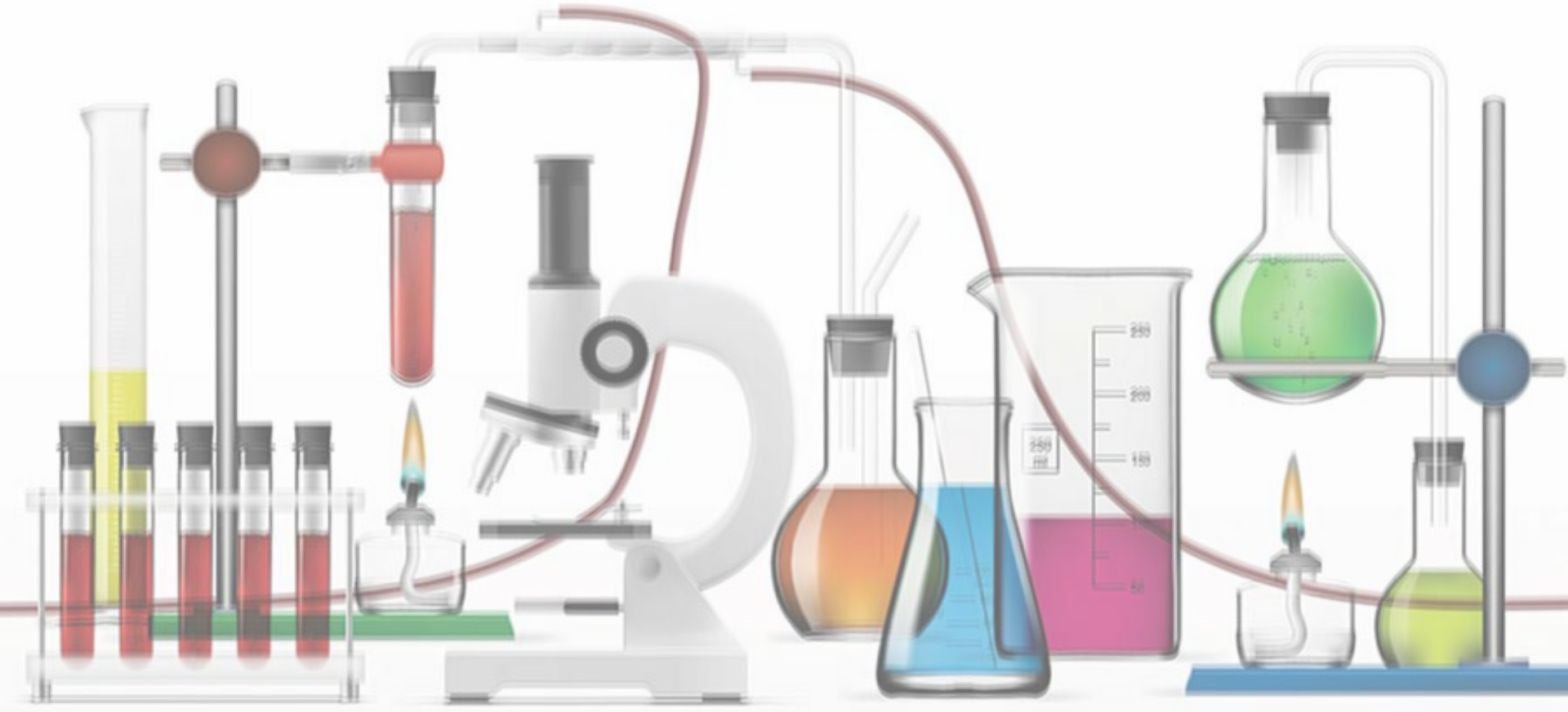
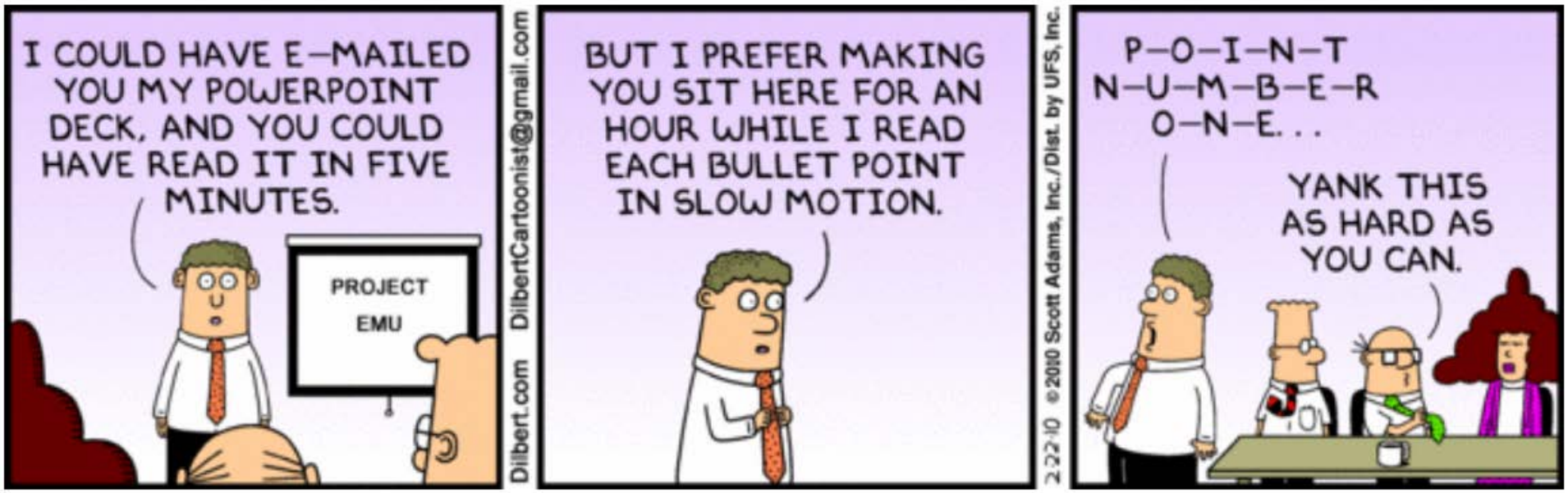
Lab Basics

Janelle Nehs
Patty Doerflinger
October 6, 2022



Written by Autumn Farrell


I COULD HAVE E-MAILED YOU MY POWERPOINT DECK, AND YOU COULD HAVE READ IT IN FIVE MINUTES.
PROJECT EMU
Dilbert.com DilbertCartoonist@gmail.com
BUT I PREFER MAKING YOU SIT HERE FOR AN HOUR WHILE I READ EACH BULLET POINT IN SLOW MOTION.
2-22-10 © 2010 Scott Adams, Inc./Dist. by UFS, Inc.
P-O-I-N-T
N-U-M-B-E-R
O-N-E...
YANK THIS AS HARD AS YOU CAN.

Lab Basics Agenda

SLIDES

Sample Handling

- Preservation
- Homogenization
- Storage and hold time

Lab Support Equipment

- Glassware
- Balances
- Desiccators

Standard Operating Procedures

Data Entry

- LOD calculations
- eDMR completion

Extra

HANDS-ON STATIONS

Sample Handling

- Checking pH

Lab Support Equipment

- Using volumetric flasks
- Using pipettes
- Reading graduations

Data Entry

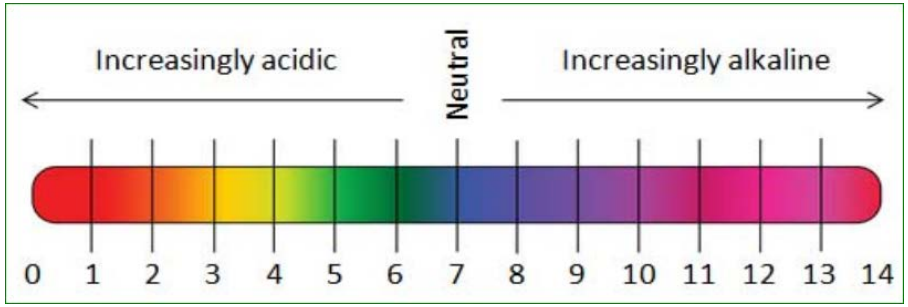
- LOD calculations
- eDMR completion

Sample Handling:
Preservation

- **Why must samples be preserved?**
  - Physical, chemical, and biological processes can alter the concentration of the analyte we want to measure.
  - Adding acid (or base) and chilling the sample help to stabilize the analyte for a limited time.

Sample Handling:
Chemical Preservation

- Chemical Preservation
  - Some analytes require chemical preservation. This information can be found in NR 219 Table F.
  - If samples for these analytes cannot be run within 15 minutes of collection, the samples must be preserved.
  - For wastewater samples that need acid preservation, generally, about 0.5 mL of acid per 200 mL will be enough to get the pH to <2.
  - Use narrow range pH paper to check that the samples are <2.
  - Record that the sample was preserved to <2.

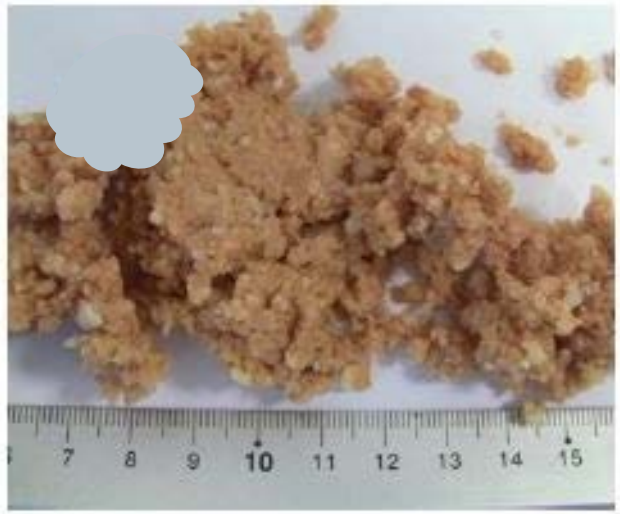


Sample Handling:
Thermal Preservation

- Thermal Preservation
  - Some analytes require thermal (cold storage) preservation. This information can be found in NR 219 Table F.
  - If samples for these analytes cannot be run within 15 minutes of collection, the samples must be thermally preserved.
  - When preservation and storage indicates 4°C, acceptable temperatures are from above freezing (usually 0°C) up to 6°C.

Sample Handling:
Homogenization (representative sample)

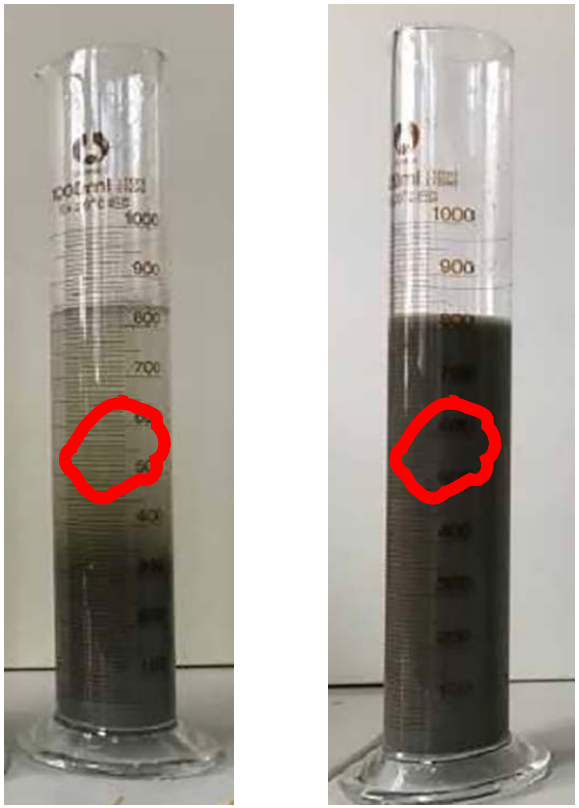
whole pizza



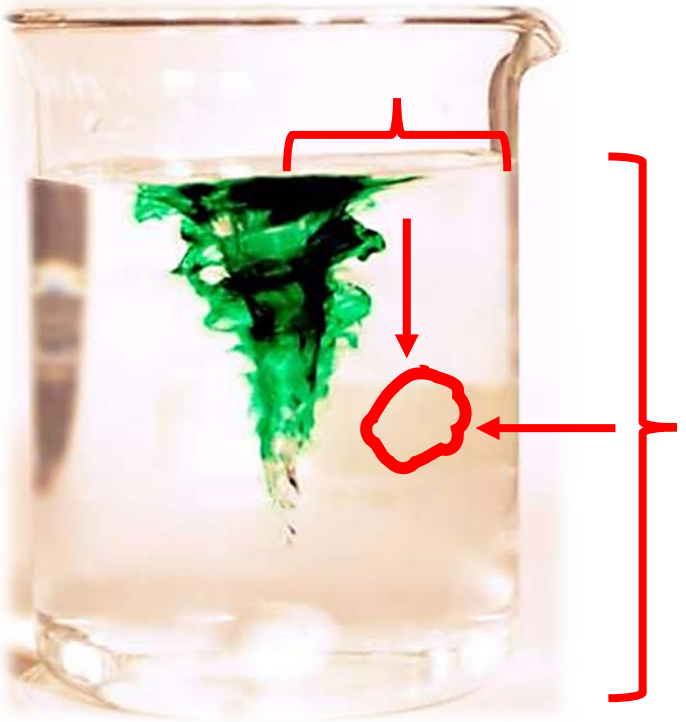
homogenized pizza

If you took one bite of this pizza, you may not get a piece of each topping. To get a bite with some of each topping, you could homogenize it. The same goes for your samples. Mix (homogenize) them well to make sure the portion you take to analyze is representative of the sample.

