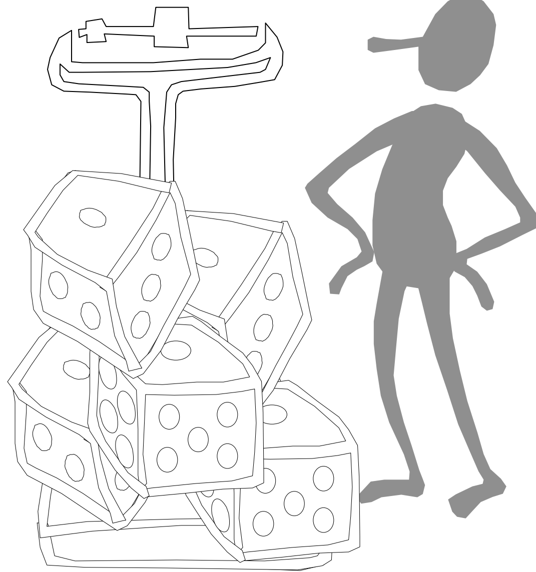
Phosphorus levels and effects	
mg/L as P	**Effects**
0.003-0.010	Amount of phosphate-phosphorus in most uncontaminated lakes
0.008	Accelerates the eutrophication process in lakes
0.033	Recommended maximum for rivers and streams

Phosphorus Critical Levels



Deleterious effects

…which leads to something like this



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.

> *[WI] 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..."

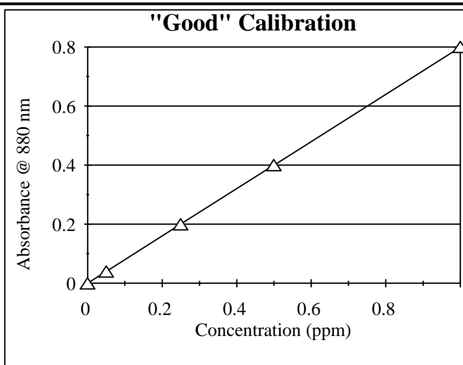
Mandatory
Ongoing calibration checks

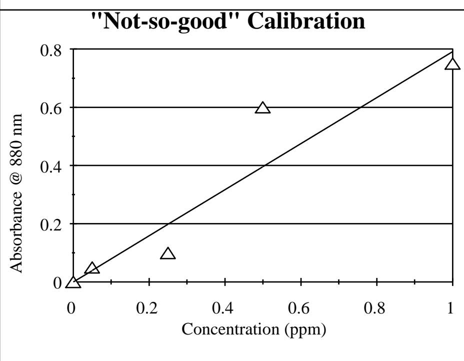
Optional
Correlation coefficient (if using linear regression)
Visual
Linearity check (response factors)
Residual analysis ("*back-calculation*")

☆☆☆ **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

$r^2 \neq r$

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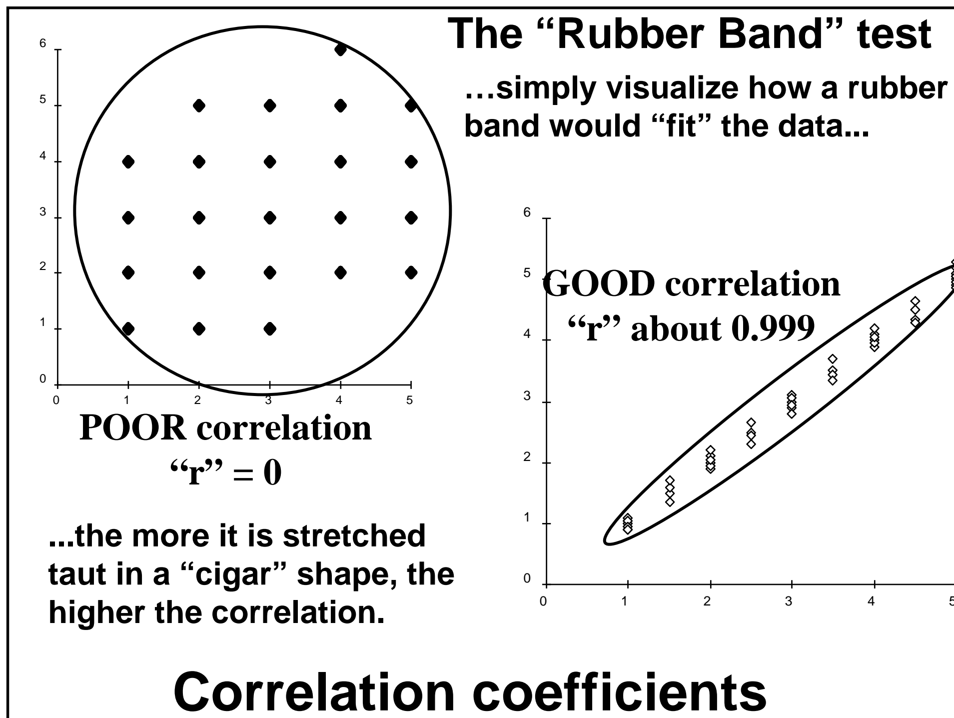
if your instrument/software provides r^2, then:

- 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



Linearity check

- 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) = $\frac{\text{Response } (= \textit{Absorbance})}{\text{Concentration}}$

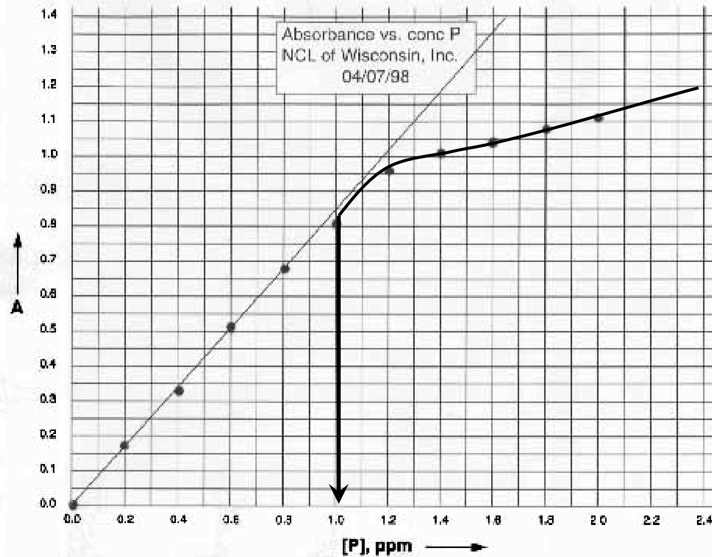
*** If you identify **non-**linearity, reduce the calibration range ***

Linearity check - response factors

mg/L	Abs.	RF (Abs / mg/L)		Rise in Abs. Per 0.2 mg/L
0	0			
0.20	0.175	0.875	Low end variability	0.175
0.40	0.325	0.813		0.150
0.60	0.500	0.833	Midpoint: stability	0.175
0.80	0.675	0.844		0.175
1.00	0.825	0.825		0.150
1.20	0.950	0.792	Steady decline in RF characteristic of exceeding linear range	0.125
1.40	1.000	0.714		0.050
1.60	1.050	0.656		0.050
1.80	1.075	0.597		0.025
2.00	1.150	0.575		0.075

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

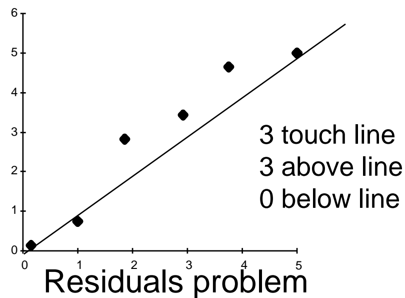
Phosphorus is non-linear above about 1.0 mg/L



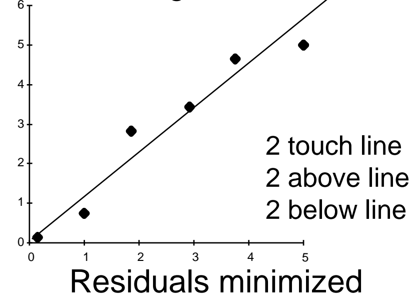
Source: North Central Labs at www.nclabs.com

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 $\pm$ 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….

