

Overview of Basic Methods and Analysis Tips

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By Amy Starkey
Stark County Sanitary Engineers

Significant Figures & Rounding Off Numbers

What determines if a digit is Significant?

- All non-zero digits are significant
 - 1233 = 4 significant digits
 - 23 = 2 significant digits
 - 24,000 = 2 significant digits
- All zeros sandwiched between non-zero digits are significant
 - 105 = 3 significant digits
 - 2005 = 4 significant digits

- All zero and non-zero digits are significant when to the right of number and decimal place.

- 1.280 = 4 significant digits

- 0.053 = 2 significant digits

Calculating with Significant Digits

Addition & Subtraction

When adding or subtracting it is the number with the least decimal places that determines the number of places that may be carried in the sum or difference.

- Example: (Addition)

$$\begin{array}{r} 51.01 \\ 783 \\ 8.556 \\ 0.05 \\ 38.6 \\ \hline 881.216 \end{array}$$

The correct answer rounded off would be 881, since "783" used in the calculation does not have any decimal places.

Multiplication & Division

When multiplying or dividing, the answer is dependant on the least amount of significant figures in the calculation

- Example: $206 \times 12 \times 52.04 = 128642.88$

Correct Answer based on sig figs = 130,000

This is because "12" only has two sig figs.

Rounding Numbers Off 3 Rules

Rule #1

If the digit being dropped is greater than 5,
round the preceding digit up

– Example: $48.7 = 49$

where 7 is dropped off and since it is greater than 5, the preceding digit is rounded up

– Example: $406 = 410$

where the 6 is dropped off

Rule #2

If the digit being dropped is less than 5, leave the preceding digit alone

- Example: $48.3 = 48$
where 3 is dropped off and since it is less than 5, the preceding digit is left alone
- Example: $48.0 = 48$
where 0 is dropped off

Rule #3

If the digit being dropped is equal to 5, round off the preceding digit to the nearest even number

-- Example: $48.5 = 48$

-- Example: $43.5 = 44$

**Methods
&
Helpful Analysis Tips**

- Solids

- TS, TSS, TDS

- BOD/CBOD

- Microbiology

- Fecal Coliform

- Organics

- Miscellaneous Info & Tips

- Orthophosphate (PO_4)

- TKN

- ICP Metals Prep

SOLIDS

Solids

- Total Suspended Solids, TSS
 - Non-filterable
 - Portion that is retained on a 2.0 μm pore size filter
 - Dried at 103 -105°C

Solids

- Dissolved Solids, TDS
 - Filterable
 - Portion that passes through a 2.0 μm pore size filter
 - Dried at 180°C

Solids

- **Total Solids, TS**

- Includes both TSS and TDS
- Amount of residue left after evaporation of a sample dried at 103 -105°C

For TSS, why limit sample size so that it yields results of 200 mg/L

- A water-entrapping crust may be formed if there is excessive residue on the filter.
 - Results in a prolonged filtering period
 - Colloidal materials will be trapped on the filter because it may have become clogged.

Can dissolved solids add weight to your TSS results?

YES!

Water containing high amounts of dissolved solids will add weight to the filter. Therefore it is necessary to "wash" the filter and sample with deionized water.

A 3-Place Manifold System

- Makes analyzing solids move along more quickly.
 - I find it takes me only about 20 - 30 minutes to filter about 20 samples.



TSS CALCULATION

$$\text{TSS, mg/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

- Where A = weight of filter + dried residue, mg
- Where B = weight of filter, mg

$$\frac{\text{mg}}{\cancel{1\text{mL}}} \times \frac{\cancel{1000\text{mL}}}{1\text{L}} = \frac{\text{mg} \times 1000}{\text{L}}$$

TDS CALCULATION

$$\text{TDS, mg/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

- Where A = weight of dried residue + dish, mg
- Where B = weight of dish, mg

**Biochemical Oxygen
Demand (BOD)
and
Carbonaceous BOD
(CBOD)**

What is BOD?

- It is a measure of the amount of oxygen consumed by bacteria during the decomposition of organic materials.
 - Organic materials from the wastewater treatment facility act as a food source for the bacteria.
- Directly related to Dissolved Oxygen
 - The bacteria require oxygen in the form of dissolved oxygen to decompose or eat the food source. Through a calculation, the amount of DO depletion between the initial day and final day of the analysis determines the BOD. Thus, BOD directly affects the amount of Dissolved Oxygen
 - The greater the BOD = more rapid oxygen depletion = less oxygen available to aquatic life.

BOD vs. CBOD vs. COD

- The BOD represents the oxidation of carbons and nitrogenous compounds present in the water. Whereas, the CBOD measures the oxidation of carbons present in water.
 - Nitrification is inhibited in the CBOD.
 - TCMP
 - 2-chloro-6-(trichloromethyl)pyridine
 - Should add at the beginning of the test because nitrification will begin almost immediately if the right organisms are present (Baird and Smith, 2002).

- COD measures of the total amount of oxygen required to oxidize all organic material into carbon dioxide and water
 - results will always be greater than BOD results.
 - analysis only takes a few hours compared to the 5-day BOD analysis.
 - Useful in determining an unknown BOD range for a sample but it can **NOT** replace the BOD test.

Composite Holding time?