Sample Handling:
Homogenization (representative sample)



The sample on the left is starting to settle. The same sample on the right has been thoroughly mixed and is homogenized. When taking a portion of sample, be sure the sample is thoroughly mixed.



SM 2540D for TSS says “While stirring, pipet a measured volume onto the seated glass-fiber filter. For homogenous samples, pipet from the approximate midpoint of the container but not in the vortex. Choose a point both middepth and midway between wall and vortex.”

Sample Handling:
Storage and hold time

- **Sample Storage**
  - Store samples at 0 – 6°C.
    - If a sample requires thermal preservation of 4 °C, the acceptable range is not frozen to 6 °C.
- **Store Samples and Standards Separately**
  - Total phosphorus and ammonia samples must be stored separately from standards.
- **Sample Hold Time**
  - Samples can be stored for only a certain amount of time from collection to analysis if it is preserved as required. Different analytes have different hold times. See NR 219 Table F for hold times.

Lab Support Equipment:
Glassware

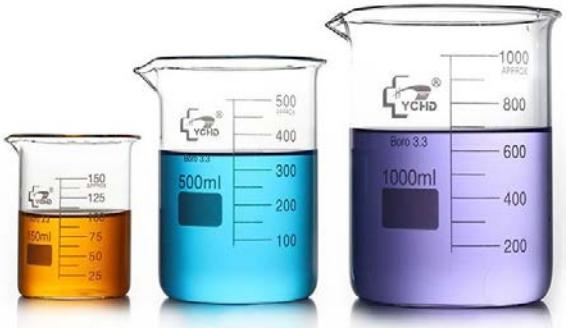
Use to make specific concentration solutions and measure sample volumes.

Volumetric Flasks

Graduated Cylinder

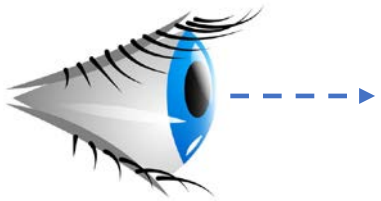
Use to mix or perform reactions. Do not use for measuring volumes – markings are not accurate.

Erlenmeyer Flask

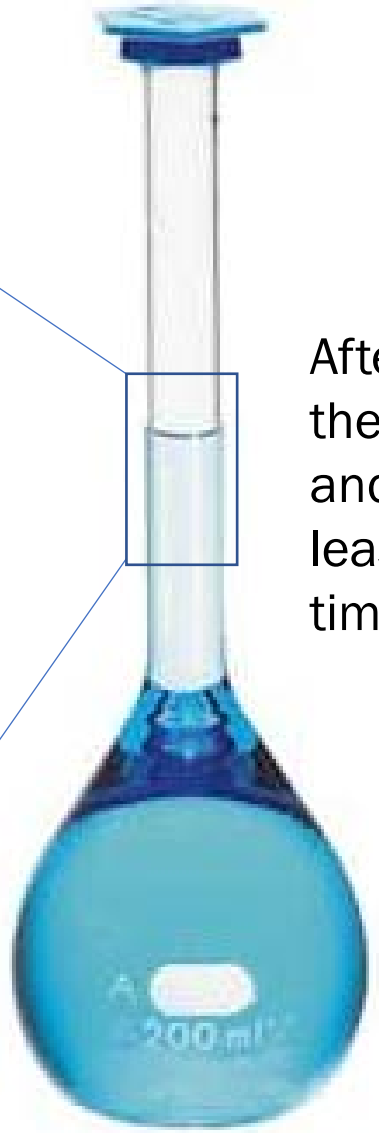


Beakers

Lab Support Equipment:
Volumetric Flasks

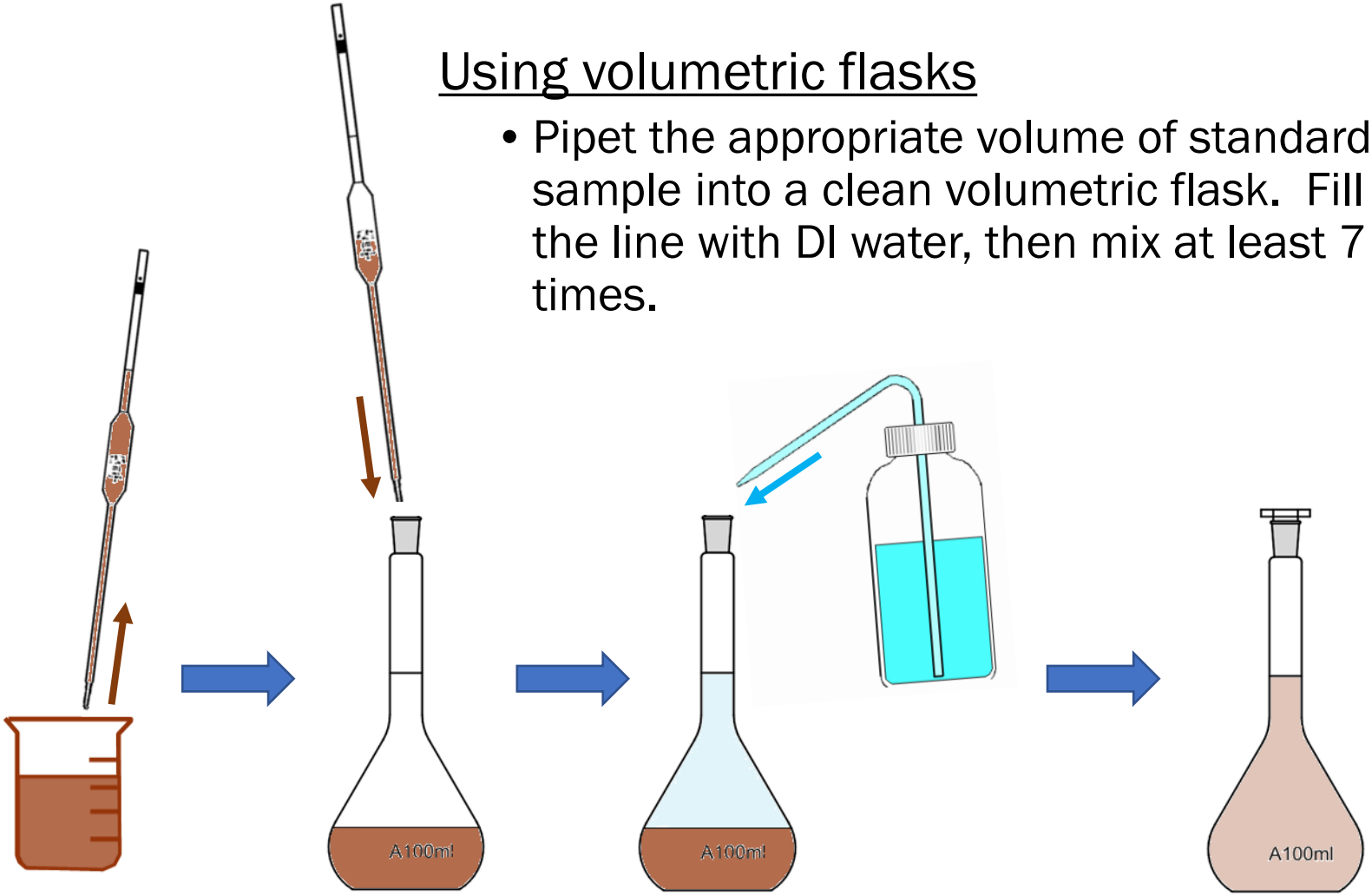


Fill until the bottom of the meniscus is at the line. View this at eye level. If you fill over the line, start over.



After filling to the line, cap, and invert at least seven times to mix.

Lab Support Equipment:
Volumetric Flasks



Using volumetric flasks

- Pipet the appropriate volume of standard or sample into a clean volumetric flask. Fill to the line with DI water, then mix at least 7 times.

Lab Support Equipment:
Pipettes

Mechanical, autopipettor or micropipette

Volumetric or transfer pipettes

Graduated, measuring, or serological pipettes

Lab Support Equipment:
Pipettes

<u>Using mechanical pipettes</u>

- Set to the desired volume if the pipet is adjustable.
- Depress the plunger to the first stop (loading stop).
  - Many pipettes have 2 stops – the first is where there is resistance; the second is where you can no longer depress the plunger.
- Immerse the tip below the surface of the liquid.
- While keeping the tip below the surface, gently release the plunger.
- Wait a second to ensure the liquid finishes gettin sucked up.

Lab Support Equipment:
Pipettes

Using mechanical pipettes (continued)

- Remove the tip from the liquid. If there are any droplets on the tip, carefully wipe away trying to avoid the tip.
- Hold the pipette at an angle and touch the tip to the inside of the receiving flask.
- Smoothly depress the plunger to the second stop (unloading stop).
- Remove the pipette. Gently allow the plunger to return to its rest position.
- Discard the tip.

Lab Support Equipment:
Glass Volumetric Pipettes

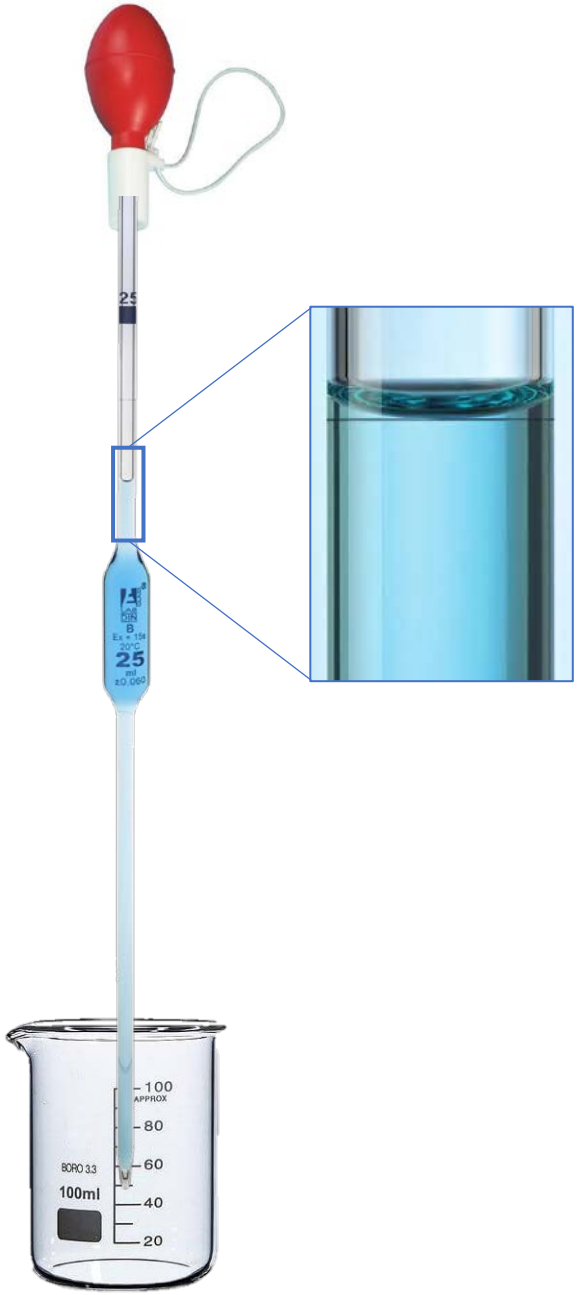
<u>Using a volumetric pipette</u>

- Use a clean pipet
  - Rinse the pipet two or three times with the liquid you want to transfer. Do this by drawing a small amount of liquid in the pipet, turn horizontally and rotate to contact all surfaces. Discard the liquid to waste.
- Squeeze the bulb and place the bulb on the top end of the pipet just enough to create a seal.
- Place the end of the pipet well below the surface of the liquid, and slowly release the bulb, drawing the liquid up the pipet. Suck enough liquid to go above the line but DO NOT suck the liquid into the bulb.