Absorbance = *slope* x Concentration + *intercept* (crossed out)
- intercept ***- intercept*** (crossed out)

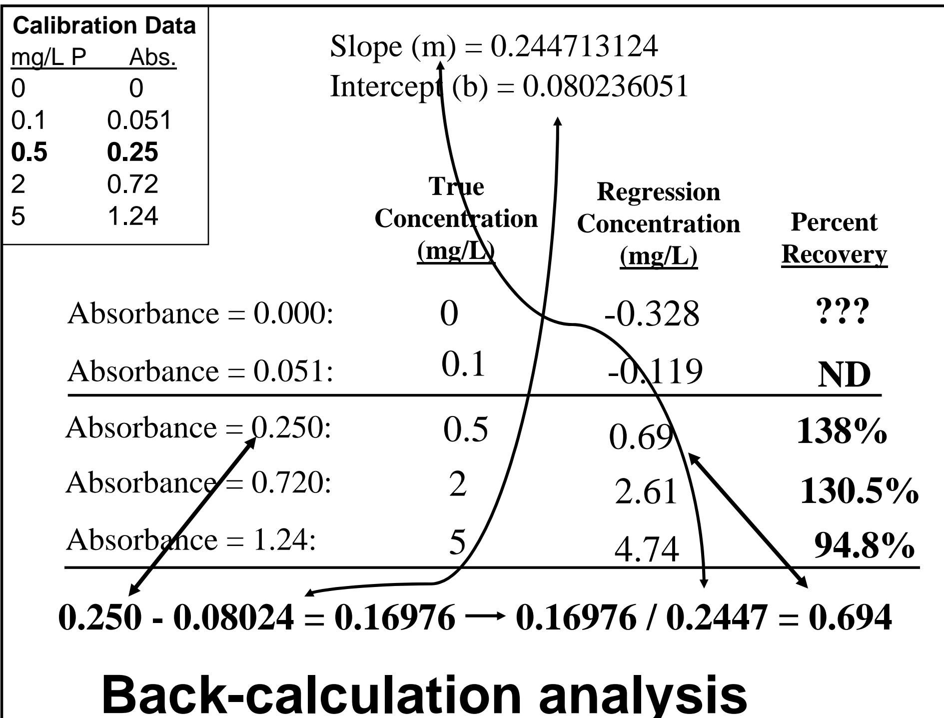
$$\frac{\text{Absorbance} - intercept}{slope} = \frac{slope}{slope} \times \text{Concentration}$$

(*slope* crossed out)

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

NOTE: some calculators and Excel switch Concentration & Absorbance

First…a little math lesson



Analytical Evaluation - ongoing

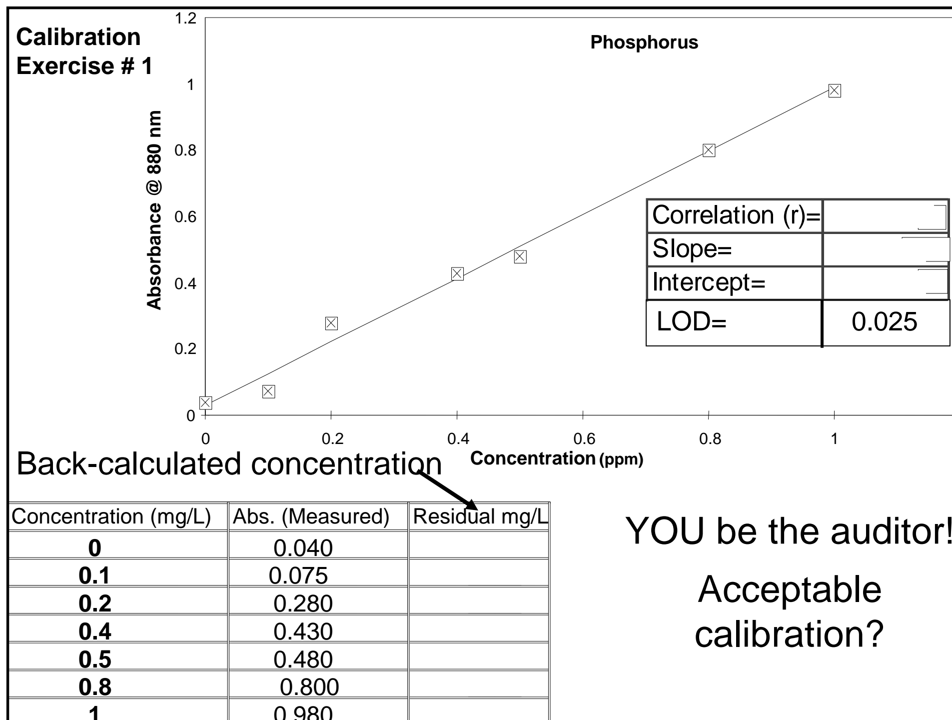
- Periodically confirm that response has not changed from initial.
- Use a midpoint standard (check standard) and blank.
- Calculate % recovery.
- Check at <u>beginning</u>, <u>every 10</u> samples and <u>end</u> of each batch.

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

  Recovery must be within 90-110% for phosphorus and ammonia. (in WI)

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

Calibration - daily checks



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?

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

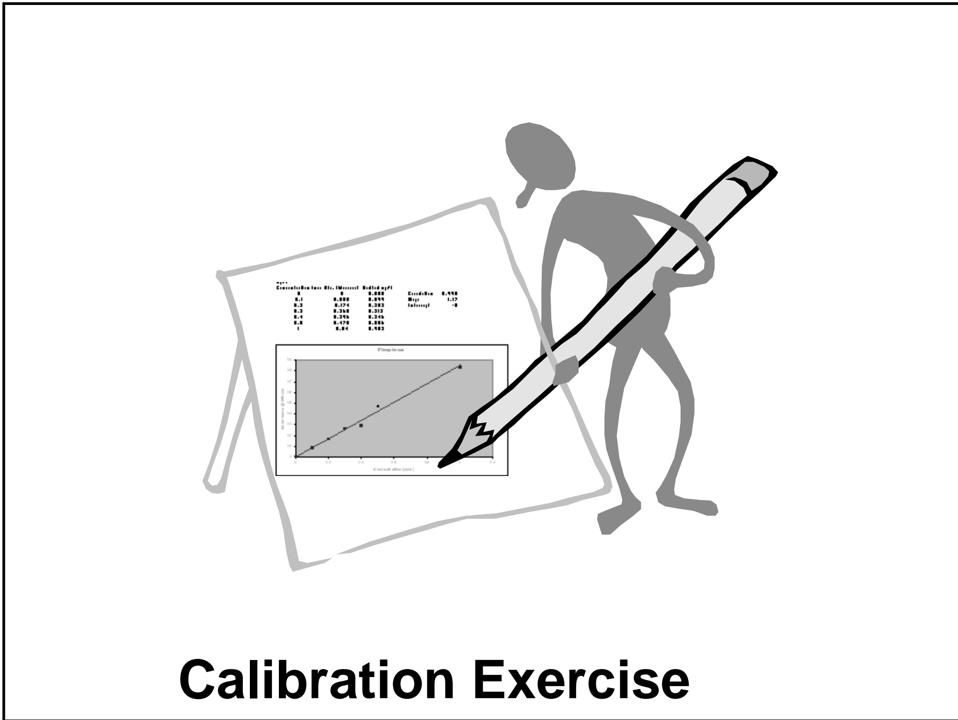
Obtain the calibration evaluation information

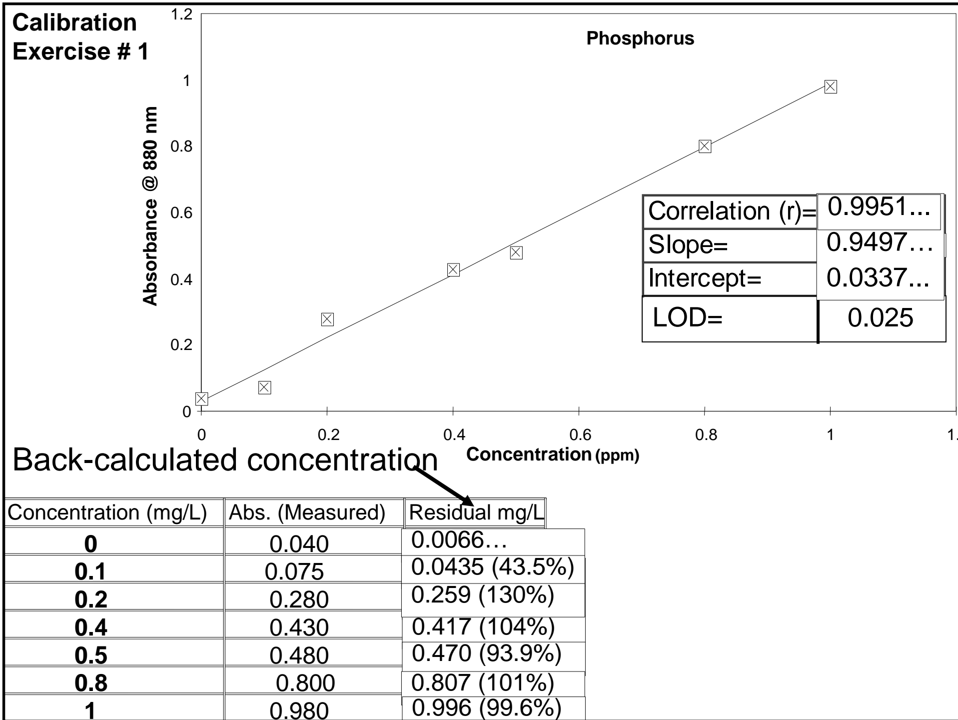
- ✓ Get the Correlation coefficient..: RCL ÷ r = 0.9951…..
- ✓ Get the Slope..........................: RCL) b= 0.9497…..
- ✓ Get the Intercept: ....: RCL (a = 0.03368…..