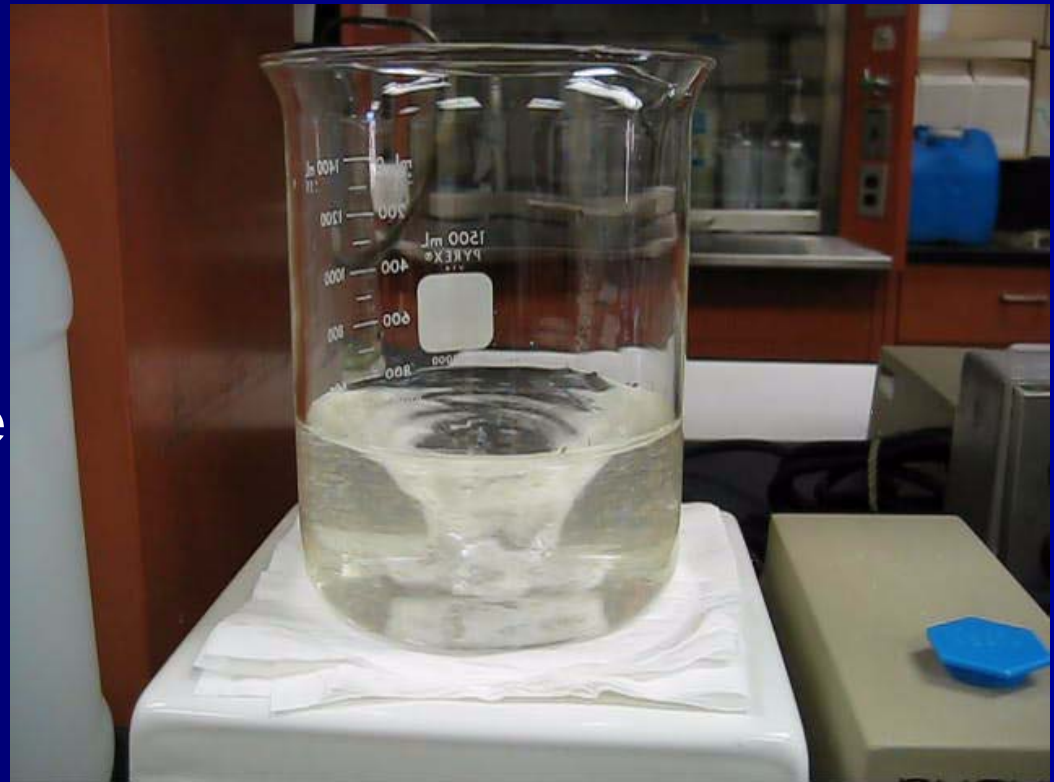
- start the measurement of holding time from the end of the compositing period.
 - For example if the compositing was started at 8:30 am on Tuesday and ended at 8:30 am on Wednesday, then the 48-hour holding time would start from the end of the compositing period which would be 8:30am on Wednesday

GGA and CBOD

- Generally will see lower results
 - Around 160 mg/L
- GGA Needs to be pH adjusted
 - Initially the pH is around 4
 - Adjust to between 6.5 -7.5, just like your samples
 - I adjust mine as close as possible to 7.0 and I have to say my GGA controls have been coming out between 160 – 190 mg/L.

Over mixing the Polyseed!

- Never let the vortex touch the stir bar
- Micro-organisms in the seed will be too tired to get the job done in your samples and may see low results in the seed factor.

