INTRO Lab Exam Review

April 20, 2015

Why are we here?

A brief history of lab exam results

How to best prep for an exam?

Why should we care?

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WI DNR Lab Certification

INTRO 2.3.1

Historical: 72%

Identify the maximum holding times and preservation methods for the following samples:

Holding Times and Preservation

Test	Holding Time	Preservation
BOD	48 hrs after compositing	Cool to 6°C or less, without freezing
TSS	7 days	Cool to 6°C or less, without freezing
Ammonia	28 days	Add sulfuric acid (H_2SO_4) to pH < 2 and cool to 6°C or less, without freezing
Total P	28 days	Add sulfuric acid (H_2SO_4) to pH < 2 and cool to 6°C or less, without freezing
Fecal Coliform	6 hours	Add sodium thiosulfate if sample was chlorinated and cool to 6°C or less, without freezing
Samples must be refrigerated at a temperature not to exceed 6°C and must not be frozen.		

INTRO 3.3.11

Historical: 68%

Discuss the selection of weights used for verification of calibration of an analytical balance.

The laboratory certification program requires labs to verify the calibration of their analytical balance at least monthly using a minimum of one Type 1 weight in the gram range, and a second Type 1 weight in the milligram range.

Select the weight close to the weight that you typically measure.

For example:

If using a Gooch crucible, use a 20-g or 50-g Type 1 weight.

If using a filter pad and pan, use a 100mg Type 1 weight.

Which you should NOT be!

INTRO 4.1.2 **Define Reagent.**

Historical: 77%

- A reagent is any substance used in a chemical reaction to detect, measure, examine, or produce other substances

Pretty simple definition

REAgent used in a REAction

INTRO 4.2.2

Discuss the proper use of a volumetric pipet using a bulb type pipet aid.

November 2013: 67 % pass

Use a pipet bulb to draw the solution a small amount above the calibrated volume line.

Remove the bulb quickly and cover the top opening of the pipet with your index finger.

Place the tip portion of the pipet into the receiving container (flask, beaker, etc.) and allow the solution in the pipet to drain into it.

Many Class A pipets have the drain time imprinted adjacent to the "TD" designation.

When the draining is complete, touch any remaining droplet at the tip of the pipet to the inside wall of the vessel.

INTRO 5.2.14 Discuss situations when seeding is required for wastewater samples.

Historical:

- Seeding is required whenever any sample is collected downstream of any disinfection,
  whenever sample pH requires adjustment, and also
  when inhibitor is added for cBOD determination.
- Sample pH extremes will severely shock or kill microorganisms. Therefore, when sample pH adjustment is required, subsequent sample dilutions must be seeded.
- If collected downstream of chlorination, samples must be tested for chlorine residual, and if present, the residual must be quenched prior to sample dilution and analysis. Disinfection agents, such as chlorine or UV, kill or prevent microorganism populations from reproducing. Therefore, any sample which has an initial chlorine residual, or any sample collected downstream of any disinfection process must be seeded.
- Finally, the inhibiting agent used to disrupt the nitrification process for cBOD determinations may have a toxic effect on other microorganisms. Therefore, samples for cBOD determinations (to which inhibitor is added) must be seeded as well.

INTRO 5.2.24

Historical:

Discuss the smallest sample volume for BOD that can be used without performing a preliminary dilution.

If less than 3 mL of samples volume is used (<1% dilution), a preliminary dilution is required prior to pipetting the sample into the BOD bottle. This is best done by preparing a simple dilution and then pipetting a portion of the diluted sample into the BOD bottle. For example, if sample volumes of 0.5, 1.0 and 2.0 mL of sample are needed, make a 10 fold preliminary dilution. Prepare the 10 fold dilution by pipetting 10 mL of well mixed sample into a 100 mL volumetric flask (or 100 mL class A graduated cylinder) containing about 50 mL of BOD dilution water. Bring the flask to 100 mL by adding additional BOD dilution water and then mix thoroughly. Then pipet 5, 10 and 20 mL of the diluted sample into the BOD bottles which represents 0.5, 1 and 2 mL of the original sample.