Converting a sample absorbance to concentration

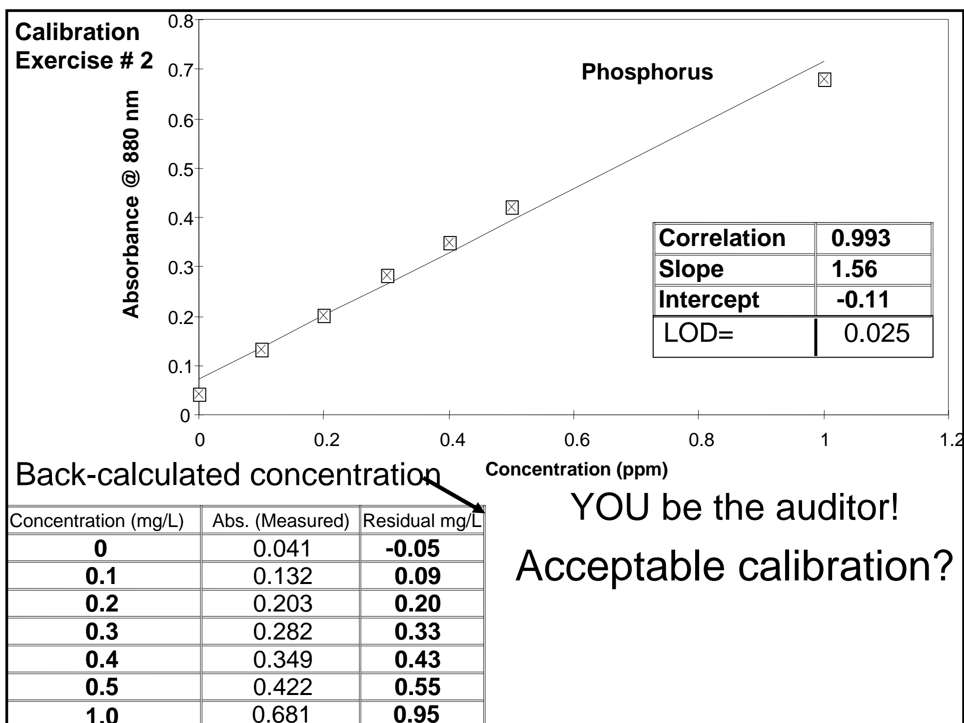
0.04:	• 0 4	2nd F (	0.007 This value should be < LOD			
0.075:	• 0 7 5	2nd F (	0.044	÷ • 1	× 1 0 0 =	44 %
0.28:	• 2 8	2nd F (	0.26	÷ • 2	× 1 0 0 =	130 %
0.43:	• 4 3	2nd F (	0.42	÷ • 4	× 1 0 0 =	105 %
0.48:	• 4 8	2nd F (	0.47	÷ • 5	× 1 0 0 =	94 %
0.8:	• 8	2nd F (	0.80	÷ • 8	× 1 0 0 =	100 %
0.98:	• 9 8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

“Back” Calculating Standards

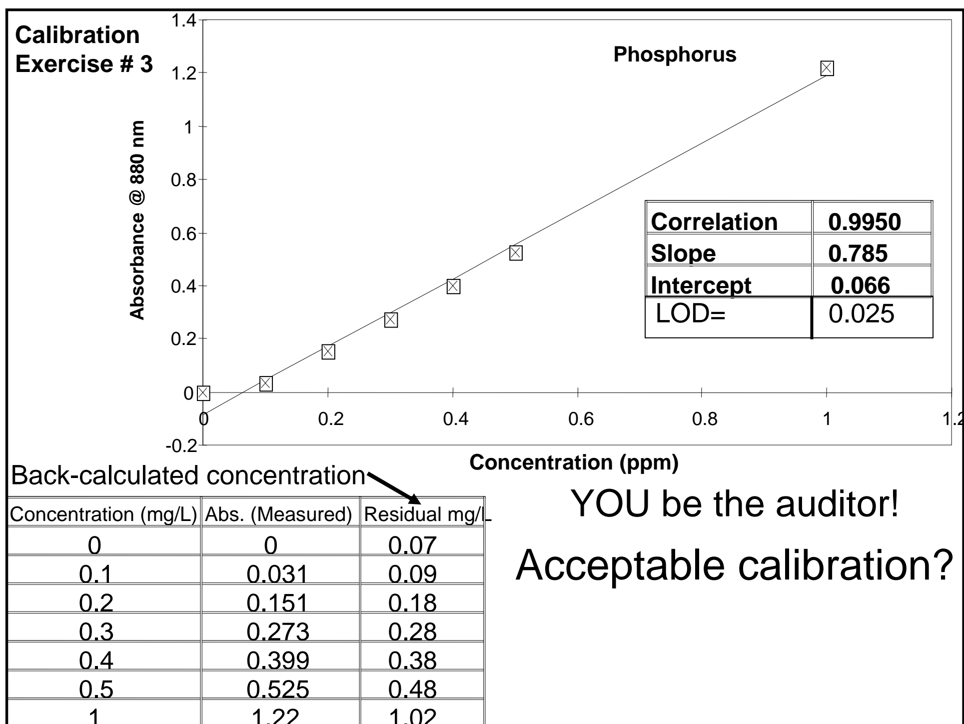




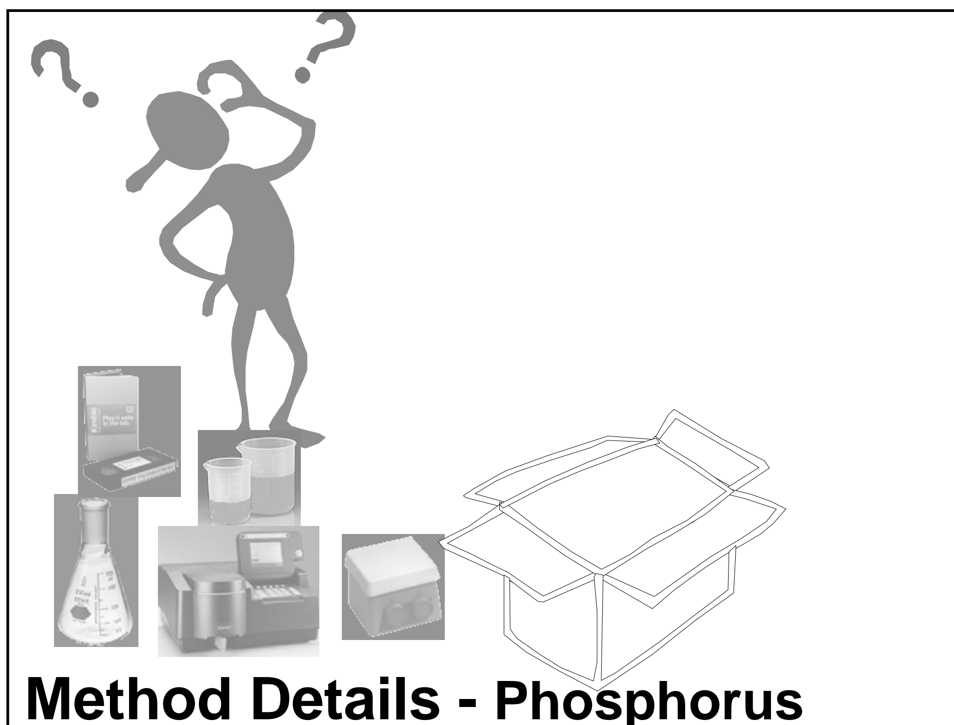
Concentration (mg/L)	Abs. (Measured)	Residual mg/L
0	0.040	0.0066…
0.1	0.075	0.0435 (43.5%)
0.2	0.280	0.259 (130%)
0.4	0.430	0.417 (104%)
0.5	0.480	0.470 (93.9%)
0.8	0.800	0.807 (101%)
1	0.980	0.996 (99.6%)