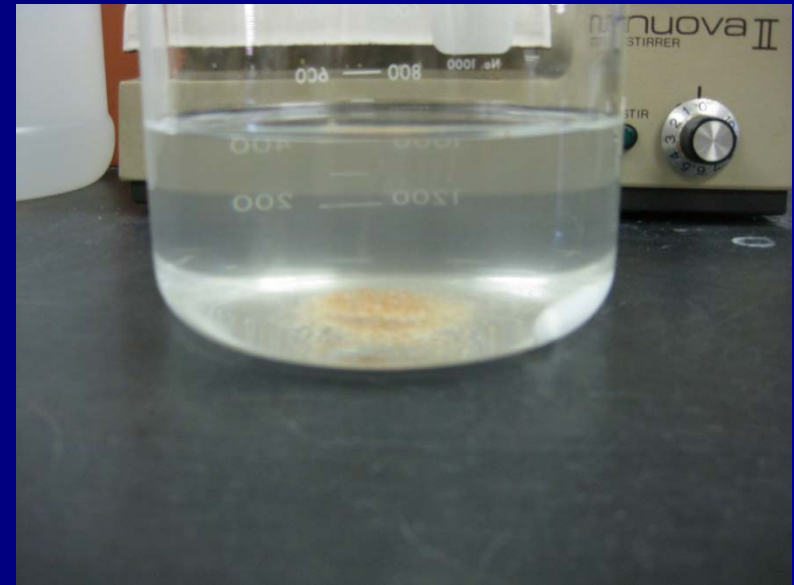


Proper way to mix the Polyseed

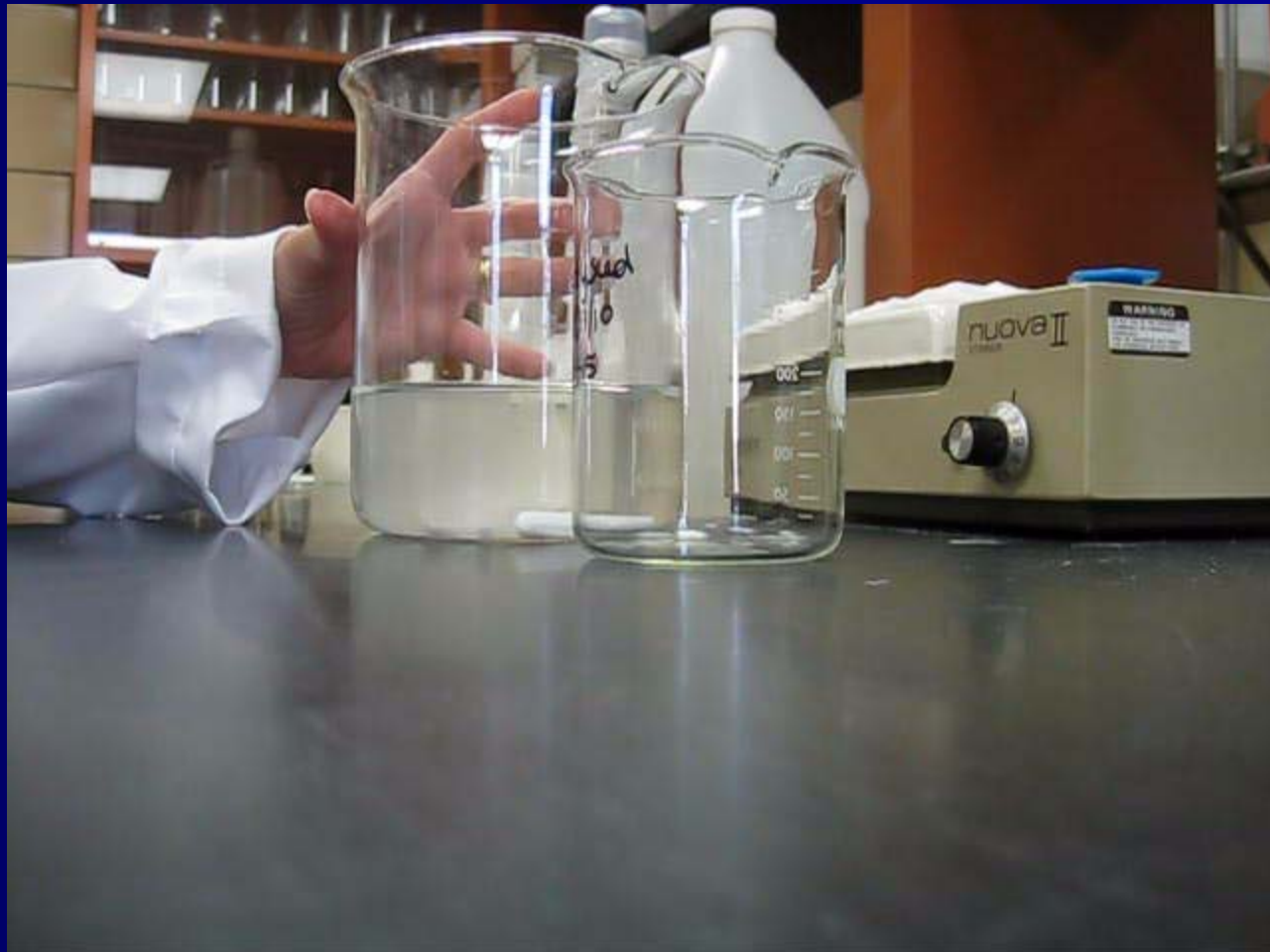
- Mix on a speed of about 5, or so that the vortex is not touching the stir bar and splashing out.
 - Mix for an hour



- Let bran settle out and transfer to another beaker to allow to mix for up to 5 hours on a speed setting between 1-2



Transferring to another beaker after bran settled out



Mix on speed between 1-2



Tip on removing Residual Chlorine

- De-chlorinating with Sodium Sulfite (Na_2SO_3)
 - To the 100 mL sample:
 - Add 1 mL of a 1:50 sulfuric acid solution
 - Add 1 mL of potassium iodide solution
 - 2 mL starch indicator
 - Titrate against 0.025N Na_2SO_3

$$\frac{X_1}{100} = \frac{X_2}{150}$$

Where, X_1 = known amount of titrant calculated to neutralize a 100 mL sample

X_2 = amount of titrant needed to neutralize 150 mL sample

EXAMPLE:

- if only using 150 mLs of sample for the BOD test, and it is known that 3 mLs of 0.025N Na_2SO_3 will remove the residual chlorine from 100 mLs of the same sample, then the equation for how much sodium sulfite the analyst would need is:

$$\frac{3.0}{100} = \frac{X_2}{150}$$

$$(3.0) * (150) = (X_2) * (100)$$

$$\frac{(3.0) * (150)}{100} = \frac{X_2}{100}$$

$$X_2 = 4.5 \text{ mLs } 0.025\text{N Na}_2\text{SO}_3$$

UNSEEDED BOD CALCULATION

$$\text{BOD}_5, \text{ mg/L} = \frac{D_1 - D_2}{P}$$

Where D_1 = initial DO of sample

D_2 = final DO of sample

P = decimal volumetric fraction of
sample used

EXAMPLE:

150 mLs of a sample was added to a 300 mL BOD bottle and the initial DO of the sample is 8.2 and the final DO is 4.2, then what is the BOD_5 mg/L? Let us break it down step by step:

$$P = \frac{150 \text{ mLs}}{300 \text{ mLs}}$$

$$P = 0.5$$

$$\text{BOD}_5 \text{ mg/L} = \frac{8.2 - 4.2}{0.5}$$

$$\text{BOD}_5 \text{ mg/L} = 8 \text{ mg/L}$$

SEEDED BOD CALCULATION

$$\text{BOD}_5, \text{ mg/L} = \frac{(D_1 - D_2) - (SF)}{P}$$

Where D_1 = initial DO of sample

D_2 = final DO of sample

P = decimal volumetric fraction of
sample used

SF = the DO uptake attributable to
the seed

EXAMPLE:

15 mLs of seed was added to a 300 mL BOD bottle and labeled as the seed control. The initial DO was 8.2 mg/L and the final DO in 5.0 mg/L. What is the seed factor, SF if 4 mLs of seed was added to the samples?

Using the calculated SF value, what would be the BOD_5 mg/L if 150 mLs of sample was added to a 300 mL BOD bottle along with 4 mLs of seed and the initial DO was 8.2 mg/L and the final DO is 4.2 mg/L?