Lab Support Equipment:
Glass Volumetric Pipettes

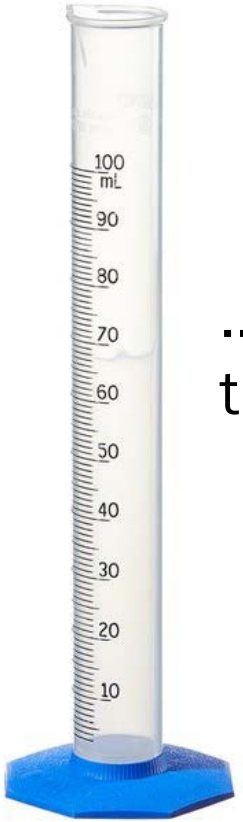
<u>Using a volumetric pipette</u> (continued)

- Quickly remove the bulb and cover the opening with your index finger.
- Remove the pipet from the liquid and gently wipe the outside of the pipet.
- Carefully release the seal your finger made until the liquid level reaches the line.
- Place the tip of the pipet in the receiving flask and remove your finger and allow the liquid to freely drain. Touch the tip once to the inside of the flask. DO NOT blow out the remaining liquid in the pipet.



Lab Support Equipment:
Graduated Cylinders

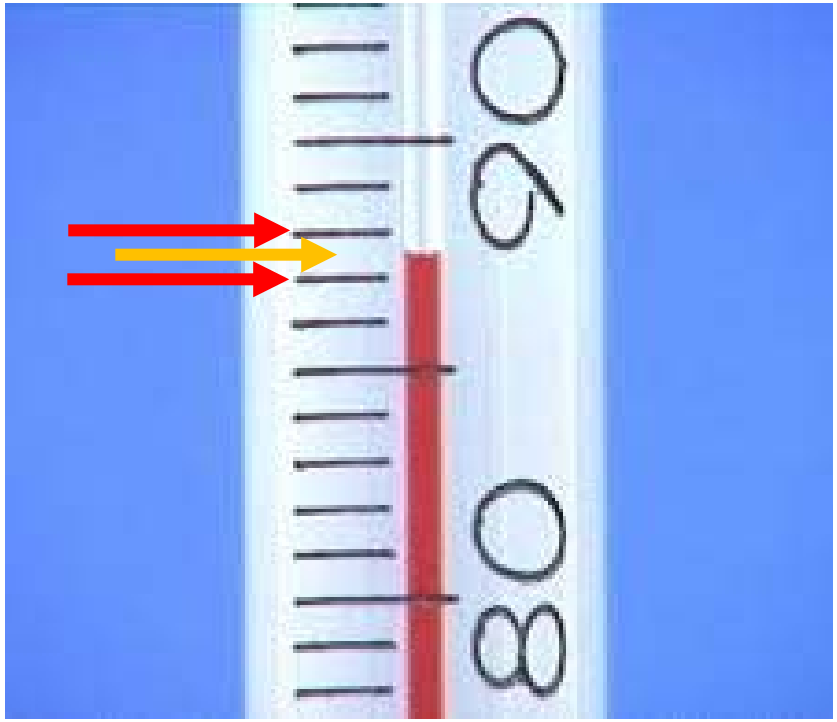
If you can read one of these…



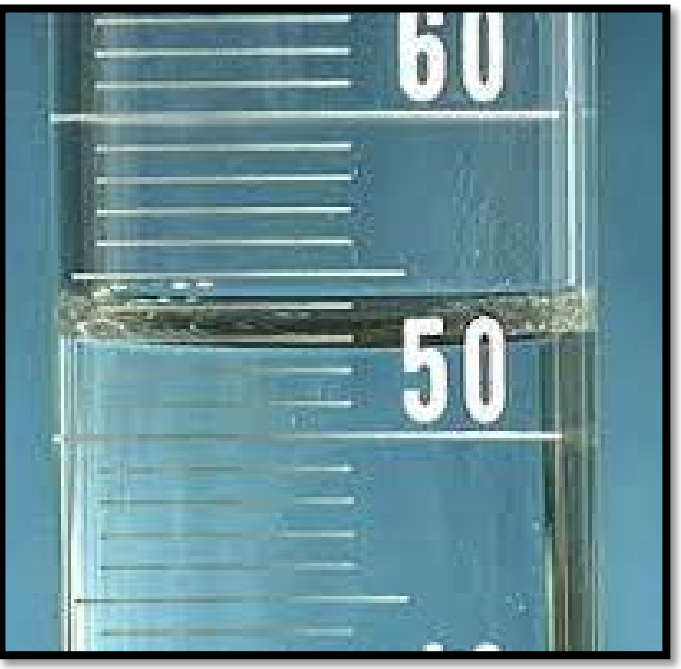
… you can read one of these.

Lab Support Equipment:
Reading Graduations

- For example, in this picture we know the temperature is for sure between 87° and 88°. It might be about halfway between the two or maybe a little less. We can only estimate the last number. This temperature would be 87.5°.

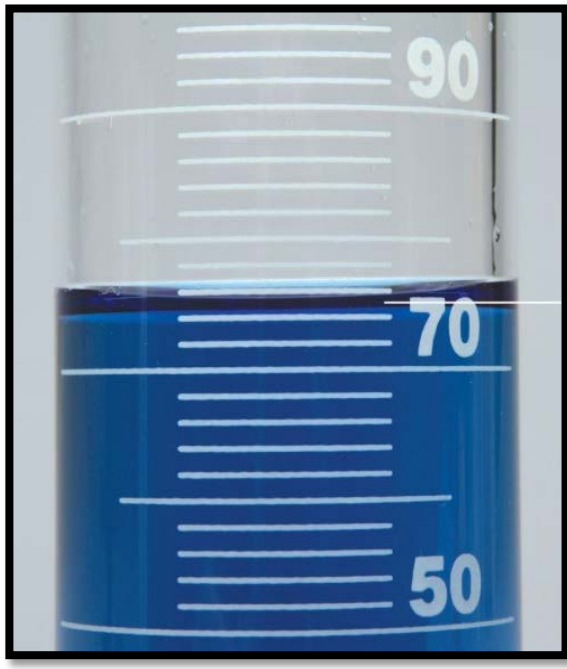


Lab Support Equipment:
Reading Graduations



52.5 mL

6.6 mL



74.0 mL

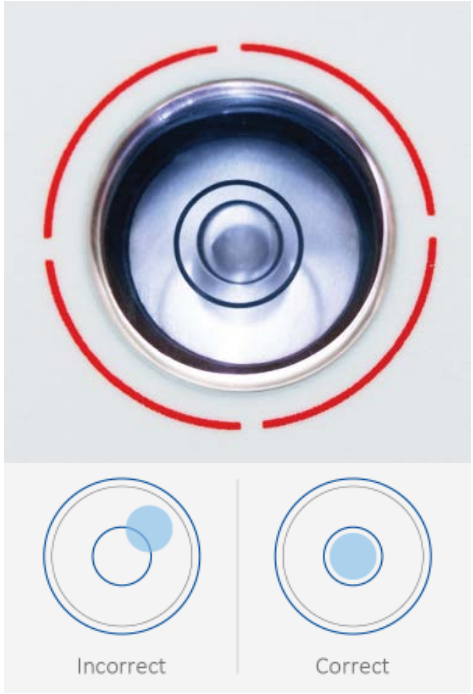
Lab Support Equipment:
Balances

Analytical balance

Able to read to four decimal places (0.0000 g)

Non-analytical or top loader balance

Able to read to one or two decimal places (0.0 g or 0.00 g)



Keep balances on a stable surface and away from drafts. Always check to make sure the bubble is centered.

Lab Support Equipment:
Balances – Weights

- Always handle weights with a forceps or gloved hands.
- Keep weights in the original container. Store in a safe place (desiccator preferable).
- Place weight in center of balance.

Lab Support Equipment:
Balances – Tare

Pressing “tare” or “zero” resets the balance’s display to zero. When you measuring the mass of something, you don’t want to include the container’s mass in the reading. To tare the container, place it on the balance, wait for a stable reading, then press the tare key to reset the display to zero.

Lab Support Equipment:
Desiccators/Desiccant

Standard Operating Procedures (SOP)

- SOPs must include some basic information. These items are required to ensure the lab can maintain quality and reproduce the test in its entirety.

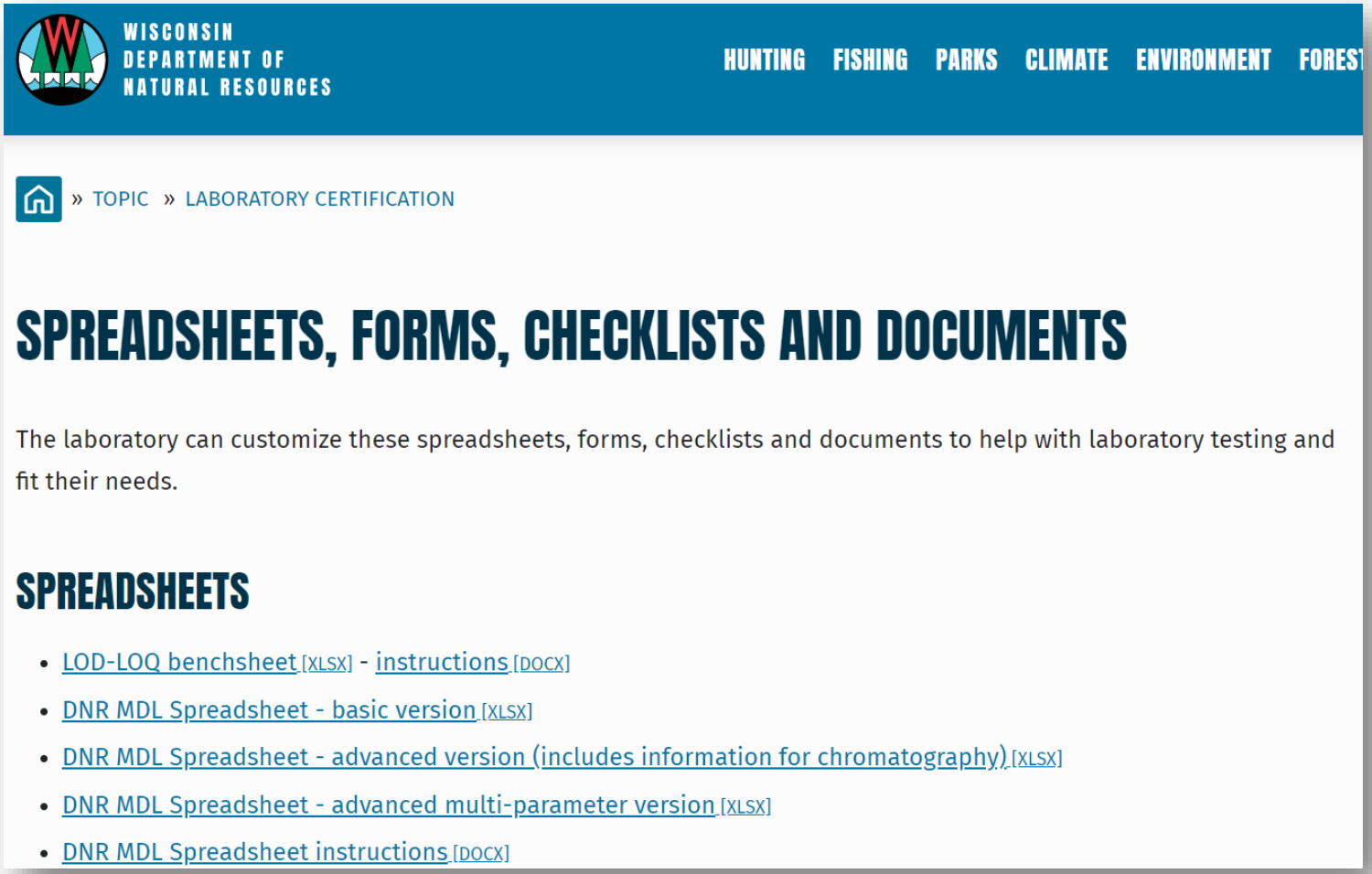
Standard Operating Procedures (SOP)

- Clearly written standard operating procedures are essential because they eliminate uncertainty about how to complete a task. Anyone with basic training should be able to produce the same results of a procedure by just following the SOP.
- The SOP can be as detailed as needed to help analysts efficiently and correctly complete a test. They are your SOPs—make them helpful.
- Some parts of the SOP won't be frequently referred to by staff but are important when needed.
- A good SOP will be the first place to go to when you have questions or concerns about the test you are doing.
- Experienced analysts likely won't look at the SOPs when doing a test. People have a tendency to become complacent or maybe even forget some details after time. It is always good to review an SOP at some frequency to ensure the test is being done as required.