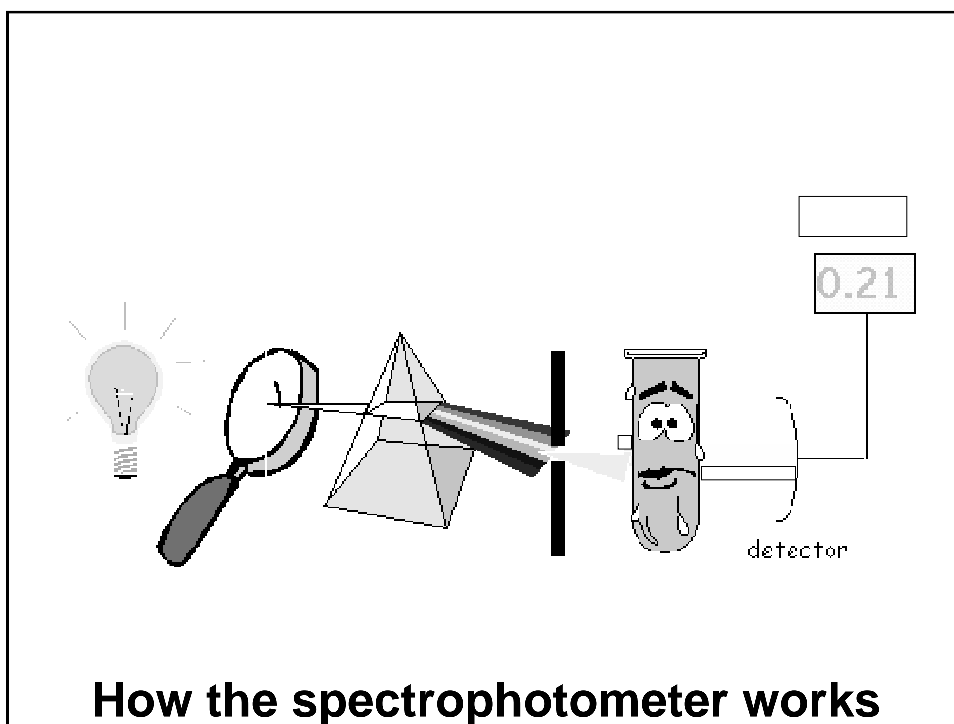


Concentration (mg/L)	Abs. (Measured)	Residual mg/L
0	0.041	**-0.05**
0.1	0.132	**0.09**
0.2	0.203	**0.20**
0.3	0.282	**0.33**
0.4	0.349	**0.43**
0.5	0.422	**0.55**
1.0	0.681	**0.95**



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


Method Details - Phosphorus


0.21
detector
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

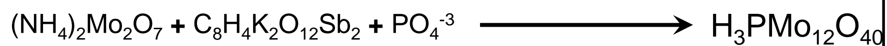
Key Steps

<u>What are we trying to do here?</u>

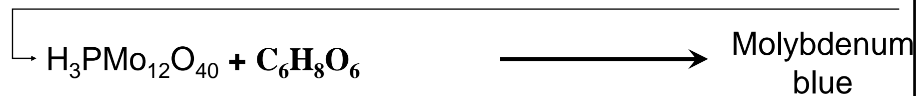
1. Convert all forms of phosphorus to phosphate (PO_4^{-3})

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

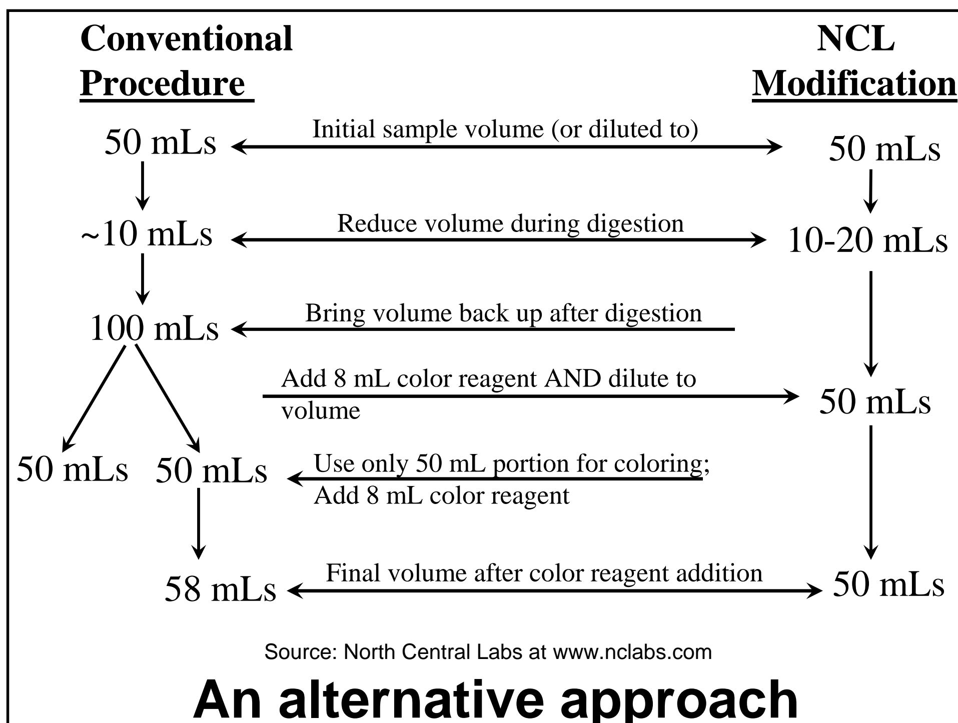
- Boil samples 30-40 mins. or until a final volume of 10 mL (whichever comes first)
- in no case should samples be boiled dry

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



Three (3) techniques approved by the EPA:

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

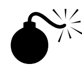
Other methods available (but not approved under NR 219)

- 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 less than 650 nm,
(2) the color of the solution you are measuring is yellow, or
(3) if you are using stannous chloride 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. <u>Discard after one week</u>.

Combined Color Reagent (100 mLs)	Color Blank Reagent (100 mLs)
--	35 ml reagent water
50 ml 5N sulfuric acid,	50 ml 5N sulfuric acid, and
5 ml potassium antimonyl tartrate,	------------------------------------
15 ml ammonium molybdate, and	15 ml ammonium molybdate
30 ml ascorbic acid	------------------------------------

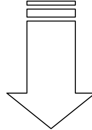
Preparing color reagent

Absorbance of sample + color reagent

—

Absorbance of sample + color blank reagent

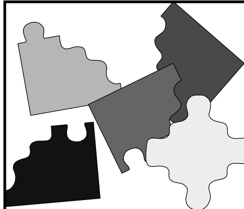
= Absorbance due to phosphorus in sample



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……*fine….but is it digested, or not?*

EPA method 365.2 [section 9.1.1.]
Process standards and blank exactly as the samples. Otherwise, silent on the issue.
Great! But what do I zero the spec with?

Zeroing the spectrophotometer

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

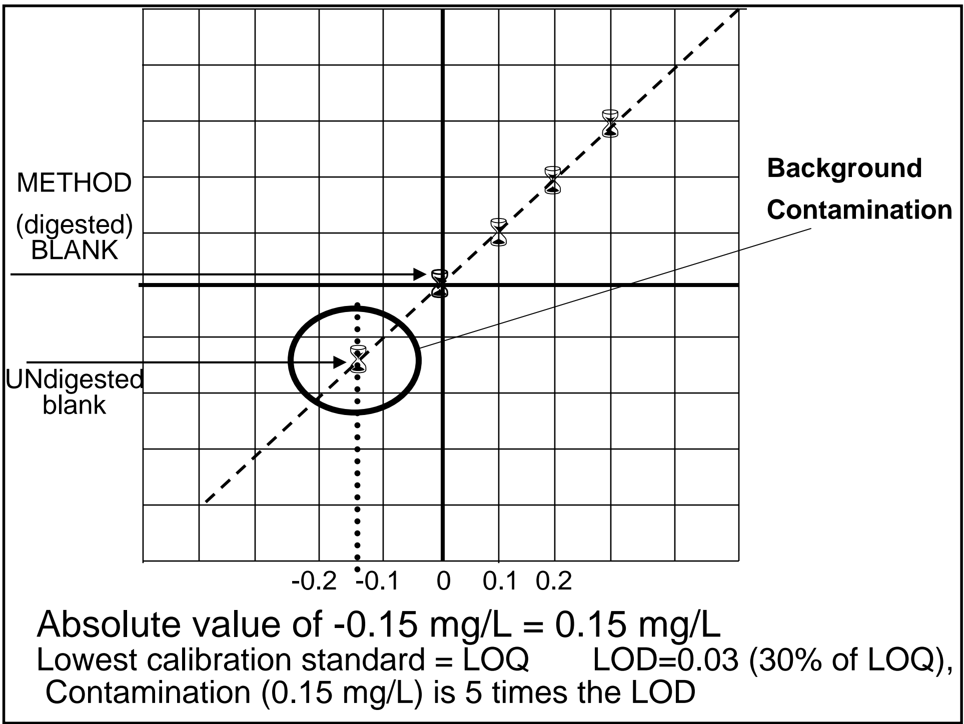
☑ Zero the spec each day with digested reagent water + color reagent.