First calculate the SF value:

$$SF = (8.2 - 5.0) \times \frac{4 \text{ mLs}}{15 \text{ mLs}}$$

$$SF = 0.853$$

**Second calculate the BOD₅
mg/L of the sample:**

$$\text{BOD}_5 \text{ mg/L} = \frac{(8.2 - 4.2) - 0.853}{0.5}$$

$$\text{BOD}_5 \text{ mg/L} = 6.3 \text{ mg/L}$$

Microbiology

Fecal Coliform

Fecal Coliform

- Fecal coliforms are non-spore forming, rod shaped, gram-negative bacteria
- Methods
 - Most Probable Numbers (MPN)
 - EPA Method 132
 - EPA Methods 1680 & 1681
 - Standard Methods 18th, 19th, and 20th edition Method 9221 C,E
 - Membrane Filtration
 - EPA Method 124
 - Standard Methods 18th, 19th, and 20th edition Method 9222 D

Most Probable Number MPN

- Positive Fecal

- Gas is produced with growth with in $24_{\pm 2}$ hours at $44.5_{\pm 0.2}^{\circ}\text{C}$ in a water bath incubator.

- Negative Fecal

- Failure to produce gas with little or no growth

Calculation for Determining the Fecal Coliform Results as MPN/100ml

$$\text{MPN/100 ml} = \frac{\text{number of positive tubes} \times 100}{\sqrt{(\text{ml sample in negative tubes} \times \text{ml sample in all tubes})}}$$

Membrane Filtration Technique

- Uses M-FC Medium & 1% Rosolic Acid to make the broth
 - When preparing it is heated to just boiling, removed from heat, and cooled to below 50°C
 - If agar is used, dispense 5 -7 mls into petri-dishes and let solidify.
 - Unused agar plates are good for 2 weeks
 - Final pH should be 7.4 ± 0.2
 - Unused broth is good for 96 hours
 - Or can purchase already made 2 mL ampules through HACH or another approved manufacturer/distributor

Membrane Filtration Technique

- **NEVER** sterilize the MFC/Rosolic Acid Broth by autoclaving
 - Decomposes the rosolic acid reagent
- Ideal range for fecal coliform count is 20 – 60 colonies on each plate

pH of the Buffer Tip

- “Fiddling” with the pH endpoint of your buffer too much can be hazardous to the microbes trying to grow on the agar plate
 - By having to correct an over shot of the pH one too many times can cause salts to build up, which are poisonous to the microbes.

MFC CALCULATIONS

$$\text{CFU} / 100 \text{ ml} = \frac{\# \text{coliform colonies on a plate} \times 100}{\text{volume (mls) sample filtered}}$$

EXAMPLE 1:

- There are 25 colonies counted on a plate where 10 ml of sample was used. Calculate the end result (coliforms/100ml).

$$\text{Coliforms / 100 ml} = \frac{(25) \times (100)}{10\text{mls}}$$

$$\text{Coliforms / 100 ml} = 250$$

EXAMPLE 2: No plate with a count in 20-60 range

- Three sample volumes were analyzed (10, 25, and 50 mls) and the coliform counts were (0, 2, 5). Calculate the Result.

$$\text{Coliforms / 100 ml} = \frac{(0 + 2 + 5) \times (100)}{(10 + 25 + 50 \text{ mls})}$$

$$\text{Coliforms / 100 ml} = 8$$

Reporting Fecals on the MOR

- Based on the geometric Mean
 - Calculated by converting coliform densities of each sample to log₁₀ values, and averaging those results

Example: On seven different days the following fecal coliform results were: 100, 50, 75, 250, 80, 5, 1000 colonies/100ml. Calculate the geometric mean & compare to the arithmetic mean.