INTRO 5.2.25 Discuss how sample volume affects the LOD for the BOD test.

Historical: 52% pass

- As a bioassay procedure, the BOD test does not lend itself well to the typical EPA procedure (40 CFR Part 136 Appendix B) for determining the limit of detection (LOD).
- Therefore, the LOD for any given sample is determined based on the method specified minimum required DO depletion (2 mg/L) and the sample volume in the least diluted (i.e., the one with the greatest volume of actual sample) sample. Detection for BOD is based is that the LEAST amount of depletion allowable is 2 mg/L. The LOD is also based on the highest volume of sample used in a dilution series. What results is the detection capability for a specific dilution series, or sample. This technique should include seed correction, because we want to identify depletion due to the sample itself, and not due to the seed material.

INTRO 5.2.25 Discuss how sample volume affects the LOD for the BOD test.

Historical: 52% pass

The calculation involved is simply:

LOD mg/L = 2 mg/L X [300 mL ÷ sample in LEAST dilution]

EXAMPLE: sample with dilutions of 100, 50, and 25 mL

LOD = 2 mg/L X [300 mL ÷ 100 mL]

= 2 mg/L X 3

= 6 mg/L LOD for that sample

highest sample volume used	Dilution	Sample LOD
300 mL	1.0	2 mg/L
200 mL	1.5	3 mg/L
100 mL	3.0	6 mg/L
75 mL	4.0	8 mg/L
50 mL	6.0	12 mg/L

In order to report an LOD of 2 mg/L, one sample dilution containing 300 mL of sample must be included.

INTRO 5.2.26 Discuss DO depletion requirements for BOD testing.

Historical: 76%

In order to be used to calculate sample results without qualification, dilutions must deplete at least 2 mg/L of DO, and the final (or residual) DO must be at least 1.0 mg/L.

INTRO 5.2.32

Historical: 58%

Discuss procedures for reporting results when all dilutions over-deplete (final DO less than 1.0 mg/L).

Each sample dilution must meet the minimum requirement of 2 mg/L DO depletion. There must also be at least 1 mg/L of DO remaining in each sample dilution after 5 days. If there is more than one acceptable dilution, these results must be averaged.

Example: Given the following data:

Sample Bottle #	A	B	C
Sample Size (mL)	300	200	100
Initial D.O. (mg/L)	8.48	8.50	8.47
Final D.O. (mg/L)	< 1.0	< 1.0	< 1.0
Depletion (mg/L)	> 7.48	> 7.50	> 7.47
Dilution Factor	1	1.5	3
BOD (mg/L)	>7.5	>11.3	>22.4

INTRO 5.2.32

Historical: 58%

Discuss procedures for reporting results when all dilutions over-deplete (final DO less than 1.0 mg/L).

In the example, all three dilutions fail to meet the residual DO requirement of 1.0 mg/L.

Report result as "> 22 mg/L"

NOTE: If this represents an unusual situation, then a comment should be included on the DMR indicating a non-routine event. Additional dilutions may be required over the next several days until the plant settles down. If, however, this situation occurs regularly, then the lab should be routinely using higher dilutions when preparing samples.

What if all dilutions over deplete?

1. Calculate BOD of each dilution assuming final DO is exactly 1.0
2. Include a “>” in front of each dilution BOD
3. Report the highest BOD (“> _____”) of the dilutions analyzed
4. Generally speaking, this will be associated with the highest dilution (least amount of sample).

INTRO 5.2.38

Historical: 59%, 87%

Explain the potential reasons why GGA results could be unacceptably high or low.

HIGH BIAS of GGA results is caused by:

- Nitrification
- Cold GGA solution
- Contamination: Organic matter (yucky stuff)
- Contamination: Microorganisms ("bugs")

HIGH GGA

NOTE: Contamination from either "bugs" or BOD material alone will cause high bias in GGA but is not likely to cause an exceedance in blanks. There must be contamination from BOTH "bugs" AND waste material for contamination to result in blank exceedances. This explains a common statement from lab analysts that "my GGA is failing high, but my blanks are fine".