☑ With each "batch" of samples digested, there must be a Method Blank, a Calibration Check, AND *we* *<u>recommend</u>* *an undigested blank*

☑ The absolute value (*from the regression*) of the undigested blank will indicate contamination.

Solution:

Zeroing the spectrophotometer



Calculations

mg/L Total P=

$$\frac{\text{mg P}}{\text{L}}\text{ (from curve)} \times V\ \cancel{\text{mL}} \times \frac{1\ \cancel{\text{L}}}{\cancel{1000\ \text{mL}}} \times \frac{FV\ \cancel{\text{mL}}}{CV\ \cancel{\text{mL}}} \times \frac{1}{SV\ \cancel{\text{mL}}} \times \frac{1000\ \cancel{\text{mL}}}{\cancel{\text{L}}}$$

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...

$$\frac{\text{mg P}}{\text{L}}\text{ (from curve)} \times \cancel{50} \times \frac{100}{\cancel{50}} = \frac{\mathbf{2}}{\mathbf{1}} \times \frac{1}{\cancel{50}}$$

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

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

Troubleshooting

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

Symptom	Possible Cause	Suggested Corrective Action
Standards turn yellow, not blue.	Bad or old ascorbic acid.	Prepare fresh ascorbic acid & re-test.
Standards & blank all turn blue.	No acid added to combined color reagent or gross contamination	Prepare fresh color reagent; wash all glassware with fresh 10% HCl.
Standards turn blue then fade	Wrong acid concentration or bad antimony solution.	Check acid strength; check post digestion pH adjustment; prepare fresh color reagent.

Some Common Phosphorus Problems

Symptom	Possible Cause	Suggested Corrective Action
Absorbances seem low or weak color intensity	Excess persulfate present after digestion	Make sure using proper amount of persulfate; heat/cook down longer.
Combined color reagent is dark yellow.	Ascorbic acid is bad.	Prepare fresh combined color reagent using fresh ascorbic acid solution
Neither standards nor samples turn blue.	Bad color reagent.	Check expiration dates, discard old solutions and prepare fresh color reagent.

Other Phosphorus Problems

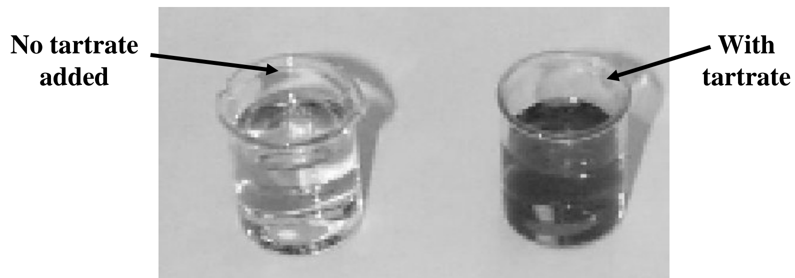
Prepared with
1 N acid
(*instead of 5N*)

Properly prepared color reagent; light pale straw color

Color Reagent: weak or no acid present

When potassium antimonyl tartrate is absent, old, or weak, the color reaction proceeds slowly.

Example of 1 ppm standard 7 minutes after addition of color reagent.



Impact of the Potassium Antimonyl Tartrate Solution

Parameter	**Phosphorus**
# Standards	3 or more
SM says:	*6 + blank*
Aprox. LOD	0.01-0.02 mg/L
Low std/LOQ	~ 0.05 - 0.1 mg/L
Linear range	~0.1- 1.3 mg/L*
High Standard	0.8 - 1.0 mg/L
Suggested range	0, 0.1, 0.2, 0.4, 0.6, 0.8, 1

** using a 1.0 cm cuvette. Range can be adjusted w/ different cell path lengths*

Calibration Range

Suggested Way to Prepare Intermediate Stock Total Phosphorus Standards

mL Stock Std. Diluted to 1000 mL	***Conc. Stock Std to use (ppm)***	***Final Conc. Stock Standard (ppm)***
5	1000	5
50	1000	50

*Note: **Always** use class A volumetric pipets to prepare standards.*

Suggested Way to Prepare Working Total Phosphorus Standards

mL Stock Std. Diluted to 500 mL	***Conc. Stock Std to use***	***Final Conc. Working Std.***
10	5	0.1
20	5	0.2
6	50	0.6
8	50	0.8
10	50	1.0

Note: ***Always*** use class A volumetric pipets or air displacement pipets (Ependroff, Rainin, Gilson, etc.) to prepare standards.

More Common Problems

Contamination!

- Wash glassware well, using a non-phosphate detergent
- THROW OUT your Alconox (~8.7% phosphorus)
- 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 <u>inside</u> 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 (*but still rotate glassware*)

Combined (color) reagent

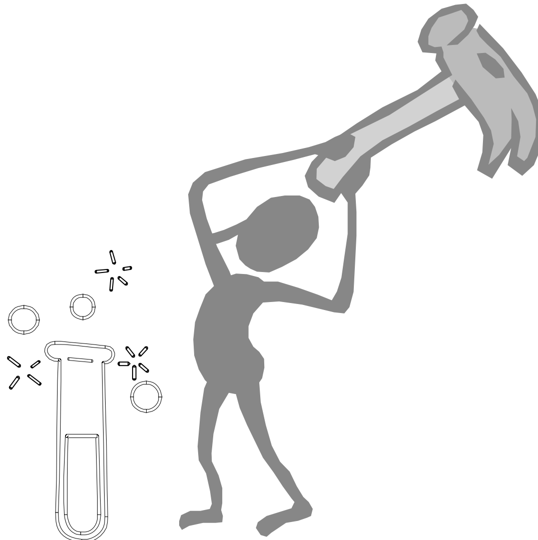
- Make your Combined Reagent fresh daily
- It should be a light straw or light yellow color.

Sample pH

Not at proper pH prior to adding the Combined Reagent.

- ✓ ***Always*** do hotplate digestions in a good laboratory fume hood.
- ✓ ***Always*** prepare acids and bases in a fume hood.
- ✓ ***Always*** use a fume hood when rinsing glassware with 10% HCl.
- ✓ ***Never*** substitute a household range hood for a laboratory fume hood.
- ✓ ***Always*** were safety glasses and gloves when rinsing glassware with HCl, and...
- ✓ ***Never*** mouth pipet.

Safety Tips for Total Phosphorus Testing.



Method Details - Test N' Tube

- ☑ Turn on COD Reactor; set at 150° C (early versions say 105°C)
- ☑ 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 at 610$^+$ nm*

** 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

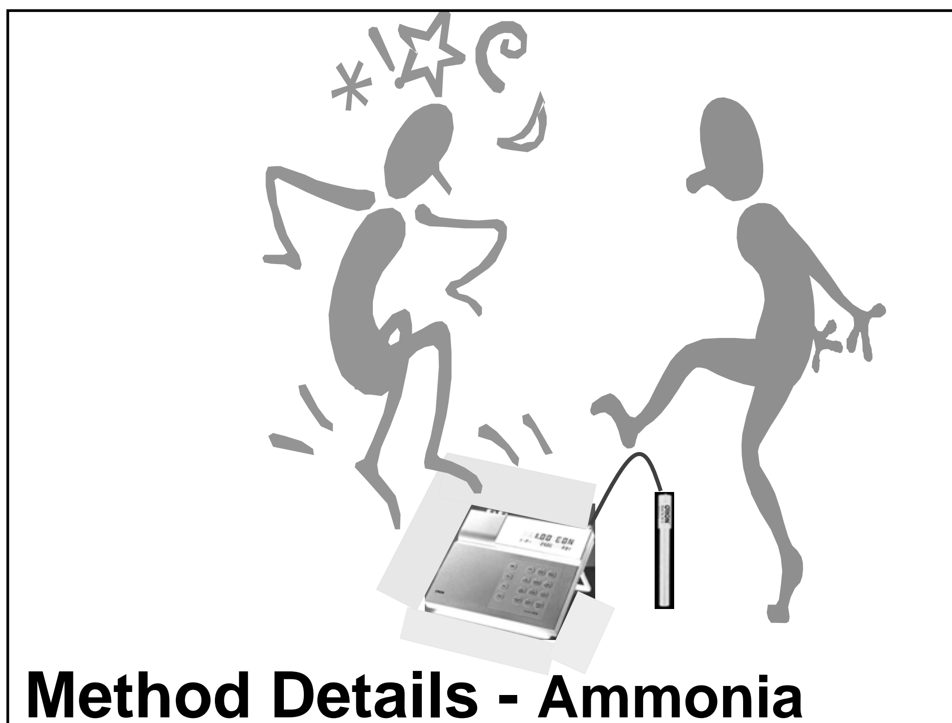
☑ Problematic for colored or turbid samples

☑ 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



Method Details - Ammonia

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

Basic Ammonia Equipment

Can I let the ISE meter software calculate my ammonia concentrations?