Arithmetic Average

50

75

250

80

5

1000

Σ 1460

$$\frac{1460}{7} = 208$$

Geometric Mean

Log 50 = 1.7

Log 75 = 1.9

Log 250 = 2.4

Log 80 = 1.9

Log 5 = 0.7

Log 1000 = 3.0

Σ 11.6

$$\text{Geometric Mean} = \frac{11.6}{7} = 1.6$$

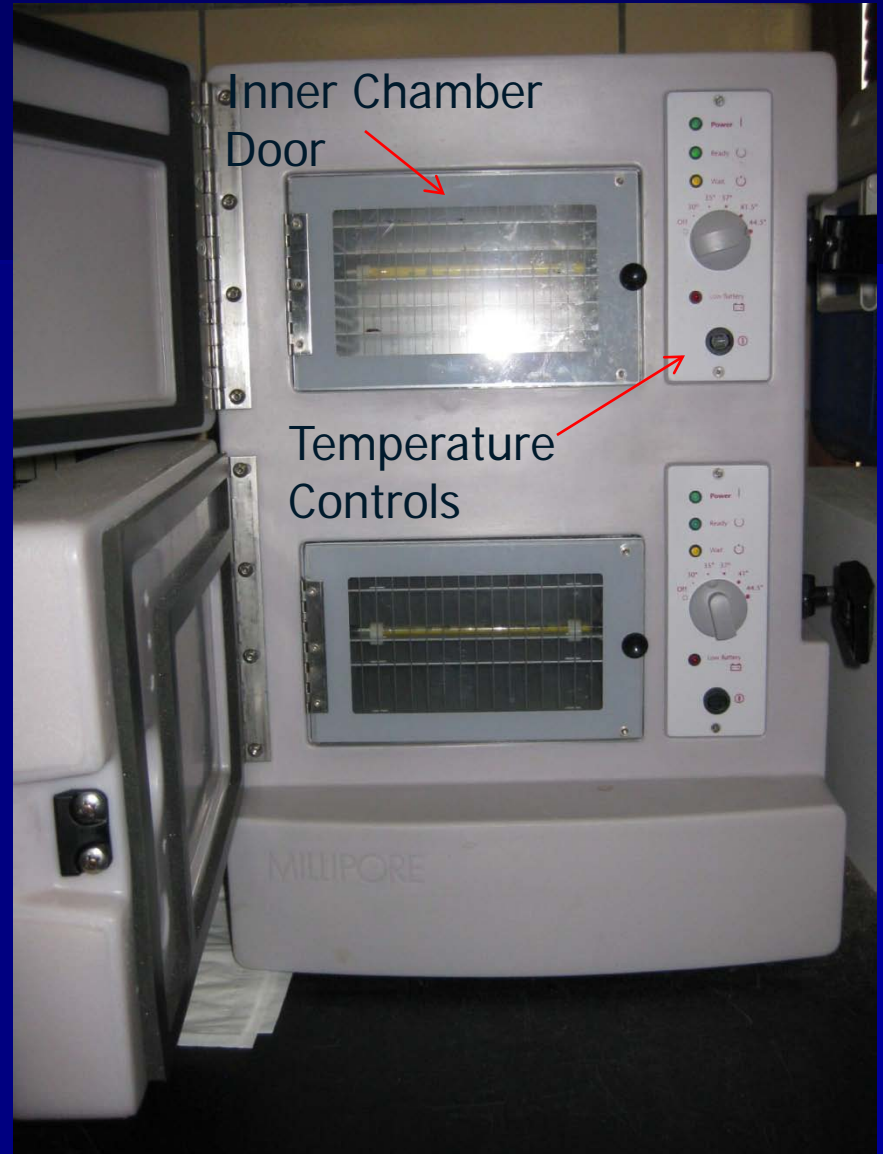
= antilog 1.6

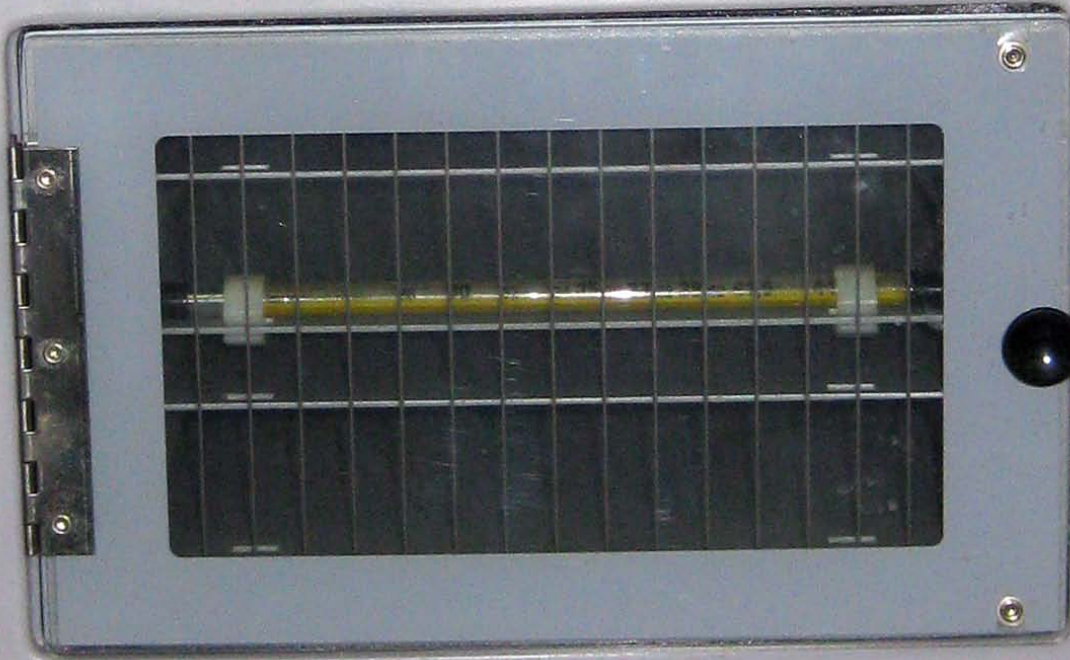
= 40

Incubator Tips

Water Bath Tip

- If using an open water bath, cover the un-occupied surface of the water with ping pong balls
 - Insulates the bath
 - Minimizes evaporation
 - Helps maintain a more stable temperature throughout the bath





Power |

Ready ○

Wait ○

30° 35° 37° 41° 44.5°

Off ○

Low Battery 



Detailed description: This control panel is vertically oriented and contains several indicators and controls. At the top is a green LED labeled 'Power' with a vertical bar icon. Below it is another green LED labeled 'Ready' with an open circle icon. The third indicator is a yellow LED labeled 'Wait' with an open circle icon. A large grey rotary knob is positioned in the center, with temperature markings at 30°, 35°, 37°, 41°, and 44.5°. The knob is currently set to 37°. To the left of the knob is an 'Off' position with an open circle icon. Below the knob is a red LED labeled 'Low Battery' with a battery icon. At the bottom is a black circular button with a power symbol icon.

ORGANICS

ORGANICS

- What is NOT an organic Compound?
 - Carbonates
 - Carbides
 - Cyanides

ORGANICS

- What is an organic Compound?
 - Carbon
 - Hydrogen, Nitrogen, Oxygen & Phosphorous
 - Halogens

Example of Organic Compounds

- Hydrocarbons
 - contain only carbon and hydrogen
- Organic compounds with other functional groups
 - carboxylic acids
 - Alcohols
 - Aldehydes
 - Ketones
 - Phenols
 - Ethers
 - Esters

Analytes of Interest

- Volatile Organic Compounds (VOC)
 - Trihalomethanes
 - Chlorinated Organic Solvents
 - Disinfection Byproduct
- Semi-Volatile Organic Compounds
 - BNA
 - Phenols
 - PAH
 - Pesticides
 - PCBs
 - Herbicides