LOW BIAS of GGA results is caused by:

- Not enough seed
- Seed materials too weak or variable
- GGA too old or contaminated

LOW GGA

INTRO 5.2.40

Historical: 60%, 65% pass

Discuss how BOD results relate to TSS results and how it also might relate to nitrification.

Generally speaking there should be a 1:1 relationship between BOD and TSS results for domestic municipal wastewater effluent. This relationship only applies to domestic wastewater effluents. This is because the majority of the BOD comes directly from the material that makes up the TSS. Industrial wastes, such as dairy and food processing wastes, contain a high dissolved BOD component. Milks and sugars from food wastes will pass through the TSS filters causing the BOD to be significantly higher than the TSS values.

If BOD is always significantly higher than TSS (e.g., TSS 10, BOD 25), nitrification is likely occurring. Confirm by performing side-by-side BOD tests with and without nitrification inhibitors. If the inhibited (carbonaceous) BOD results are significantly lower and closer to the TSS results, nitrification is occurring. Repeat side-by-side tests to confirm your findings.

NOTE: Always seed samples when nitrification inhibitor is used.

INTRO 5.3.2

Historical: 48%, 63% pass

Identify the critical requirements associated with the drying oven.

Drying ovens used for TSS determinations must be able to consistently maintain a temperature of 103-105°C. The purpose of the method temperature is to drive off water but not lose volatile solids.

Drying ovens should be vented properly as a health and safety precaution. Do not position a drying oven directly beneath an HVAC blower vent because drafts can be forced through the top of the oven. Direct contact with blowing cold air prevents the ability of the oven to maintain constant temperature. Ovens can be vented to, but should not be situated inside any hood.

Check and document the oven temperature daily when samples are being dried.

INTRO 5.3.3

Discuss the required filters for TSS testing.

Historical: 58% pass

Standard Methods 2540 D requires glass fiber filters without organic binders such as Whatman 934AH or equivalent.

All filter papers are NOT alike! Standard Methods cites the following brands of filters as being equivalent:

- Millipore AP40,
- Gelman type A/E, ED
- Scientific Specialties Grade 161.

Standard Methods 2540D states that, " Practical filter diameters are 2.2 cm [22 mm] to 12.5 cm [125 mm]".

INTRO 5.3.9 Discuss minimum and maximum solids capture weight requirements for TSS testing.

Historical: 10%, 56% pass

A residue of at least 1 mg, and not more than 200 mg, must be captured. If at least 500 mL of sample volume is filtered and 1 mg of residue is not obtained, the analyst is not required to repeat the analysis using more sample volume.

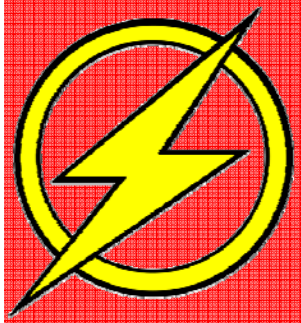
A minimum capture weight of 1 mg and 500 mL of sample filtered is required to report an LOD of 2 mg/L, the required LOD for Discharge Monitoring Report (DMR) reporting for effluent samples.

Residue amounts greater than 200 mg on a filter can lead to "flash" surface drying and the formation of a salt crust layer that traps moisture beneath it. This can cause sample results to be biased high. This is generally expected to be a problem related to process control samples with heavier solids loading.

INTRO 5.3.9

Historical: 10%, 56% pass

Discuss minimum and maximum solids capture weight requirements for TSS testing.



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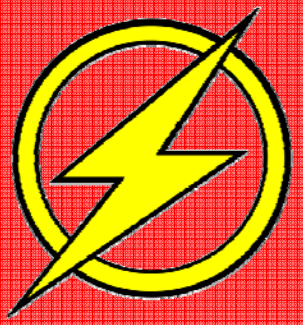


MUST capture at least 1 mg, and not > 200 mg solids

A minimum capture 1 mg and 500 mL of sample filtered is required to report an LOD of 2 mg/L,

2 mg/L = the required LOD for Discharge Monitoring Report (DMR) reporting for effluent samples.