- **Yes…. providing you:**
  - Measure and record the mV readings on samples and standards
  - Your standards are a decade apart (0.2, 2.0., 20)
  - You determine the slope between each standard
  - The slope between each standard must be within the -54 to -60 mV range

WI 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,
- Dairy or paper mill effluents (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 WI 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

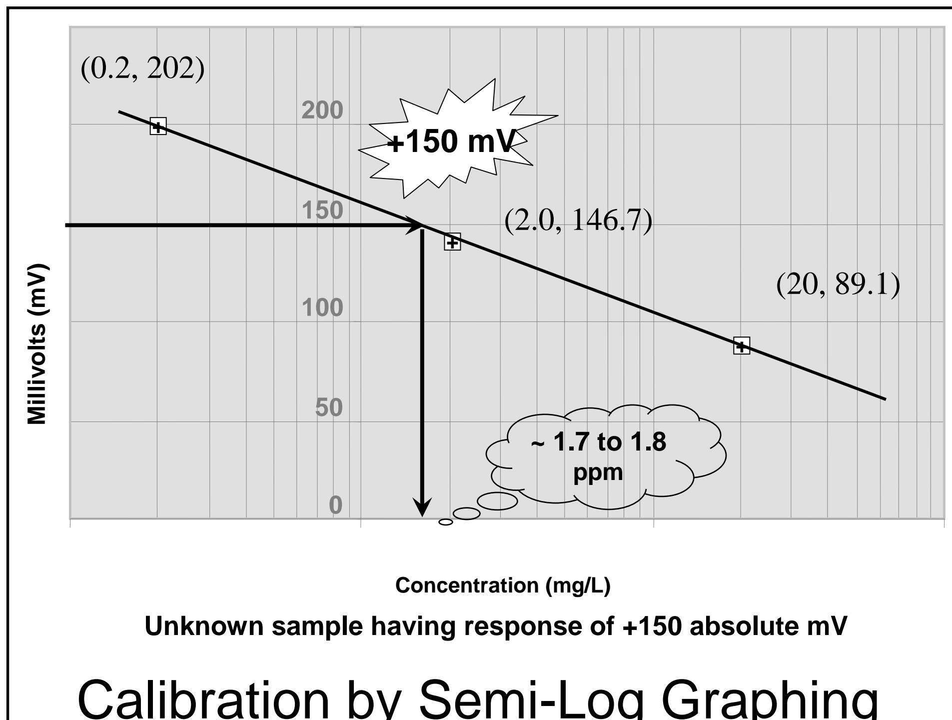
Determinative Method Options

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



Unknown sample having response of +150 absolute mV

Calibration by Semi-Log Graphing

Relative mV mode
Ammonia electrode calibration

Concentration mg/L	mV		Relative mV
0.2	202	- 146.7 =	**55.3**
2.0	146.7	- 146.7 =	**0**
20	89.1	- 146.7 =	**-57.6**
Average slope =			56.45

Some older probes can use this technique to allow 3-point calibrations

Calibration using Relative mV

- 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 *except*:

….MUST first convert concentration to logarithm
…. Then, a second conversion is required to convert to mg/L

Log conversions

Unknown sample: +205.6 absolute mV

intercept= 162.9264 slope = -56.45

Sample #	mV	log concentration	Concentration (mg/L)
Final effluent	205.6	-0.755953	0.1754072

Step 1. Subtract the intercept from the sample mV

205.6 - 162.926= 42.674

Step 2. Divide the result of Step 1 by the slope

42.673 / -56.45 = -0.7559526

This gives you the log of the sample concentration

Step 3. Take the inverse log (ANTIlog) of the result in Step 2

10 ^-0.75595 = $10^{-0.75595}$ = 0.1754072 mg/L

This is the same thing as 10 raised to the power of the result from Step 2

Quantitation by Linear Regression

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

	Keystrokes	Result
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

Parameter	**Ammonia**
No. of standards	3
****** SM says:***	***5*** ***

Aprox. LOD	0.05-0.1 mg/L
Low std/LOQ	~ 0.2 mg/L
Linear range	0.03-2000 mg/L
High Standard	20 - 50 mg/L
Suggested range	0.2, 2, 20 mg/L

NOTE: Calibration ranges for ammonia may change with the season

Calibration Range

Suggested Way to Prepare Intermediate Stock Ammonia Standards

mL Stock Std Diluted to 500 mL	***Conc. Stock Std. to Use (ppm)***	***Final Conc. Working Std. (ppm)***
5	1000	10
50	1000	100

Note: ***Always*** use class A volumetric pipets and flasks to prepare standards

Suggested Way to Prepare Working Ammonia Standards

mL Stock Std Diluted to 500 mL	***Conc. Stock Std. to Use (ppm)***	***Final Conc. Working Std. (ppm)***
10	10	0.2
10	100	2
10	1000	20

Note: ***Always*** *use class A volumetric pipets and flasks to prepare standards*

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 ($\leq$ 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 ($\leq$ 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

Symptoms: low slopes, erratic readings, error messages during calibrations, and incorrect results.

First: run an "**inner-body check**".

- This test will tell whether the sensor system itself is operating correctly.
- Dealer/Manufacturer will ask for results of this check before they will consider an in-warranty replacement.
- Even if the probe is older (outside warranty), this simple test will save you a lot of headaches.

If the probe fails this test, it will not work regardless of how many times you recalibrate or remake your standards.

Note: If probe has been dry for more than a few days,
- disassemble the probe
- soak the inner-body in regular pH 7.00 buffer overnight.

If you neglect to do this the probe will probably fail the test regardless of whether it is actually good or not!

Source: North Central Labs at www.nclabs.com

Ammonia probe problems

You will need two reagents to run this test:

pH 7.00 buffer with 0.1 M NaCl (Sodium Chloride) ~ $10.00/500 ml
pH 4.00 buffer with 0.1 M NaCl (Sodium Chloride) ~ $10.00/500 ml

1. Pour 50-100 ml of each into separate beakers; label each beaker.
2. Disassemble your Ammonia probe. *Be careful--the glass at the bottom of the inner body is very fragile. If you break it, you WILL be buying a new probe.*
3. Connect probe to meter and select millivolt (mv) direct-read mode.
4. Place the beaker containing the pH 7 buffer with NaCl on a stir plate, add a 1" stir bar and stir at slow to medium speed.
5. Clamp the inner body into a probe holder and immerse the probe in the beaker, being careful to NOT lower it so far that it is hit by the stir bar.
6. The reading should stabilize within two minutes.
A "stable" reading is a reading which drifts 0.1 mv/minute or less.
Record this reading paying attention to the minus sign (-), if present.
7. Remove inner body from solution, rinse with DI water
8. Repeat steps 4-6 above using the pH 4.00 buffer with NaCl.
9. Calculate the difference, in millivolts, of the two readings.
Remember to take any minus signs into account.

Inner-body check

Source: North Central Labs at www.nclabs.com

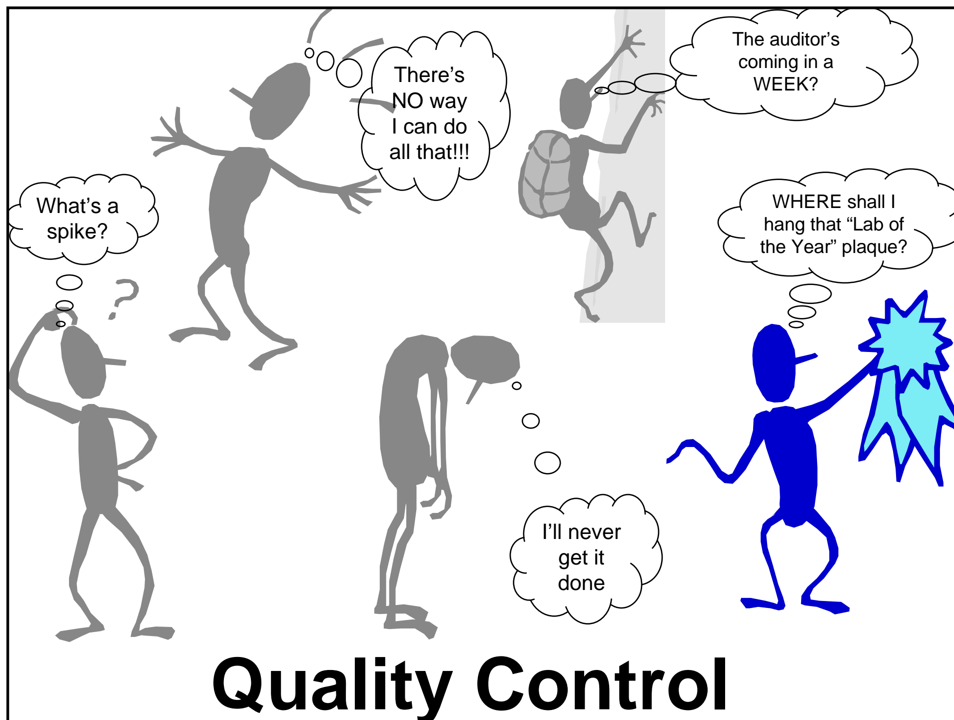
pH 7 buffer	pH 4 buffer	Difference	
-25 mV	145	170 mV	-25 to 0= 25; 25 + 145 = 170