Volatile Organic Compounds

VOC

- Have a high vapor pressure and low water solubility
- Typically are industrial solvents
 - trichloroethylene; fuel oxygenates, such as methyl tert-butyl ether (MTBE)
- Or by-products produced by chlorination in water treatment
 - such as chloroform

Rough Classification of VOC's Determined by Boiling Points

- Very Volatile Organic Compounds (VVOC)
 - boil or volatilize at $<0^{\circ}$ - 50° C
 - However, there are some that boil between 50° - 100° that are also considered VVOCs
- Volatile Organic Compounds (VOC)
 - boil between 50° - 260° C
 - However, the majority that are analyzed by VOC methods fall in the 100° - 240° C range

Rough Classification of VOC's Determined by Boiling Points

- Semi Volatile Organic Compound (SVOC)
 - anything that boils between 240° and 400°
 - majority on the SVOC list between 260° and 380°
 - include the 8270 (Base Neutral Acid extraction), the pesticides and PCBs, the Herbicides and PAHs

Keep your VOC and SVOC rooms separate!

- The semi-vols are usually extracted (shaken vigorously or sonicated) with methylene chloride, and will be exchanged over during concentration with hexane or acetone or toluene or ether or any number of other lovely compounds
 - Most of these are on the VOC list, and so the 2 rooms should be kept VERY SEPARATE, with no personnel traipsing back and forth.