Data Entry

LOD (MDL) Calculations and Instructions

Spreadsheets, forms, checklists and documents | | Wisconsin DNR



WISCONSIN DEPARTMENT OF NATURAL RESOURCES

HUNTING FISHING PARKS CLIMATE ENVIRONMENT FOREST

» TOPIC » LABORATORY CERTIFICATION

SPREADSHEETS, FORMS, CHECKLISTS AND DOCUMENTS

The laboratory can customize these spreadsheets, forms, checklists and documents to help with laboratory testing and fit their needs.

SPREADSHEETS

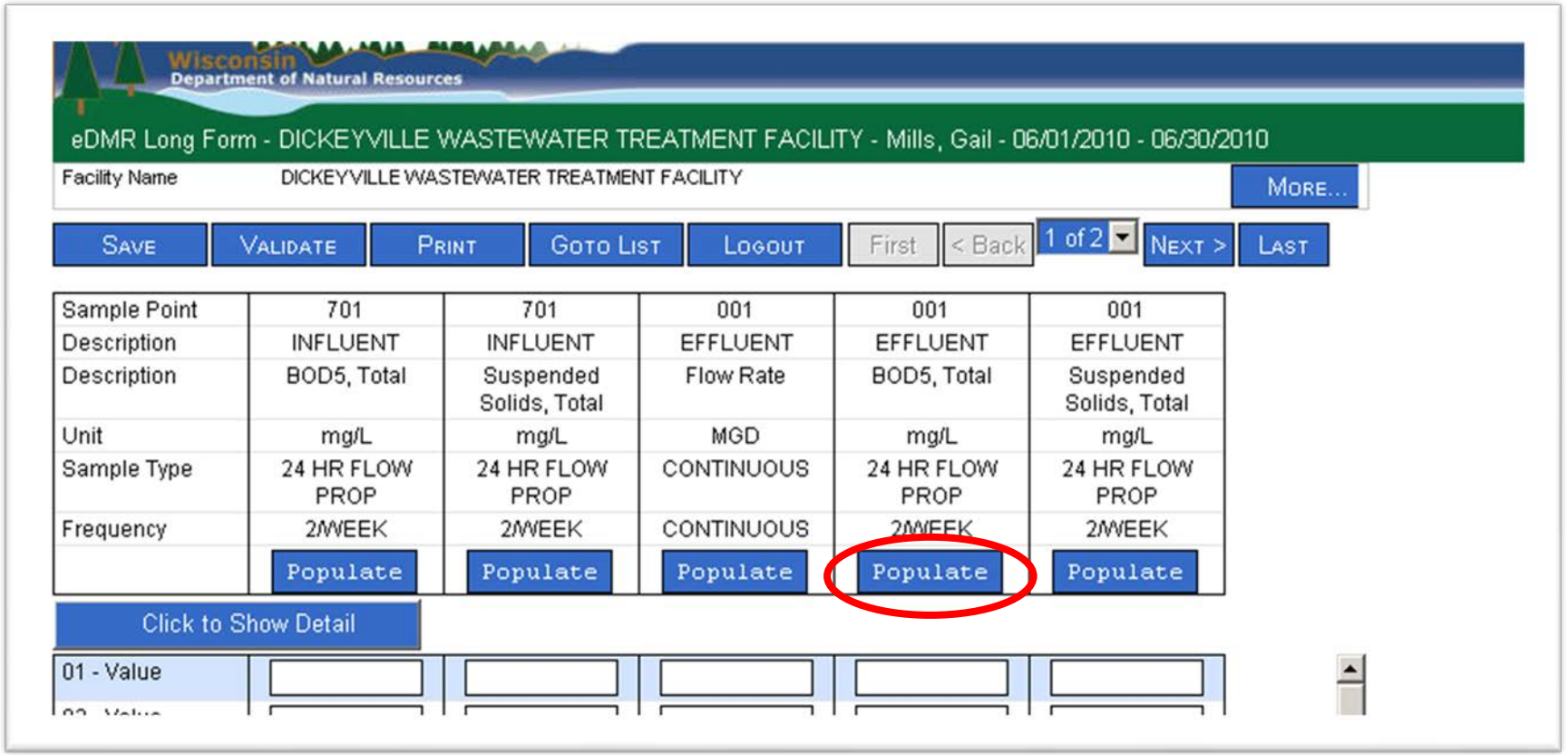
- LOD-LOQ benchsheet [XLSX] - instructions [DOCX]
- DNR MDL Spreadsheet - basic version [XLSX]
- DNR MDL Spreadsheet - advanced version (includes information for chromatography) [XLSX]
- DNR MDL Spreadsheet - advanced multi-parameter version [XLSX]
- DNR MDL Spreadsheet instructions [DOCX]

Data Entry
eDMR Reporting – Qualifiers

- The specific items to include are as follows:
  - What QC sample type failed? (e.g., GGA, BOD blank, etc.)
  - What was the value of the failed QC sample? (e.g., 150 mg/L)
  - What was the analysis date of the failed QC sample? (e.g., samples analyzed on 9/30/22)
  - What were all of the sample dates that were analyzed with the failed QC sample? (e.g., influents and effluents on 9/26/22, 9/28/22, 9/30/22)
- Complete examples:
  - The GGA analyzed on 9/30/22 failed at 150 mg/L. The samples analyzed with this GGA were the effluents with sample dates of 9/26/22, 9/28/22, and 9/30/22.
  - The BOD blank analyzed on 9/30/22 failed at 0.31 mg/L. The samples analyzed with this BOD blank were from sample dates of 9/26, 9/28, and 9/30.
  - The TSS oven temperature on 9/30/22 was 106°C. Samples could not be recollected. The samples affected had sample dates of 9/28 and 9/30.

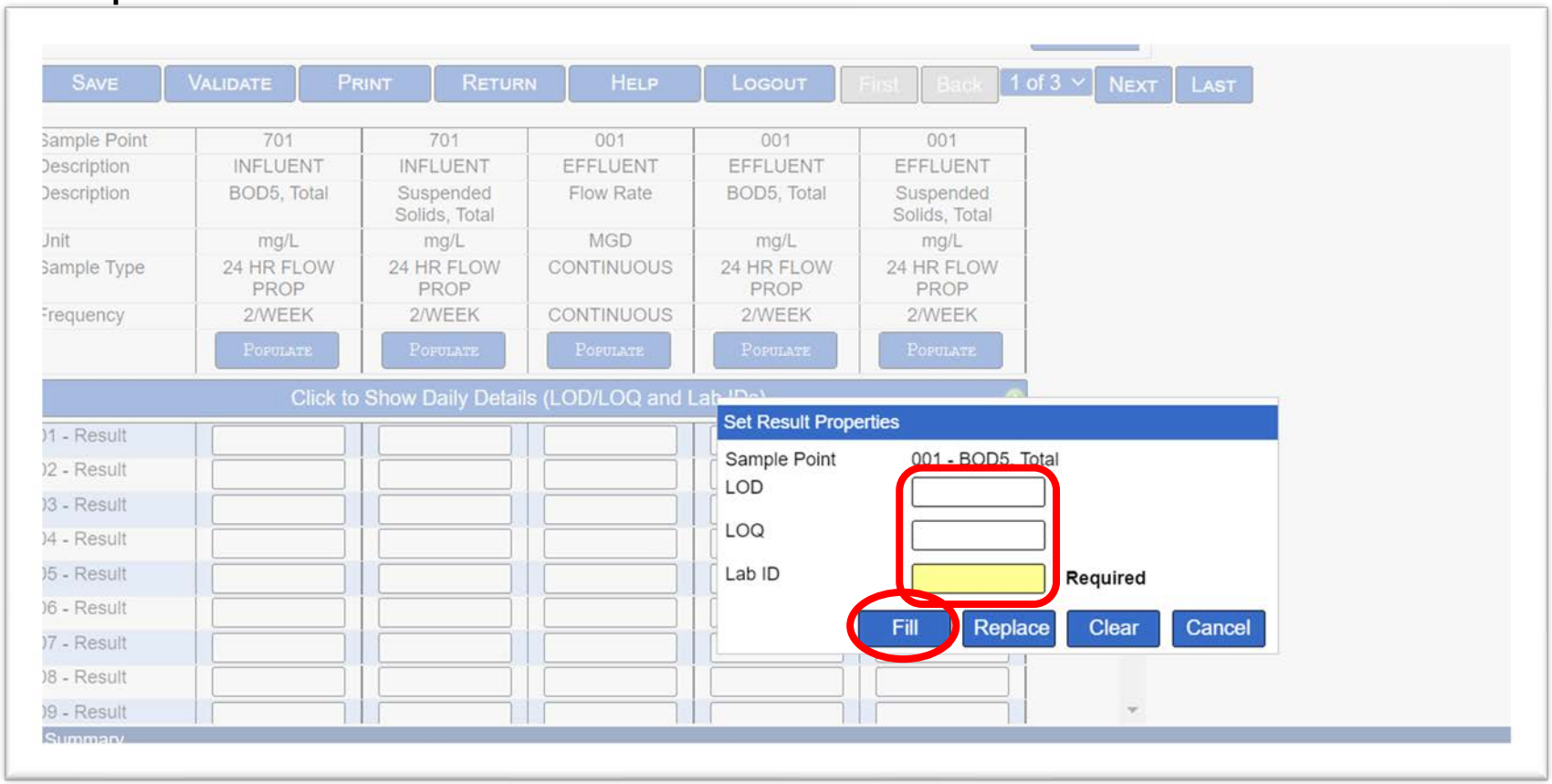
Data Entry
eDMR Reporting – LODs and Lab FIDs

- To enter the LOD, LOQ, and Lab ID data that applies to all or most samples for a test, click on the “Populate” button to enter data that will apply to all samples for that test.



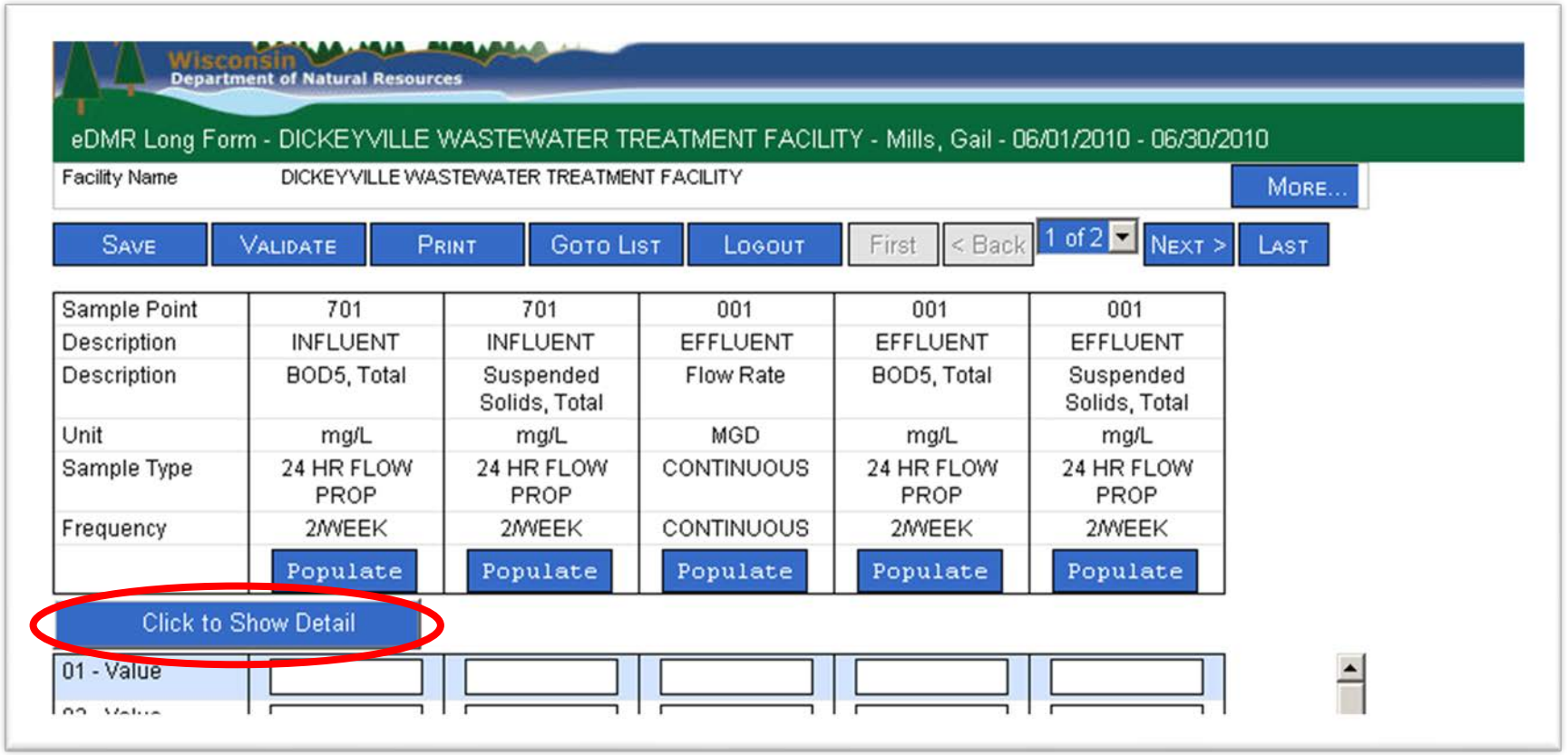
Data Entry
eDMR Reporting – LODs and Lab FIDs

- In the window that opens, enter the LOD, LOQ and Lab ID. Click "Fill." This will apply that LOD, LOQ, and Lab ID to all the samples for that test.