WHY? > 200 mg residue can lead to formation of a salt crust layer that traps moisture beneath it. This can cause sample results to be biased high.

INTRO 5.3.12

Historical: 38% pass

Discuss the required LOD for TSS and what to do if you can't achieve it.

In order to achieve an LOD of 2 mg/L (as required for DMR reporting), one must filter 500 mL and fail to achieve a residue of 1 milligram of solids. More volume can be filtered, but as long as you filter at least 500 mL and you do not obtain at least 1 milligram of residue, then a result of "< 2 mg/L" can be reported.

If you are not capturing at least 1 milligram of residue and you are filtering LESS than 500 mL of sample, you will be required to filter a larger sample volume, up to a maximum of 500 mL.

INTRO 5.4.1

Historical: 63%, 72%

Discuss the significance of the ammonia test in wastewater testing.

- Ammonia is a major excretory product of animals and is toxic to organisms at high pH levels.
- Approximately 60% of the nitrogen entering a wastewater plant is ammonia.
- Ammonia toxicity is based on pH and temperature.
- Ammonia affects wastewater plants differently based on type or design.
- High levels of ammonia **and** the presence of nitrifying organisms will result in increased BOD values (i.e, biased high).

INTRO 5.4.7

Historical:

Discuss the importance of allowing a proper amount of time to obtain a stable ammonia measurement on low level samples and standards.

If the response of the low concentration calibration standard is slow, the millivolt response can be biased low relative to that of more concentrated calibration standards which respond much more rapidly. The net result is that the millivolt difference between calibration standards that are a factor of ten different in concentration falls outside of the acceptable range.

As ammonia concentration decreases electrode response time takes longer. The lower the concentration of ammonia in the sample, the fewer the number of ammonia molecules as gas that pass across the membrane to react with water and trigger a pH change observed as a millivolt increase on the meter. Most meters come equipped with circuitry that identify the point when the reading is stable as the point at which the increase in millivolts slows appreciably.

INTRO 5.4.7

Historical:

Discuss the importance of allowing a proper amount of time to obtain a stable ammonia measurement on low level samples and standards.

Unfortunately, many of these computer chips "jump the gun" and lock in on a millivolt response when the millivolts are actually continuing to rise… although quite slowly. For this reason, many manufacturers will suggest techniques such as pressing the "READ" button twice, or allowing an initial minute or two of measurement to be recorded before actually pressing the "READ" button.

This phenomenon explains why blank measurements are often incorrectly believed to be above the limit of detection for ammonia. It also explains why some people have trouble meeting calibration acceptance criteria.

INTRO 5.4.17

Historical: 4% pass

Discuss the purpose of the NaOH buffer solution added to samples and standards and why it should not be added until after the probe is immersed in the sample.

In aqueous solutions, ammonia exists in an equilibrium between two forms, the ionized form (NH4+, ammonium ion) and the un-ionized form (NH3, ammonia gas). The relative amount of each form present in any sample is controlled solely by pH and temperature. As temperature and pH increase, the predominant form becomes the NH3 (gaseous) form.

This is, of course, the form which can cross the gas permeable membrane of the ammonia probe. Since ammonia is a gas, however, it can dissipate quickly from solution. To prevent these rapid losses of ammonia from being measured, the probe is placed into the solution and readings initiated BEFORE the buffer solution is added to a sample or standard.

NOTE: *At least one manufacturer sells a buffer solution which remains blue in color as long as the solution pH is above 11.*

INTRO 5.5.7

Historical: 15% , 34% pass

Discuss critical calibration requirements for the total phosphorus test.

- Lab certification code requires at least three calibration standards plus a calibration blank.