Miscellaneous Info and Tips

Precision vs. Accuracy

Precision vs. Accuracy

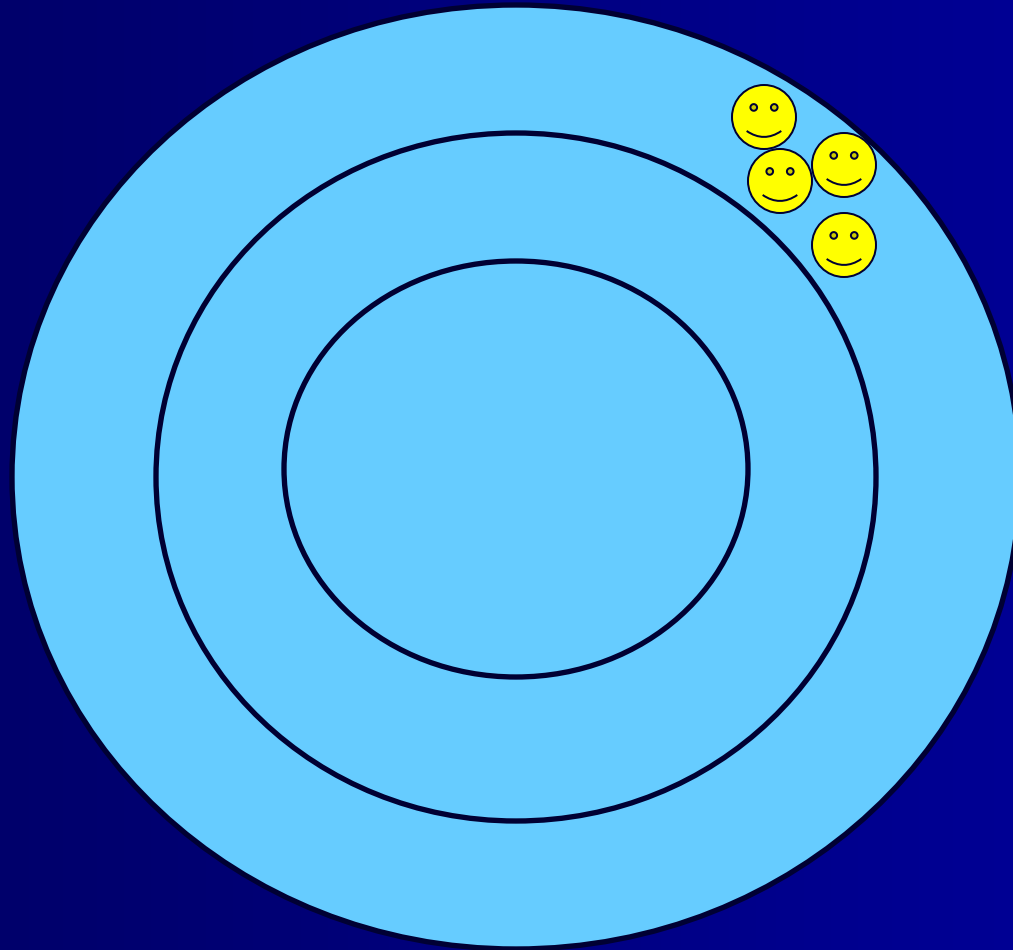
- Precision

- Measures the reliability or reproducibility of data points

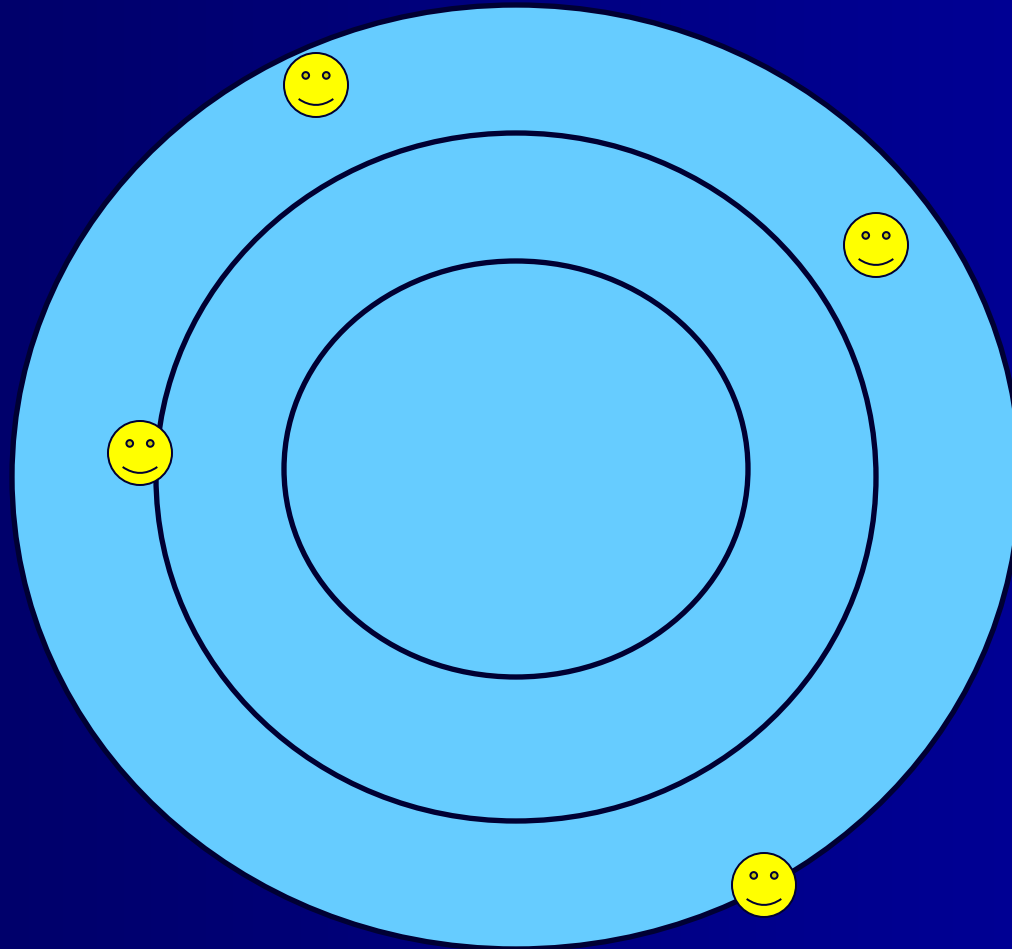
- Accuracy

- Measures how closely the data points agree with the true or accepted value

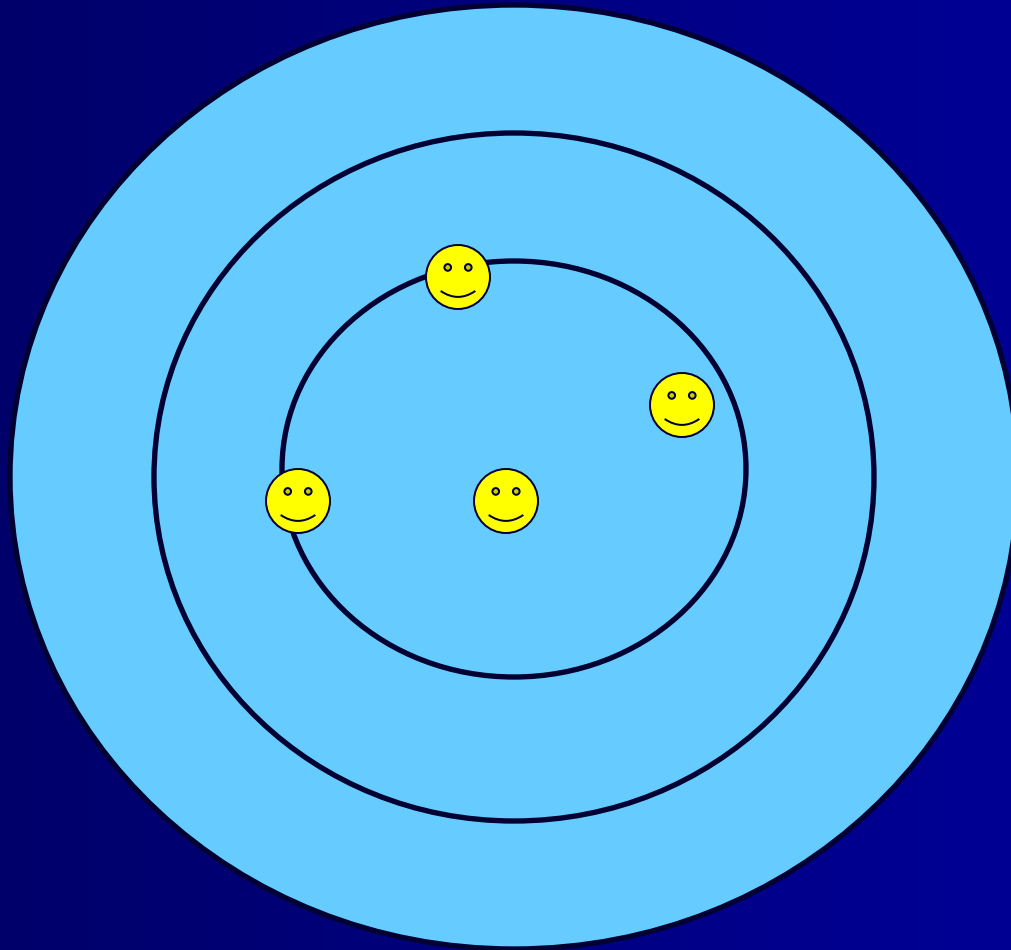
High Percision Low Accuracy



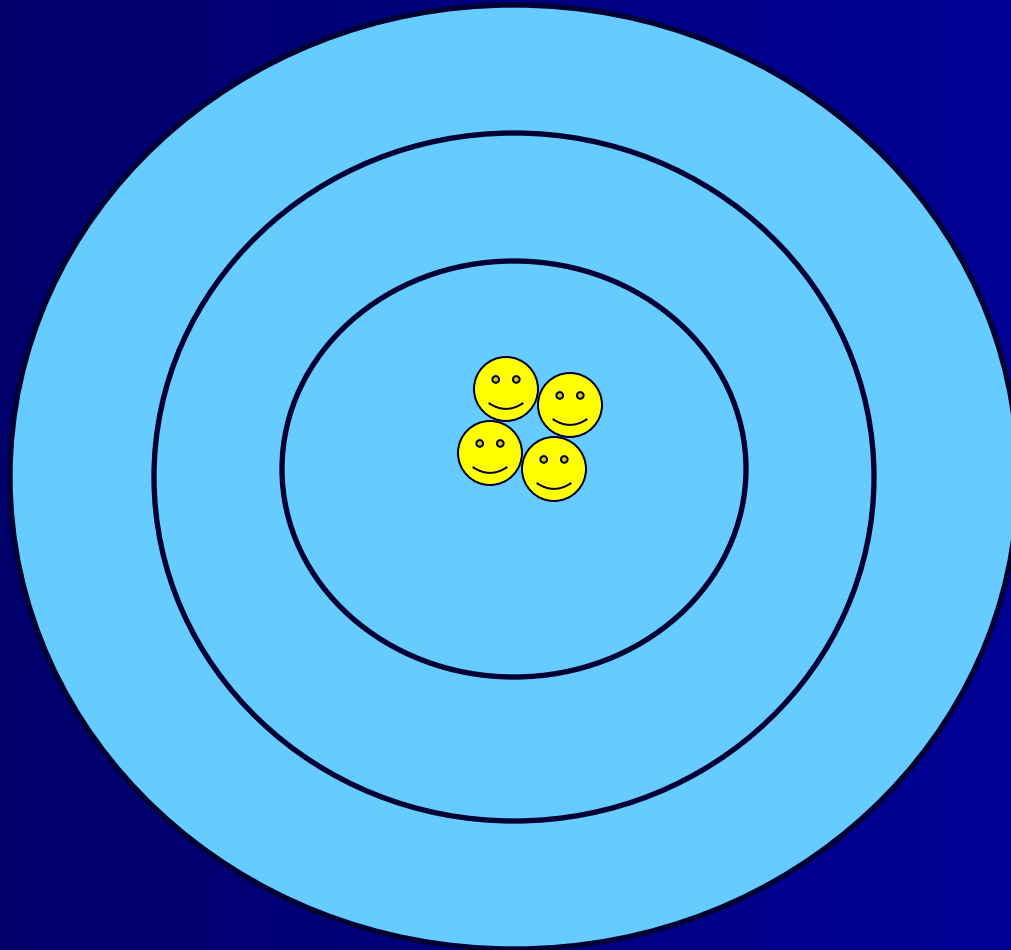
Low Precision and Low Accuracy



Good Accuracy, Low Precision



Good Accuracy and Good Percision



Qualitative vs. Quantitative Data

■ Qualitative Data

– Descriptive data

- Color
- Texture
- Smell
- Appearance

■ Quantitative

– Measurable Data

- Length
- Height
- Weight
- Volume
- Temperature

Orthophosphate (PO_4)

Orthophosphate (PO_4)

Ascorbic Acid Colorimetric Method, Std Methods 4500-P E

Basic Method Steps:

- 50 mLs sample measured out (after the sample digestion)
- 0.05 mL (1 drop) phenolphthalein indicator is added to sample
 - If red color develops, add 5N H_2SO_4 dropwise til color disappears
- Add 8 mL combined reagent and mix for 10-30 minutes
- Measure absorbance at 880 nm, using reagent blank as reference

This method requires a pH of 8.3 for the blue color complex to develop. The combined reagent contains sulfuric acid. So if all the pink is removed from the sample after the phenolphthalein step, then when the combined reagent is added the pH will be too acidic for the blue color to form properly and low percent recoveries may be seen in the standards.

Orthophosphate (PO_4) Tip

Ascorbic Acid Colorimetric Method, Std Methods 4500-P E

Nancy Taylor (City of Newark) suggest not removing all the pink.