Data Entry
eDMR Reporting – LODs and Lab FIDs

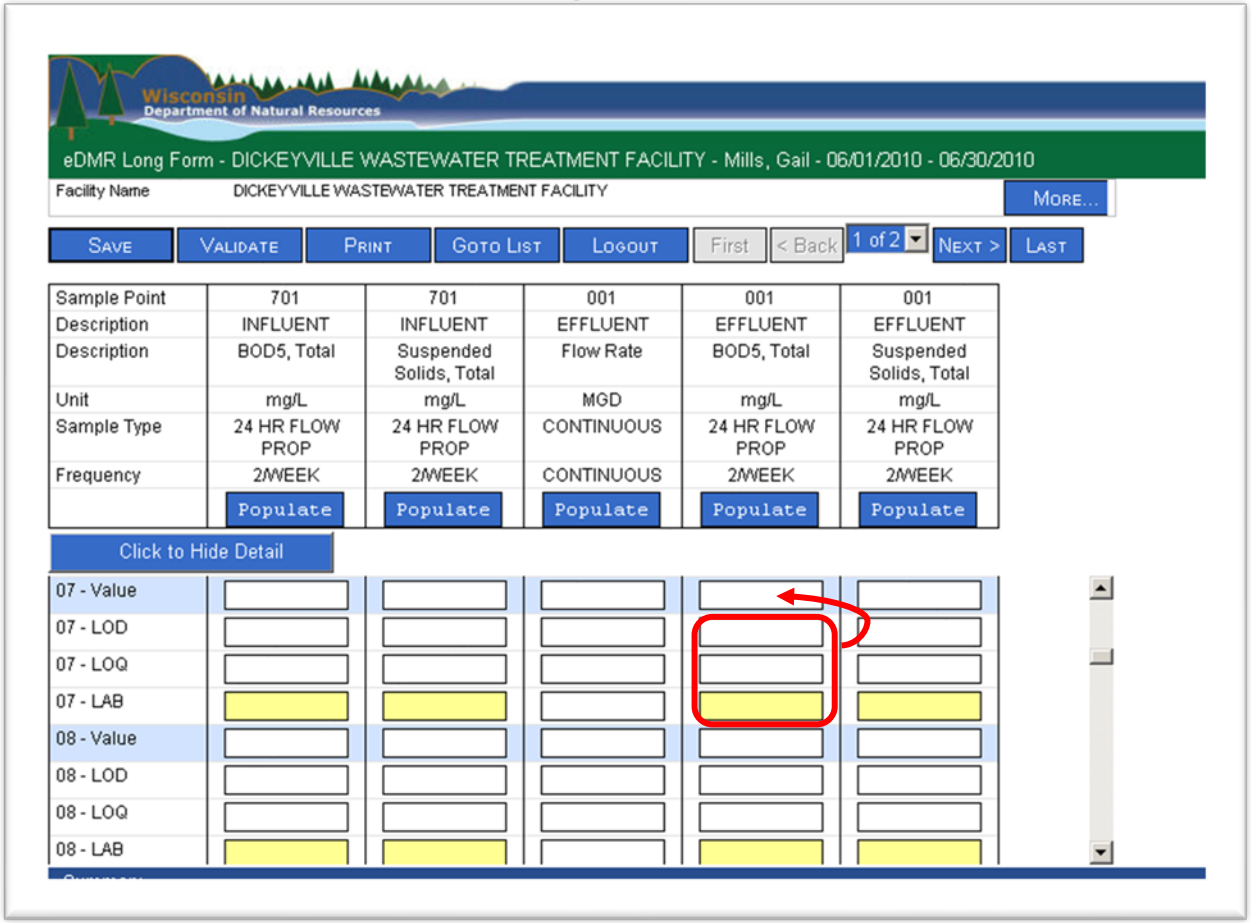
- To enter data that only applies to a few samples, after populating, click on the "Click to Show Detail" button to update information for those specific samples.



Data Entry
eDMR Reporting – LODs and Lab FIDs

- When the “Click to Show Detail” button is clicked, a cell for LOD, LOQ, and Lab ID for each individual sample result is shown.

This would be used for occasional samples that were diluted in the lab and need the LOD adjusted. Also, for sample sent to a subcontract lab instead of analyzed in the facility, you would enter in the subcontract lab ID for those specific samples.




IF IT WASN'T DOCUMENTED
IT WASN'T DONE
makeameme.org

More information

- **Lab Accreditation Code, NR 149:** (000001.ildoc) (wisconsin.gov)
  - Contains the requirements for registered and certified laboratories
- **Lab Accreditation website:** Laboratory Certification | Wisconsin DNR
  - Contains lists of accredited labs, BOD help and information, documents to help labs, application information, training, etc.

- **Wastewater Analytical Test Methods and Procedures Code, NR 219:** (000001.ildoc) (wisconsin.gov)
- **Wastewater website:** Wastewater | Wisconsin DNR
  - Contains a listing of WPDES permits, guidance, information on operator certification, etc.

The equipment for this session was provided by…

- GBMSD (NEW Water)
- Plymouth Utilities Commission

Thank you to Lab Cert staff and Wastewater staff for helping with the hands-on stations.

Questions?

Read all about it!

More lab information is included in the following slides. Reach out to your auditor with any questions.

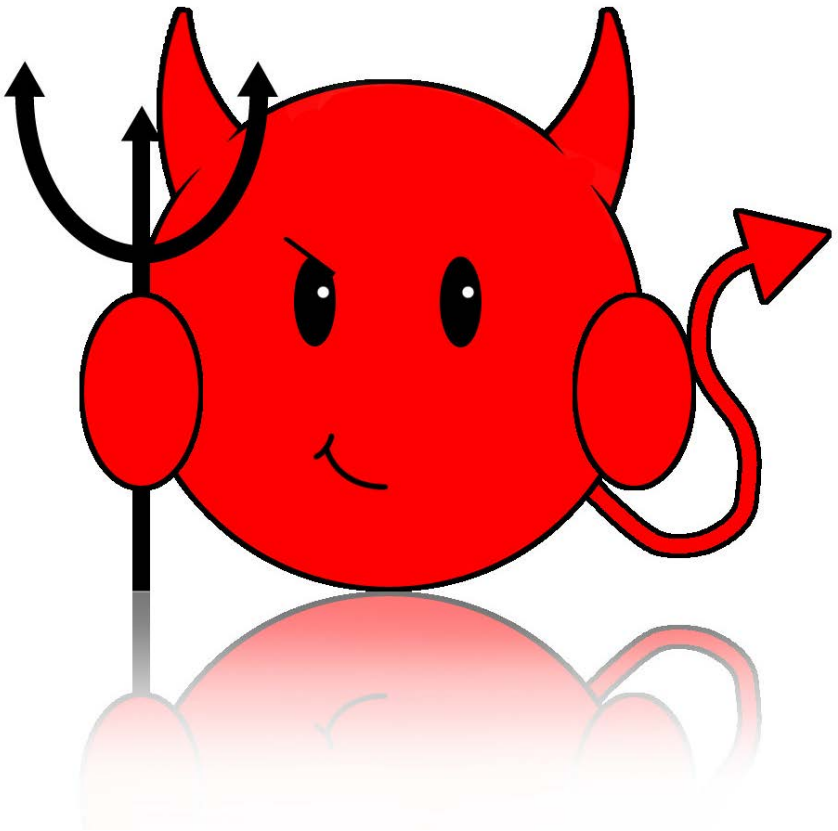
Standards
Definitions

- **Standard:** Standards are solutions for which the analyst knows the true value before running the test. Standards can be made in-house or purchased from a supply company. Standards are often used to calibrate instruments and to evaluate the accuracy of an analysis.
- **Calibration Standards:** A series of solutions with known amounts of analyte. Calibration standards are used to construct calibration curves from which the concentrations of analytes are determined.
- **Stock Standard:** Concentrated standard used to prepare working, intermediate, or other standards.
- **Working Standard:** Standard solutions prepared by dilution of the stock solution or intermediate solution. Working standards are the standards at the concentrations to be analyzed.
- **Intermediate Standard:** A dilution of the stock standard prepared that will be used to prepare the working standards.
- **Verification Standard:** A standard of known concentration used to assess the accuracy of the primary standards of the accuracy of the initial calibration. This includes the ICV and CCV.

Samples
Definitions

- **Environmental Sample or Field Sample:** This is just typically called a sample. This is the liquid or solid material collected to be analyzed. Examples are influent and effluent.
- **Quality Control Sample:** These are often called quality control standards or quality control samples. These solutions have a known amount of analyte and are used to assess the quality of the testing method. Examples are proficiency testing samples and method blanks.

And then Satan said, "Put the alphabet in math."



Solutions

Calculations

$$C_1 \times V_1 = C_2 \times V_2$$

C_1 - 1st (initial) concentration

V_1 - 1st (initial) volume

C_2 - 2nd (final) concentration

V_2 - 2nd (final) volume

If you know three of the four variables, you can solve the equation to determine the last variable.

Need C_1? $C_1 = C_2 \times V_2 / V_1$

Need V_1? $V_1 = C_2 \times V_2 / C_1$

Need C_2? $C_2 = C_1 \times V_1 / V_2$

Need V_2? $V_2 = C_1 \times V_1 / C_2$

Solutions

Calculations

You want to make a 5 mg/L standard.
You have a 100 mL volumetric flask.
Your purchased stock is 50 mg/L.
How much stock should you pipette into the volumetric flask?

Stock Standard
50 mg/L

Working Standard
5 mg/L

$C_1 \times V_1 = C_2 \times V_2$

50 mg/L x 10 mL = 5 mg/L x 100 mL

Solutions

Calculations

	A	B	C	D	E
1	**Calibration Standards Preparation**				
2					
3	Concentration of the stock ammonia standard:		10	mg/L	mg/L = ppm = ug/mL
4					
5		**Concentration of Standard to prepare (mg/L)**	**Final volume (mL)**	**Amount of stock standard to pipete (mL)**	**Amount of DI water to add (mL)**
6					
7	Standard 1	0.2	5	0.10	4.90
8	Standard 2	0.4	5	0.20	4.80
9	Standard 3	0.8	5	0.40	4.60
10	Standard 4	1	5	0.50	4.50
11	Standard 5	1.4	5	0.70	4.30
12	Standard 6	1.8	5	0.90	4.10
13	Standard 7	2	5	1.00	4.00
14	Standard 8				
15					
16					
17					
18					
19					
20					
21					
22					
23					