  The more calibration standards used to develop the relationship between phosphorus concentration and absorbance, the more accurate the calibration.
- Calibration standards must bracket sample concentrations.
- Phosphorus calibrations are evaluated using the correlation coefficient which must be greater than or equal to 0.995.

INTRO 5.6.4

Historical: 22% pass

Discuss the Department permit reporting requirements and LOD requirements for total residual chlorine.

- Permits are written to include a detection limit requirement of 38 ug/L residual chlorine.
- However, laboratories are considered to be in compliance as long as their reporting limit for residual chlorine does not exceed 100 ug/L

Permit limit: 38 ug/L (0.038 mg/L)

Acceptable REPORTING limit: 100 ug/L (0.1 mg/L)

INTRO 5.7.3

Historical: 76% pass

Discuss the importance of dissolved oxygen (DO) in process control.

Dissolved Oxygen (DO) field analysis is used to monitor the amount of dissolved oxygen in various areas of the facility. A dissolved oxygen meter with a submersible field probe is used. Some facilities also have in-line DO monitors.

Aerobic organisms require a minimum DO level of about 2.0 mg/L for proper health. When a facility is accomplishing biological nitrogen and phosphorus removal, the oxygen levels in the anaerobic, anoxic, and oxic sectors must be carefully monitored.

INTRO 6.1.3 Define Bias and Precision

Historical: 67%, 72% pass

Bias (Accuracy) is the systematic or persistent error in an analysis which results in the expected sample measurement being consistently different than the sample's true value.

A systematic bias is a bias resulting from a flaw integral to the system within which the bias arises (for example, an incorrectly calibrated thermostat may consistently read - *that is, 'biased'* - several degrees hotter or colder than actual temperature). As a consequence, systematic bias commonly leads to systematic errors, as opposed to random errors, which tend to cancel one another out.

INTRO 6.1.3 Define Bias and Precision

Historical: 67%, 72% pass

Precision is a measure of how closely multiple determinations performed on the same sample will agree with each other.

Accuracy is the degree of closeness to the actual value while precision is the degree of reproducibility.

Using the typical "target" analogy, if a large number of arrows are fired at the target, the size of the arrow cluster on the target represents the shooter's precision. When all arrows are grouped tightly together, the cluster is considered precise since they all struck close to the same spot. Note that the cluster may be very precise, by virtue of a tight grouping, but if the cluster is nowhere near the bullseye, then the shooter is precise but not accurate.