- "What I do is pour my samples into a 50 mL graduated cylinder, bring them to a 45 mL volume and look down on them from the top to see as much of the pink color as possible. Then I adjust the pH to 1 drop above removing all the pink. If I over shoot then I raise the pH with NaOH to return to the pink color and start over. When I then add the combined reagent the last bit of pink dissipates and I am achieving recoveries between 95-105% on my standards. Before, my recoveries were between 50-75%."

TKN (Micro Method) Tip

TKN (Micro Method) Tip

- Try cooking the 50 mLs of sample down overnight in a 200°C oven before boiling to fumes on the Labconco Micro-Kjeldahl Digestion Unit.
 - Pre-baking in an oven reduces the water volume, and a smaller volume placed on a burner is less likely to spatter out of the micro-Kjeldahl flask (which would result in sample loss and low standard recovery).
 - The acid is what is left behind, and goes to fumes.

– Nancy Taylor, City of Newark

ICP Metals Prep Tip

- Use ultra pure reagents
 - Double distilled acids are a must!!!!
- Equipment is pre-cleaned, disposable or HAND cleaned
 - Absolutely **NO** tap water or general lab grade acids should be used for cleaning
- “Kitchen area” of the ICP is cleaned regularly
 - Again **NO** tap water or general lab grade acids
- Tubing is changed after sludge samples are analyzed or if they appear crimped
 - Denise Seaman, City of Youngstown

Laboratory Temperature

- The temperature of the lab itself can be detrimental to some analyses!
 - It can throw off the calibration of your spectrophotometer

Maintenance Log Books

- Keep a log book for all laboratory instruments
 - Maintenance logs
 - Last calibration date and results
 - Gas detectors very important for safety reasons
 - Cleaning if necessary
 - Instrument repairs
 - Why it was needed and what was done, best to be specific so when problem occurs a year from now you can fix it easily.

Questions or Comments?