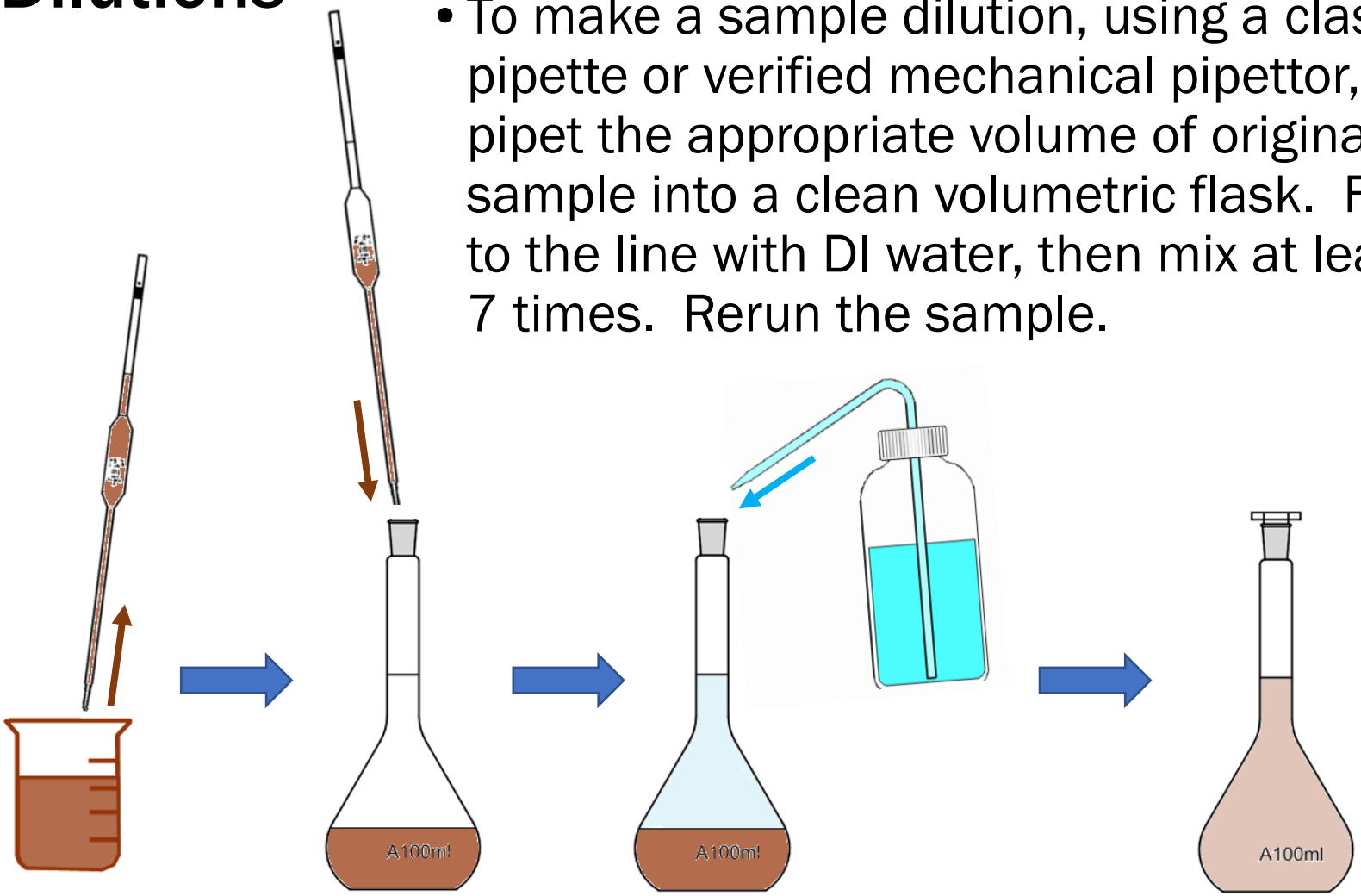
Benchsheet | Standards Prep

There is a tab on the WDNR spreadsheets that will help determine how to prepare the calibration standards. Fill in the green cells.

Solutions

Dilutions

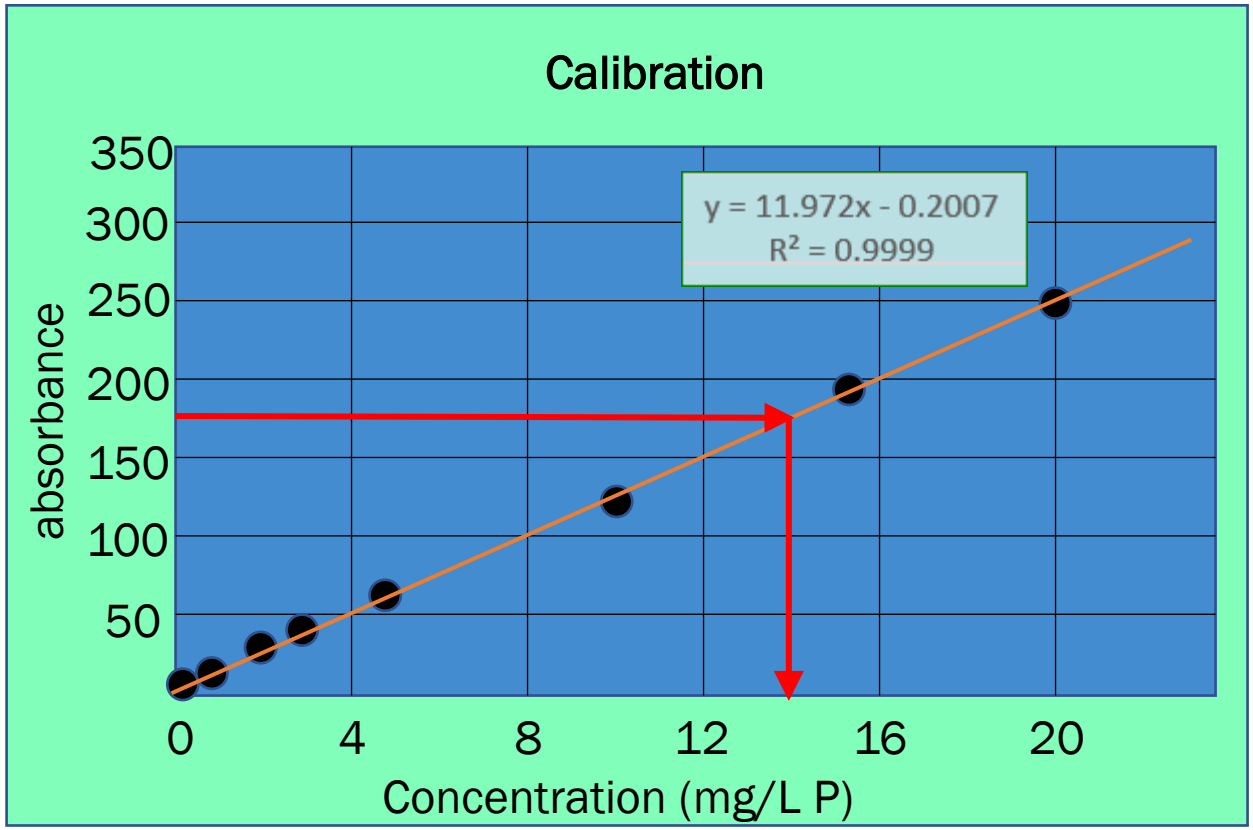
- To make a sample dilution, using a class A pipette or verified mechanical pipettor, pipet the appropriate volume of original sample into a clean volumetric flask. Fill to the line with DI water, then mix at least 7 times. Rerun the sample.



Solutions

Calibration

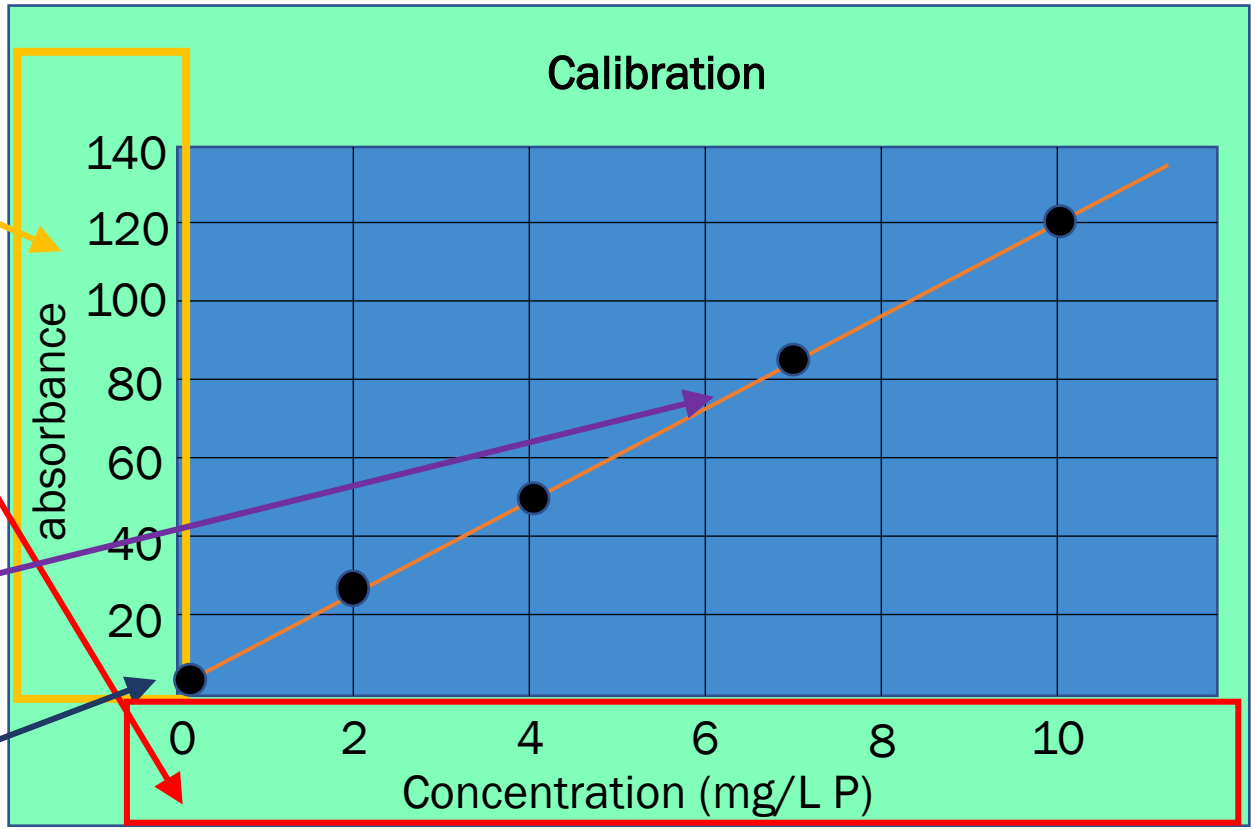
By graphing the relationship between the instrument signal (e.g., absorbance) and the concentration of the calibration standards we prepared, we can calculate a line. From that line, we can determine the concentration of samples at any absorbance.



Solutions
Calibration

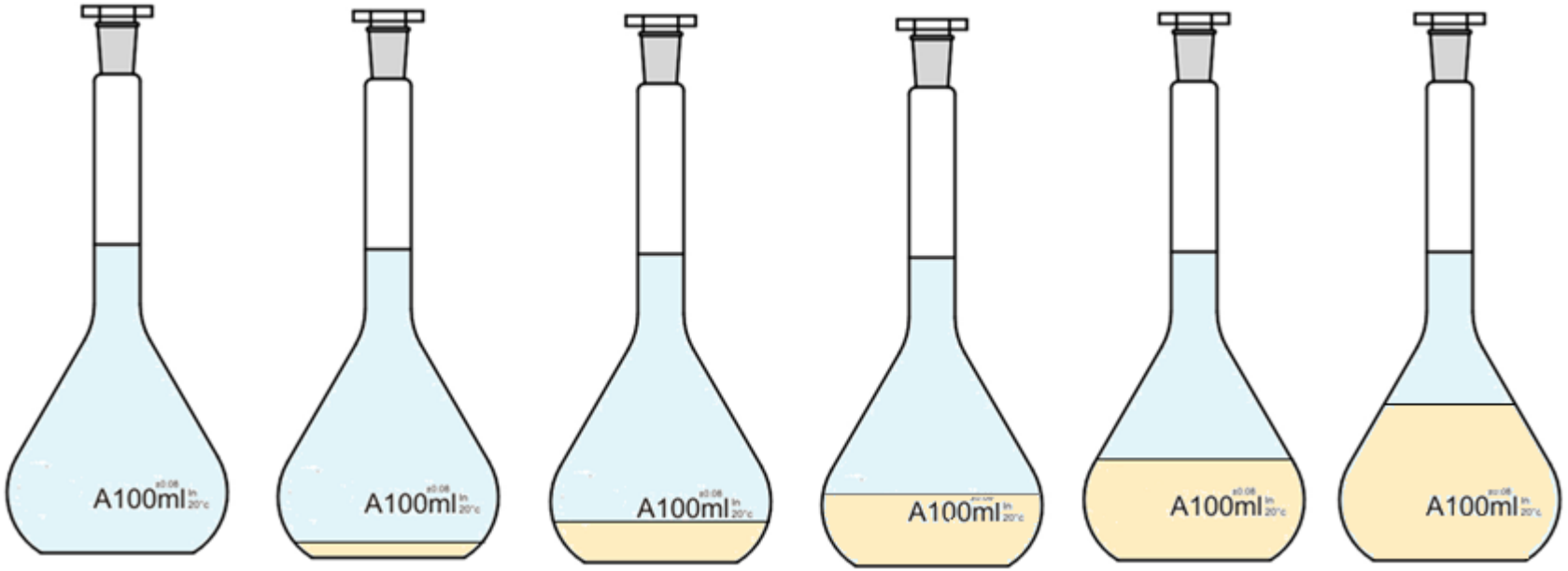
$$y = mx + b$$

- "y" is the uncontrolled variable (we can't control what the instrument reads)
- "x" is the variable we can control—we made the standards
- "m" is the slope, or the steepness of the line
- "b" is the y-intercept, or where the line crosses the y-axis



Solutions
Calibration

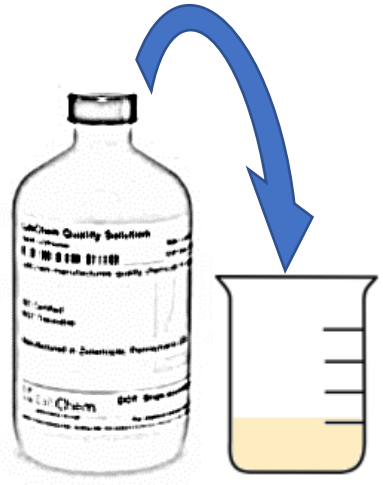
- Preparing Standards
  - A very accurate way to prepare standards is to do dilutions of the stock standard in volumetric flasks.