- The ideal difference is 175 mv.
- Normally, any difference over 150 mv is satisfactory.
- Usually, if the sensor is bad, the difference will be 2 mv or less,
- If the inner body check FAILS, your options are VERY limited.
  - If brand new, call your dealer for a replacement as an out-of-box failure.
  - If you have used the probe for some period of time, but it's still under warranty, contact the manufacturer for an in-warranty replacement.
  - If it is out of warranty, you still MUST replace it. You are wasting your time if you continue to play with it.
- If the inner body check PASSES, the problem lies elsewhere:

  ...bad membranes, filling solution, reagents, or standards
  or even improper lab technique.

Inner-body check

Source: North Central Labs at www.nclabs.com



Quality Control

1. Determine a spike concentration
   (should be close to the expected LOD (MDL))
   Ammonia: Try 0.5 to 1.0 mg/L
   Phosphorus: Try 0.1 to 0.2 mg/L
2. Prepare at least 7 replicates at this concentration
   Use reagent water; make a large volume--split from that
3. Calculate the mean (**X**) and standard deviation (**SD**)
4. Obtain the "**t**"-value for the number of replicates
5. Calculate the LOD (MDL): **SD** x **t**
6. Perform the "5-point check" of the LOD (MDL)
   First 3 checks are mandatory!
7. **Repeat annually, or with any "major" changes**

LOD (MDL) - calculations

Phosphorus Example

Spike level = 0.1 mg/L

Rep. 1	0.104
Rep. 2	0.082
Rep. 3	0.096
Rep. 4	0.1
Rep. 5	0.087
Rep. 6	0.114
Rep. 7	0.108
mean	**0.099**
st dev.	**0.01135**
t-value	**3.143** ← from table based on # replicates

# replicates	t-value
7	3.143
8	2.998
9	2.896
10	2.821

LOD (MDL) = **0.035684** = t-value x std deviation

LOQ (Reporting Limit)= **0.118948** = 3.333 x LOD (MDL)

LOD (MDL) - calculations

The 5-point check (mandatory checks) LOD= 0.036

1. Is LOD (MDL) greater than 10% of the spike level? **yes**

Spiked at **0.1**, so LOD must be > 0.01
If LOD < 10% of spike level, re-do at lower spike level

2. Is the spike level greater than the LOD (MDL)? **yes**

Common sense: if LOD > spike level, couldn't detect it

3. Is the LOD (MDL)? below any relevant permit limit? **???**

If Phosphorus,	Permit limit =	~1.0 -1.4 mg/L
If Ammonia,	Permit limit =	varies

LOD (MDL) - calculation checks

The 5-point check (additional checks)

Though not specifically required by the EPA method....
these checks help you obtain the best estimate of the LOD (MDL).

4. Is the signal-to-noise ratio (S/N) between 2.5 and 10? **yes**

$$S/N = \text{Mean/std dev.} = \frac{0.099}{0.01135} \quad S/N = 8.69$$

S/N >10 suggests you could spike lower;
S/N <2.5 suggest you may have spiked too low

5. Is mean recovery within reasonably expected limits? **yes**

Mean recovery= mean/spike level x 100 = 98.7%

Expected range is approximately 80 to 120%

LOD (MDL) - calculation checks

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

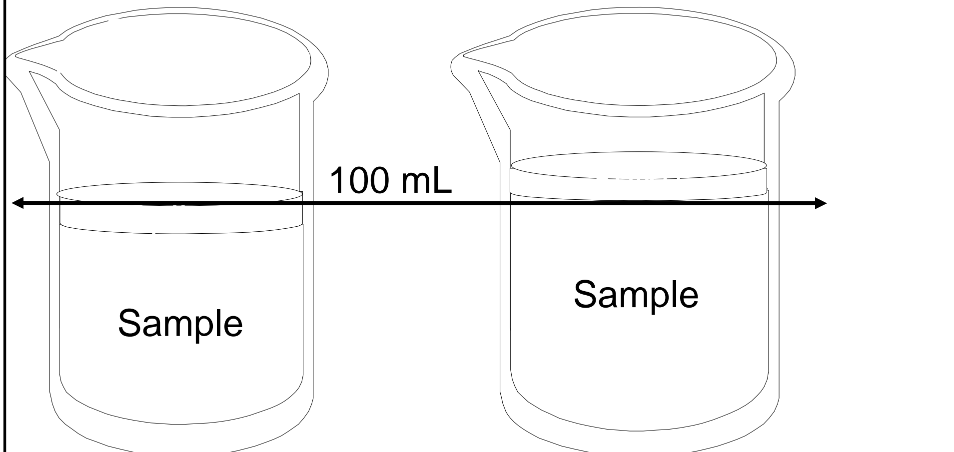
(i.e., 0.5 mL standard to 5 mL with sample)

- The spike faces less matrix than in the unspiked sample

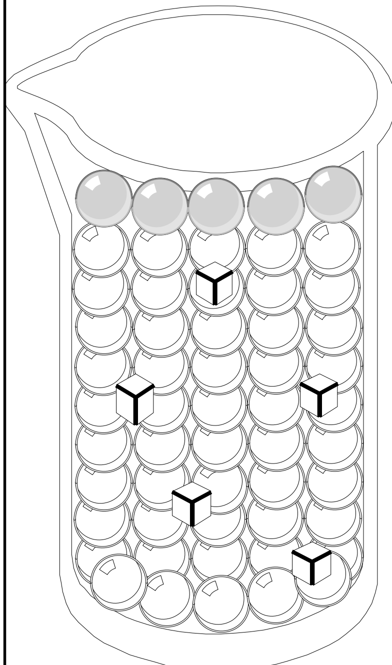
Matrix Spikes: How much should you spike?

• 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 <u>must</u> 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* <u>must</u> be adjusted as well.



Understanding sample dilution

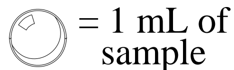


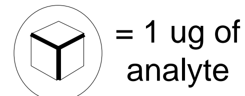
Here is 50 mLs of sample
with 5 ug of analyte dissolved in it

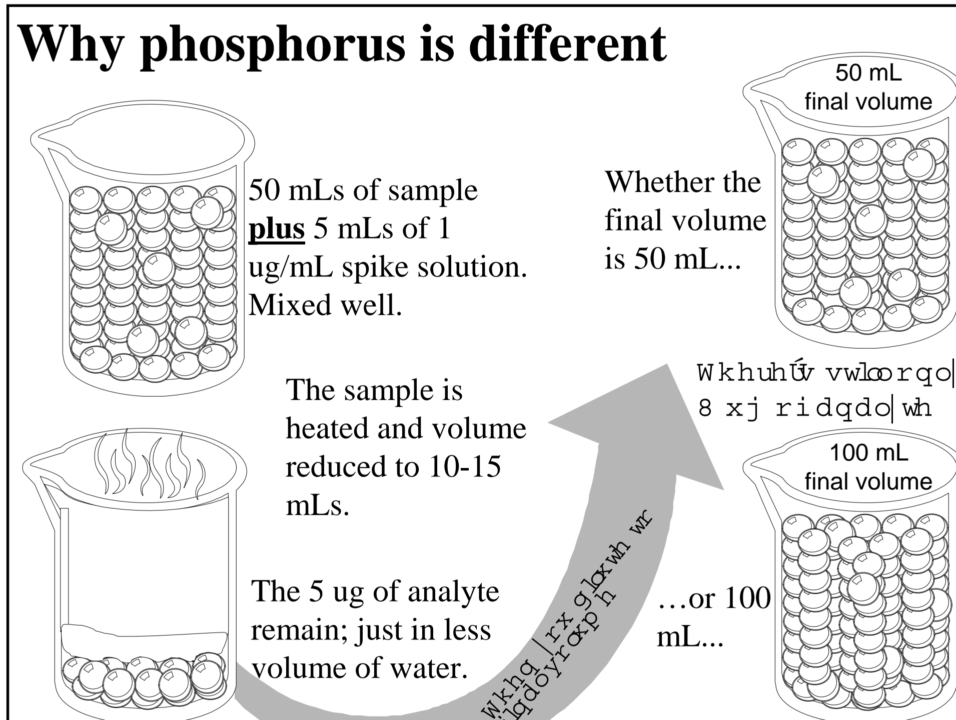
The concentration is 5 ug/50mL
equaling 0.1 ug/mL or 0.1 ppm

If we add 5 more mLs of reagent water, (*the water volume from a spike*)

The concentration of analyte is now 5 ug per 58 mLs
or 5/58 = 0.0909 ug/ml
= 0.0909 ppm







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.