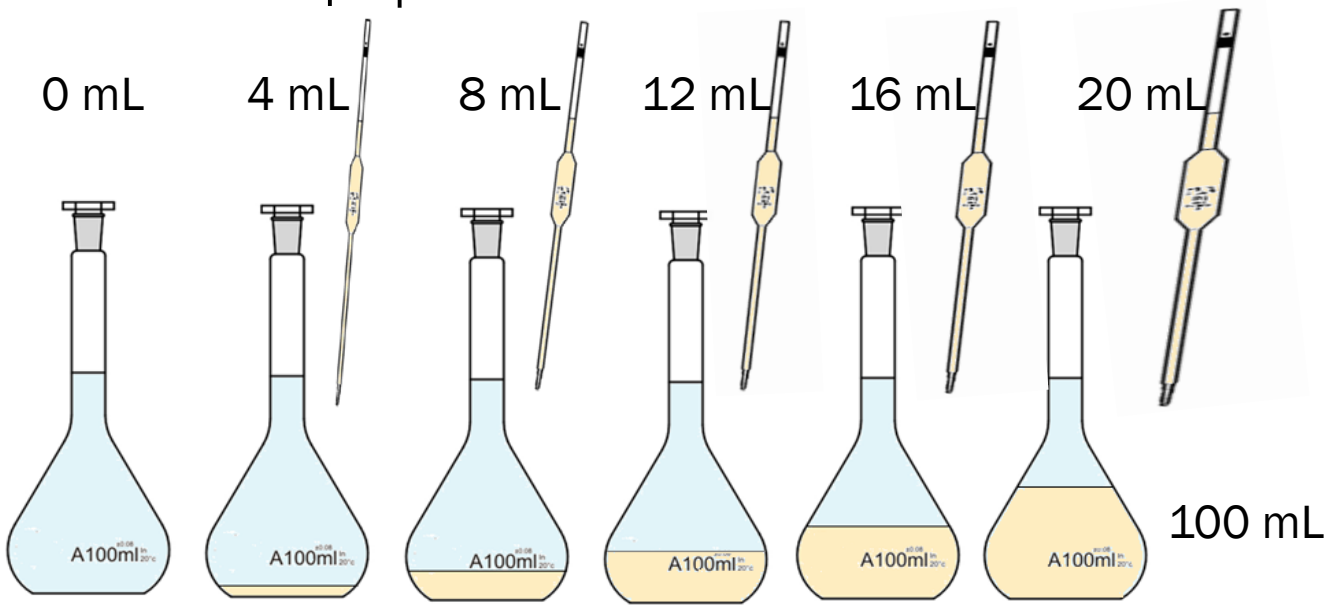
Solutions
Calibration

Standard Preparation

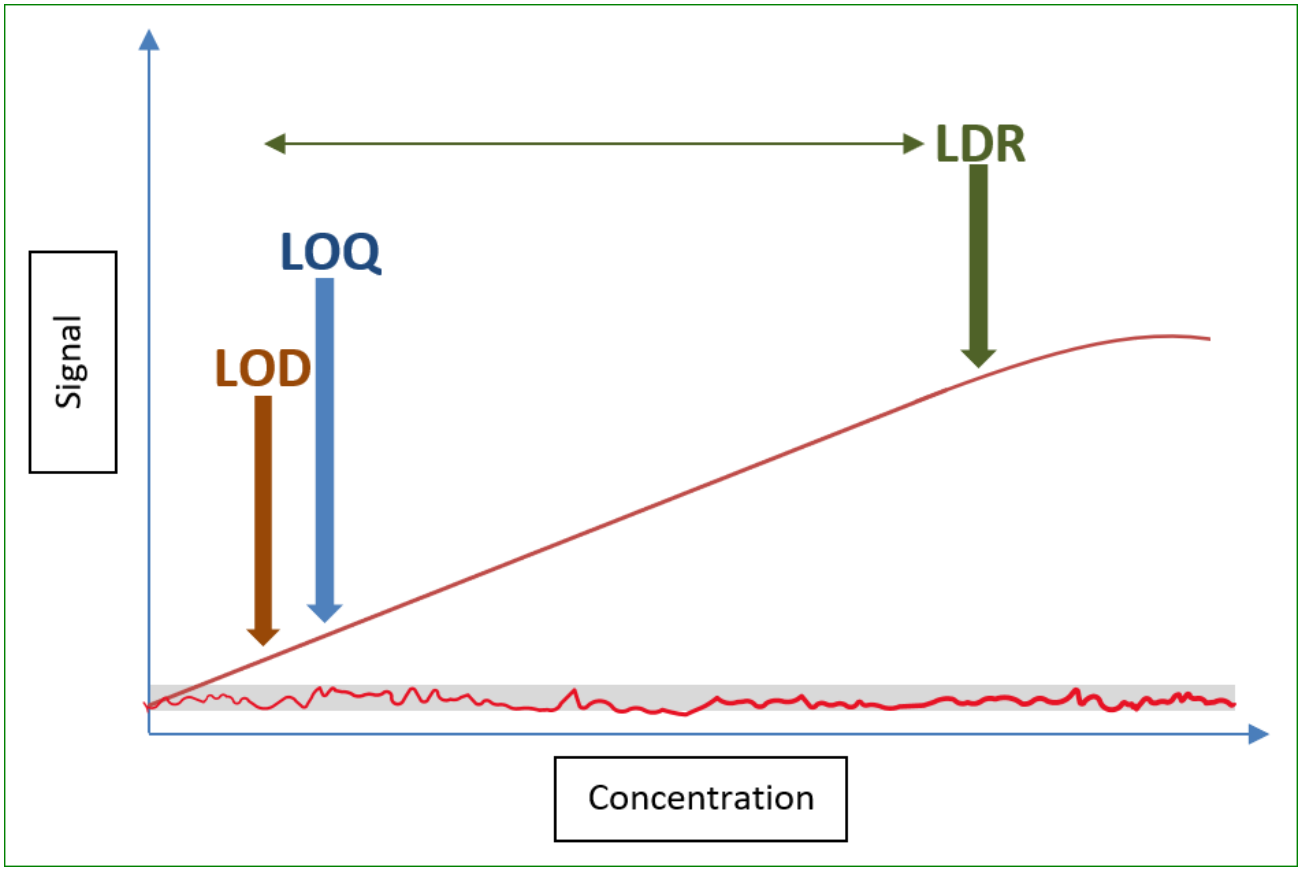
- To make the standards, using a class A pipette or verified mechanical pipettor, pipet the appropriate volume of stock standard into a clean volumetric flask. Fill to the line with DI water, then mix.
- Use a separate volumetric flask to prepare each concentration of standard.



5 mg/L-NH3

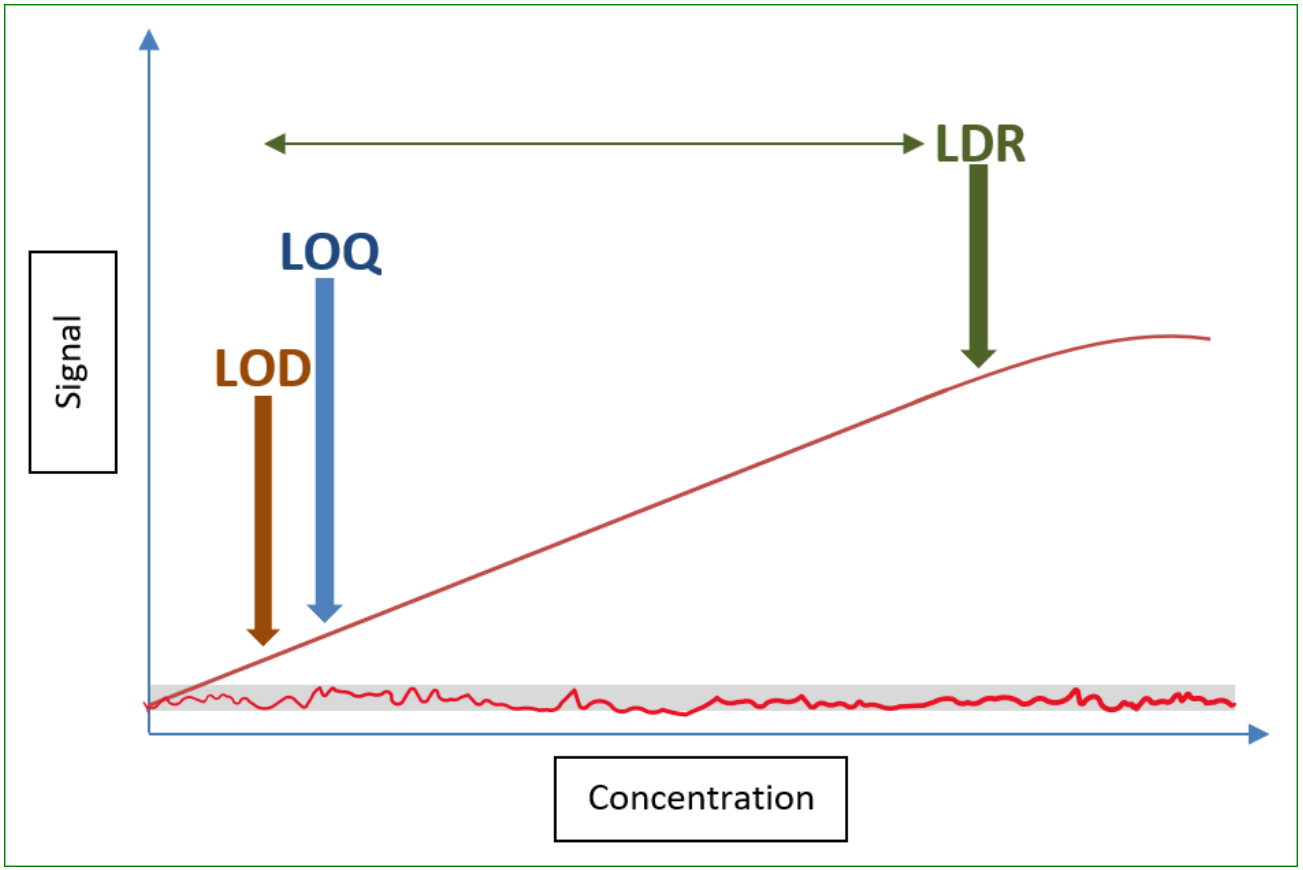


Calibration



Limit of detection (LOD):
the lowest concentration of an analyte that can be identified and reported with confidence that the analyte is in the sample and isn't just noise.

Calibration

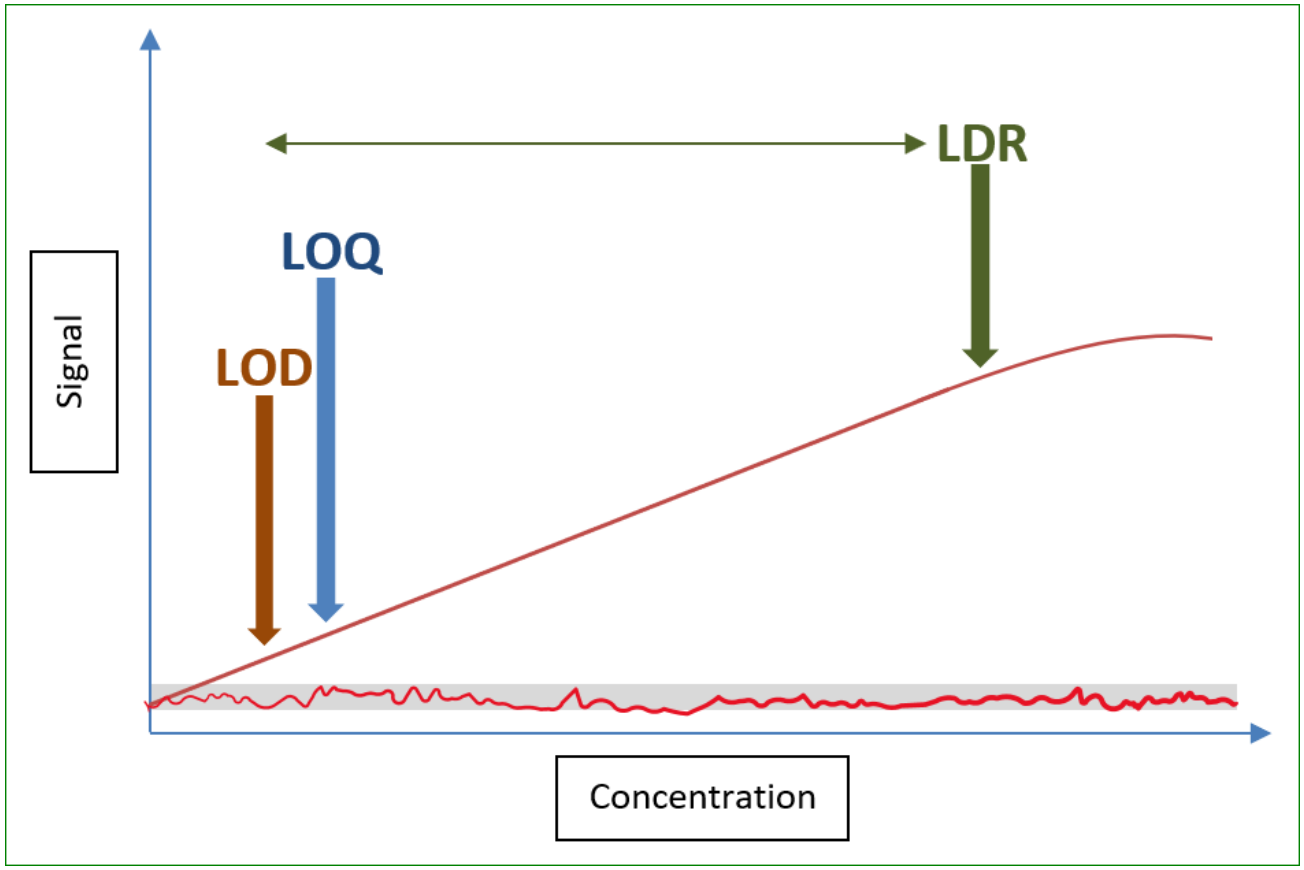


Limit of quantitation (LOQ):
the lowest concentration of an analyte for which quantitative results can be obtained.

Between the LOD and LOQ there is confidence the analyte is present in the sample.

Above the LOQ to the LDR, there is confidence the analyte is at that actual concentration value.

Calibration



Linear dynamic range (LDR):
the point where the linear equation (y = mx + b) no longer works. Above this point, the results will not be accurate using the linear initial calibration. Generally, the LDR is set at the highest calibration point in the curve. Dilute samples above this point.

Calibration Verification

- **Initial Calibration Verification (ICV)**
  - On days that you perform an initial calibration (which involves running a series of different concentration standards), you need to make sure the standards you made for the curve are at the correct concentration.
  - You will need to run what is called an initial calibration verification (ICV) standard. This standard MUST be from a different source than the standards you used to make the calibration curve standards.
  - **The ICV standard must be run after the calibration curve standards and before any samples.**

Calibration Verification

- **Continuing Calibration Verification (CCV)**
  - On days that you don't perform an initial calibration (which involves running a series of different concentration standards), you need to make sure the curve you made is still valid.
  - You will need to run what is called a continuing calibration verification (CCV) standard. This standard MAY be from the same source as the standards you used to make the calibration curve. You MAY use a different source.
  - **This must be run first.**

Quality Control

- **Method Blank**
  - You need to run a method blank with every 20 or fewer samples.
  - A method blank is just like running a sample but instead of sample, you use DI water.
  - The point of the method blank is to see if there was any contamination from anywhere in the procedure. If your blank is greater than your LOD, there may have been some contamination.

Quality Control

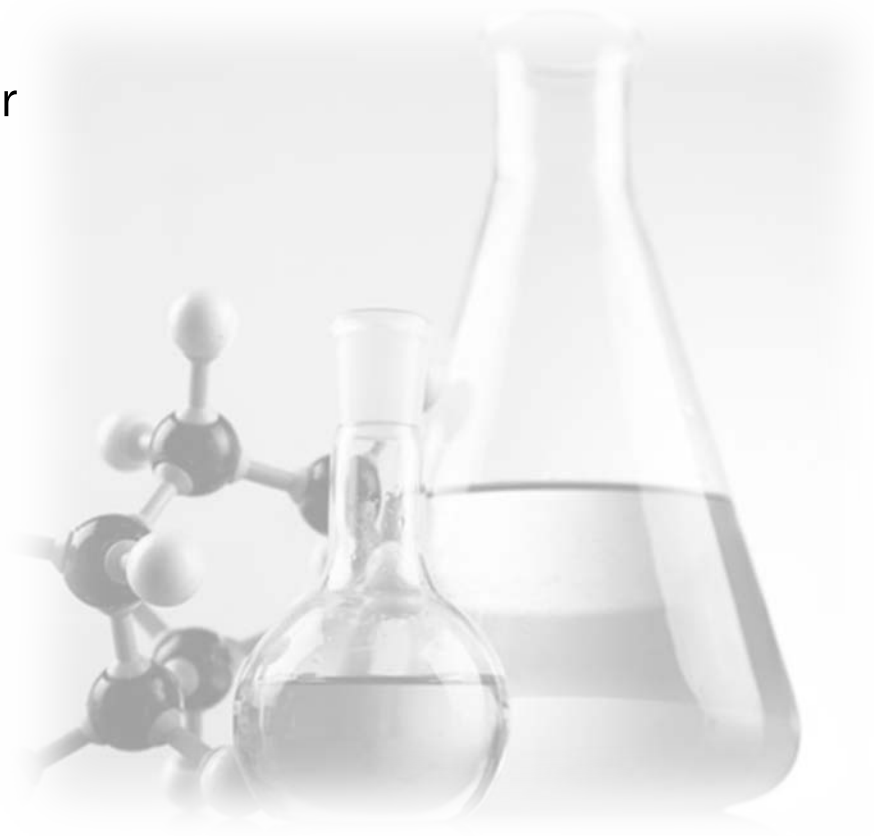
- **Method Blank**
  - The DNR requires that the method blank be less than the highest of the following:
    - LOD,
    - 5% of the permit limit, or
    - 10% of the sample concentration.
  - The LOD is the point where the test can statistically identify if the analyte is present or not. The method blank should not have any analyte—it's just water and reagents. So, if the method blank has a value above the LOD, there may be some issues going on. However, you may use a limit of 5% of your permit limit or 10% of the sample concentration.

Quality Control

- **Method Blank**
  - Possible sources of contamination
    - Soap or detergents used to clean glassware
    - Inadequate cleaning of glassware
    - Contaminated reagents (putting pipettes directly in reagents)
    - Other?
  - Zeroing on a method blank
    - If you zero the instrument of the method blank, you are virtually subtracting any response from any contamination that may be in your process.

Quality Control

- **Laboratory Control Standards (LCS)**
  - You need to run an LCS with every 20 or fewer samples.
  - An LCS is processed exactly the same way as samples.
  - It is used to determine whether the lab is capable of making accurate and precise measurements.
  - Since you know exactly what the result should be, if it's not, something is wrong
    - Reagents are bad
    - Temperature setting is wrong
    - Other?



Quality Control

- CCV or LCS?

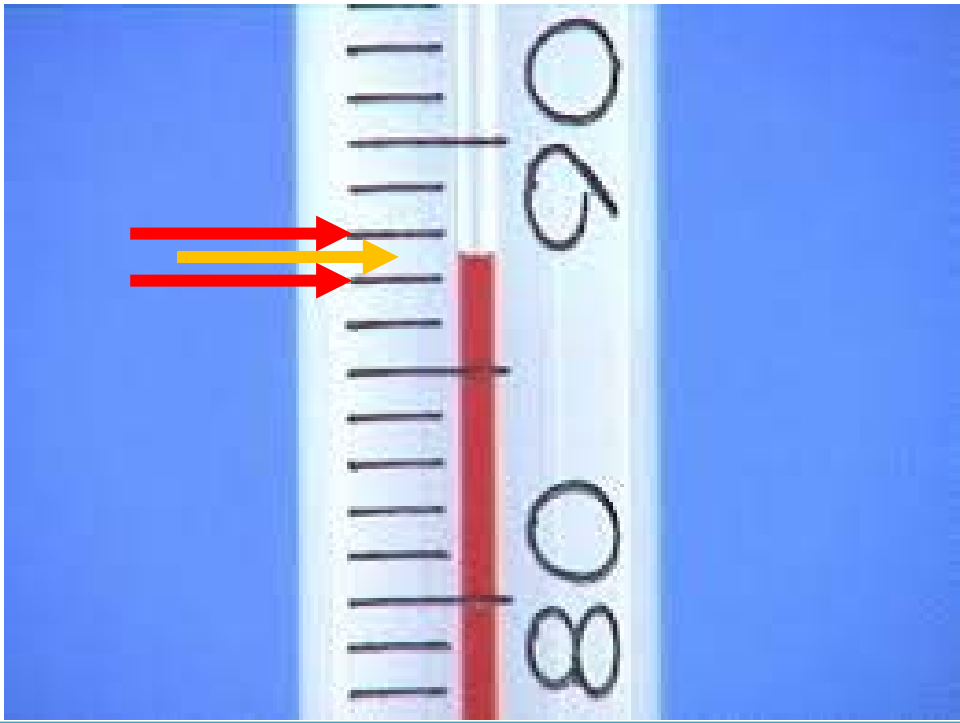
CCV	LCS
Used to assess calibration	Used to assess procedure
Processed same as calibration standards	Processed same as samples

If the calibration standards are processed (e.g., digested, distilled) the same as the samples are processed, the CCV and LCS are equivalent.

Significant Figures and Rounding

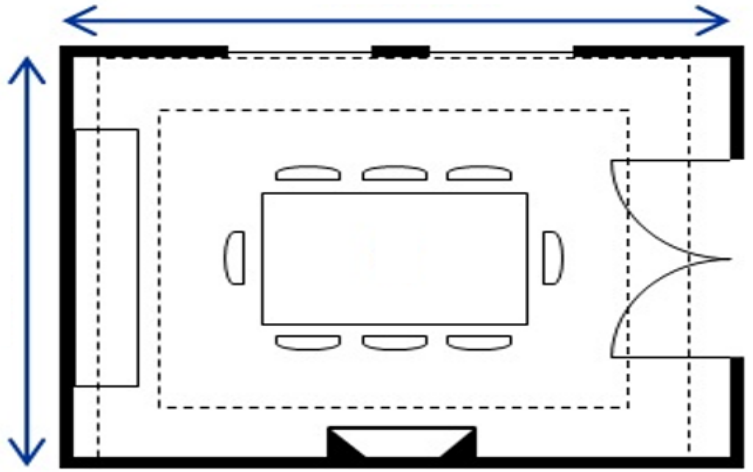
- Significant figures is a way to know which numbers are actual values and which are estimates.
- The last number in a measurement is the estimate.

- For example, we know this temperature is for sure between 8<u>7</u>° and 8<u>8</u>°. It might be about halfway between the two or maybe a little less. We estimate the last number to be 87.<u>5</u>°.
- In the temperature reading of 87.5°, we know the **87** part is definitely correct (significant), but the last number **(.5)** is just an estimate. Any numbers after the .5, like 87.55, would be nonsense!



Significant Figures and Rounding

- Different measuring tools have different levels of accuracy and precision.
  - Say you wanted to know the square footage of a large room. If you used a standard tape measure to get the length of a room and a laser tool to measure the width of a room, you are limited in the precision of the least precise tool (the standard tape measure).



Significant Figures and Rounding

- When reporting results, you may often need to round your data. Many digital instruments will give you more digits than are needed.
- When doing calculations, do all the calculations before doing any rounding.
- If the last digit that is being dropped is 6, 7, 8, or 9, then increase the preceding digit by one.
  - Ex: 24.6 rounds to 25
  - Ex: 2.97 rounds to 3.0
- If the last digit that is being dropped is 0, 1, 2, 3, or 4, then do not change the preceding digit.
  - Ex. 24.4 rounds to 24
  - Ex. 2.90 rounds to 2.9
- If the last digit that is being dropped is 5, then round off the preceding digit to the nearest even number. [*Standard Methods* 1050 B. (2) – 2006]
  - Ex. 24.5 rounds to 24
  - Ex. 2.95 rounds to 3.0

CONNECT WITH US

Janelle Nehs

Janelle.Nehs@wisconsin.gov

(920) 461-9178

Patty Doerflinger

Patricia.Doerflinger@wisconsin.gov

(608) 716-9226

/WIDNR

@WIDNR

@WI_DNR

/WIDNRTV

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OFF THE RECORD"