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**

A. Contribution (ug) from the sample in the spike = 5 9 4 13 7

6 15 9 6 *ug/mL X (* 4 3 3 *mL -* 5 3 *mL) =* 6 15 9 6 *X* ; 3

B. The # of ug (of analyte) **spiked =** 7 3 3

5 3 *ug/mL X* 5 3 *mL*

C. The # of ug (of analyte) **in the spiked sample =** 8 5 9 18

8 15 9 8 *ug/mL X* 4 3 3 *mL*

D. The # of ug (of analyte) **recovered =** 5 9 8 17 9

8 15 9 8 - 5 9 4 13 7

% Recovery = 9 9 17 **%**

(5 9 8 17 9 */* 7 3 3 *) X 100*

Example: Ammonia- dilute to known volume

Unspiked sample	**.246 X 25 = 6.15ug/mL**	**Spiked sample**	**.346x 25= 8.65 ug/ml**
Unspiked sample Volume	**2mL=>50 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 = 4 5 16

3 15 7 9 / 9 14 8 *ug/mL X (* 8 3 / 5 *mL -* 3 / 3 *mL) =* 3 15 7 9 / 9 14 8 *X* 8 3 / 5

B. The # of ug (of analyte) **spiked =** 8 13

8 13 *ug/mL X* 4 *mL*

C. The # of ug (of analyte) **in the spiked sample =** 4 : 16

; 19 8 / 3 16 7 9 *ug/mL X* 5 / 8 3 *mL*

D. The # of ug (of analyte) **recovered =** 8 13

4 : 16 - 4 5 16

% Recovery = 4 3 3 **%**

(8 */* 8 *) X 100*

Example: Phosphorus- hotplate

Unspiked effluent = 0.38 ug/mL
Unspiked sample Volume 4.5 mL

Spiked effluent = 0.81 ug/mL
Total volume= 5.0 mL

Spike volume 0.5 mL
Spike Conc. 5.0 ug/mL

A. Contribution (ug) from the sample in the spike = 4 1: 4

3 16 ; *ug/mL X (* 8 13 *mL -* 3 18 *mL) =* 3 16 ; *X* 7 18

B. The # of ug (of analyte) **spiked =** 5 18

8 13 *ug/mL X* 3 18 *mL*

C. The # of ug (of analyte) **in the spiked sample =** 7 13 8

3 1; 4 *ug/mL X* 8 13 *mL*

D. The # of ug (of analyte) **recovered =** 5 16 7

7 13 8 - 4 1: 4

% Recovery = <6 19 **%**

(5 16 7 */* 5 18 *) X 100*

Example: Phosphorus- Test N' Tube

Unspiked sample 0.625 ug/mL
Unspiked sample Volume 50mL=>100 mL

Spiked sample 0.92 ug/mL
Total volume 25 =>50 mL + 5 mL =>100 mL

Spike volume 5 mL
Spike Conc. 5 ug/mL

A. Contribution (ug) from the sample in the spike = 4 8 19 5 8

3 19 5 8 *ug/mL X (* 8 3 *mL -* 5 8 *mL) =* 3 19 5 8 *X* 5 8

B. The # of ug (of analyte) **spiked =** 5 8

8 13 *ug/mL X* 8 *mL*

C. The # of ug (of analyte) **in the spiked sample =** 7 9

3 1<5 *ug/mL X* 8 3 *mL*

D. The # of ug (of analyte) **recovered =** 6 3 16 : 8

7 9 - 4 8 19 5 8

% Recovery = 4 5 4 18 **%**

(6 3 16 : 8 */* 5 8 *) X 100*

Example: Phosphorus- autoclave

Replicates

Replicates - measuring precision

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 %

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

RPD = Range / Mean
Range = 0.4
Mean = (2.2 + 1.8)/2
= 2.0
RPD = (0.4/2.0) x 100
= 20%

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
It might be a good idea to use RPD for raw; range for final

Is RPD for you?

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

	Phosphorus
Calibration	When check fails at least quarterly At least 3 stds + blank if LSR, r $\geq$ 0.995
Calibration Check	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

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

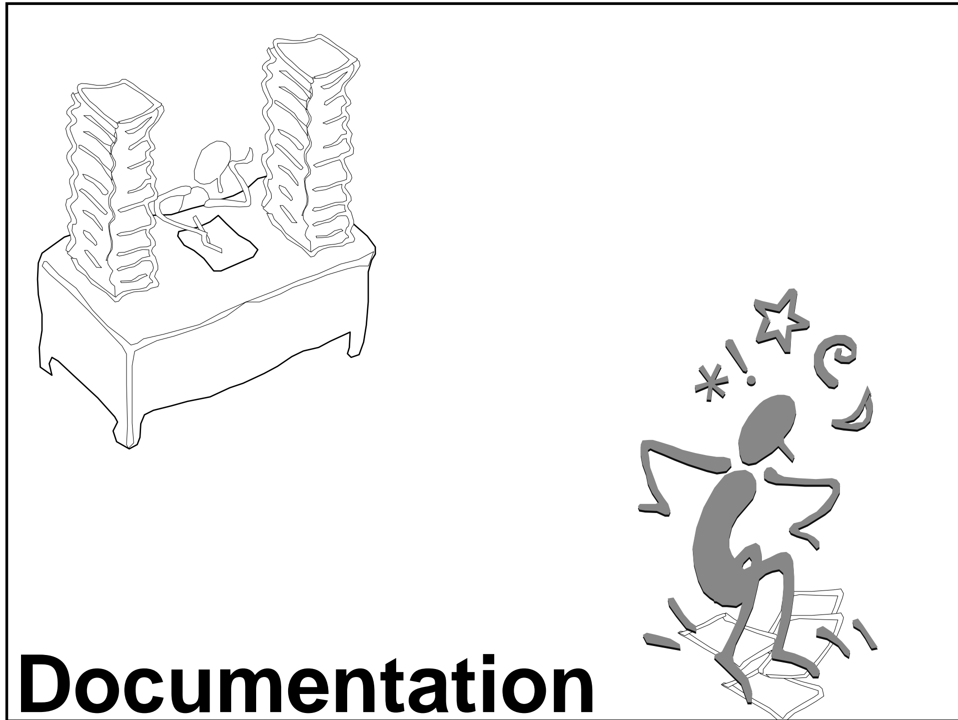
QC Requirement summary II

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

QC Requirement (WI) summary

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

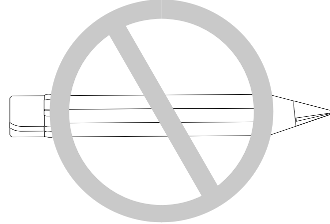
QC Requirement (WI) summary II



A laboratory is required to:

maintain records: WI **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

Setting up an effective QA Plan

- 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)

Your QA Plan

Evaluating?	Criteria	Corrective Action
Method Blank	< LOD	1) Identify contamination source 2) Correct Problem 3) Qualify data
Phosphorus Calibration check/ Known standard	90-110% of true value	1) Replace standards/reagents 2) If high…contamination? 3) Re-do calibration 4) Reanalyze/ qualify samples
Ammonia Calibration slope	-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

WI requires you to have available for any inspection

- ✍ **Digestion (or distillation information)** ***copy of WSLH 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	50/5 x 50/5 =100	50/25=2	50/25=2	50/5 x 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

0.276 x 50 = 13.8 ug

\- 0.045 x 50 = 2.25 ug

11.55 ug

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

DF = (Initial dilution final mLs / Initial dilution sample mLs) X (mLs digested for standards / mLs digested for sample) X (mLs colored for standards / mLs colored for sample)

(11.55 ug / 2 x 5 = 10 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

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

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

Ammonia Benchsheet: Data

Summary

- ☑ 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]

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