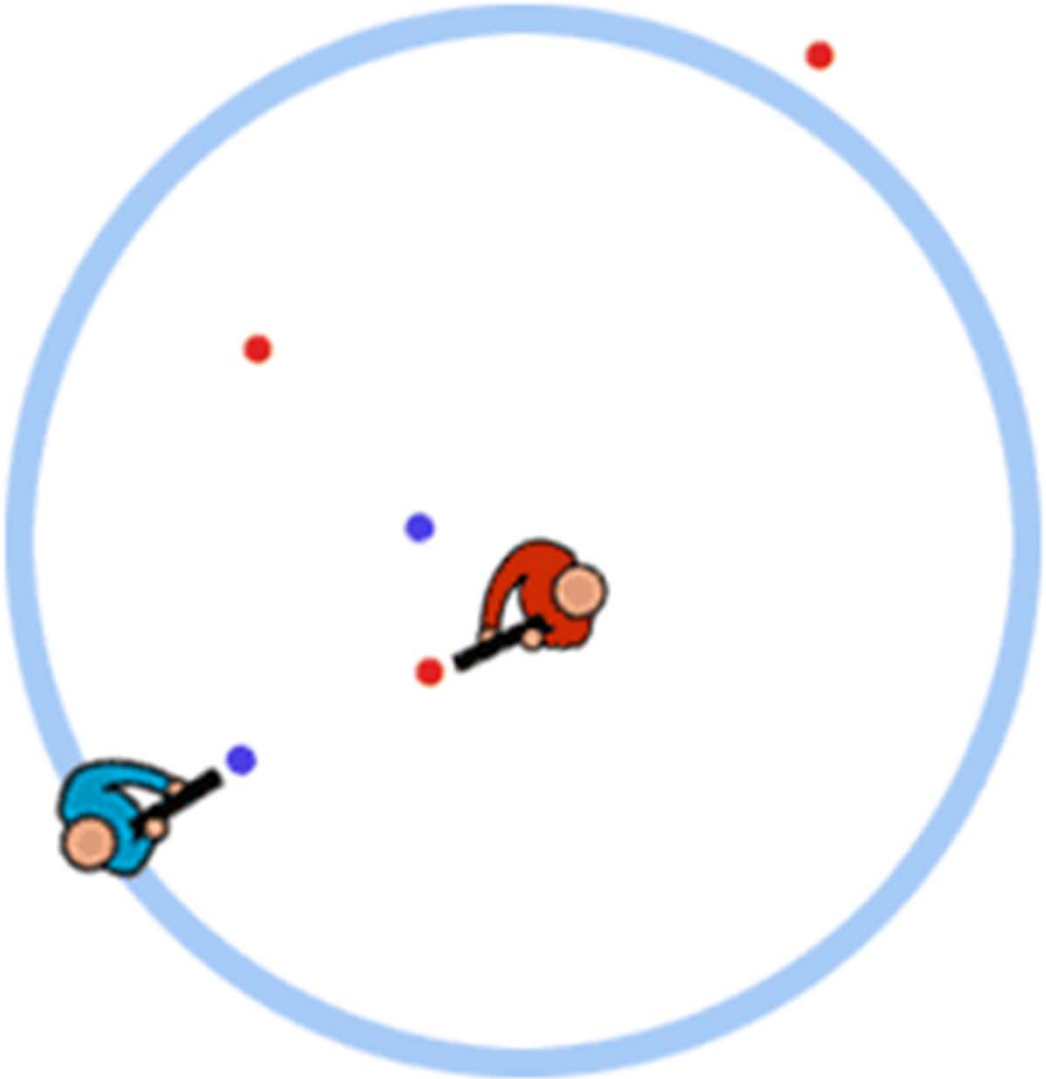
Accuracy v. Precision

- Accuracy: Can I get the right answer
- Precision: Can I get that answer again

Precision in practice

- Measuring precision requires multiple attempts….more than one check. That forces it to relate to duplicates or replicates

Is either accurate? Precise?



Accuracy & Precision recap

- We've tried the target and hunting analogy.
- Let's try something else.

	Accuracy?	Precision?
GGA = 200		
GGA = 160,231,172,225,158		
GGA = 224,226,221,228,222		
GGA = 196,202,199,203,200		

INTRO 6.1.5

Historical: 53%

Define the following terms:
A. Arithmetic Mean.
B. Geometric Mean..

A. Arithmetic Mean.

The arithmetic mean (or simply the mean) of a list of numbers is the sum of all the values divided by the number of values.

If four (4) values are 20, 10, 80, and 10,
the arithmetic mean is (20 + 10 + 80 + 10) ÷ 4 = 30.

= 120 /4

INTRO 6.1.5

Historical: 53%

Define the following terms:
A. Arithmetic Mean.
B. Geometric Mean..

B. Geometric Mean

The geometric mean of a set of positive data is defined as the 'n'th root of the product of all the values, where 'n' is the number of values. The geometric mean of a data set is always smaller than or equal to the set's arithmetic mean (the two means are equal if and only if all values of the data set are identical).

If four (4) values are 20, 10, 80, and 10, the geometric mean is the 4th root of (20 × 10 × 80 × 10) or the 4th root of 160,000. The 4th root of 160,000 is 20. [Note that the arithmetic mean is 30]

The geometric mean is used for microbiological analysis due to the unpredictability of their exponential growth rate.

TI-30X IIS

Roots

Press	Display
4 [2nd] [$\sqrt[x]{\ }$] 160000 ENTER	4 $\sqrt[x]{}$160000 = 20. DEG

ROOT / KEY / VALUE

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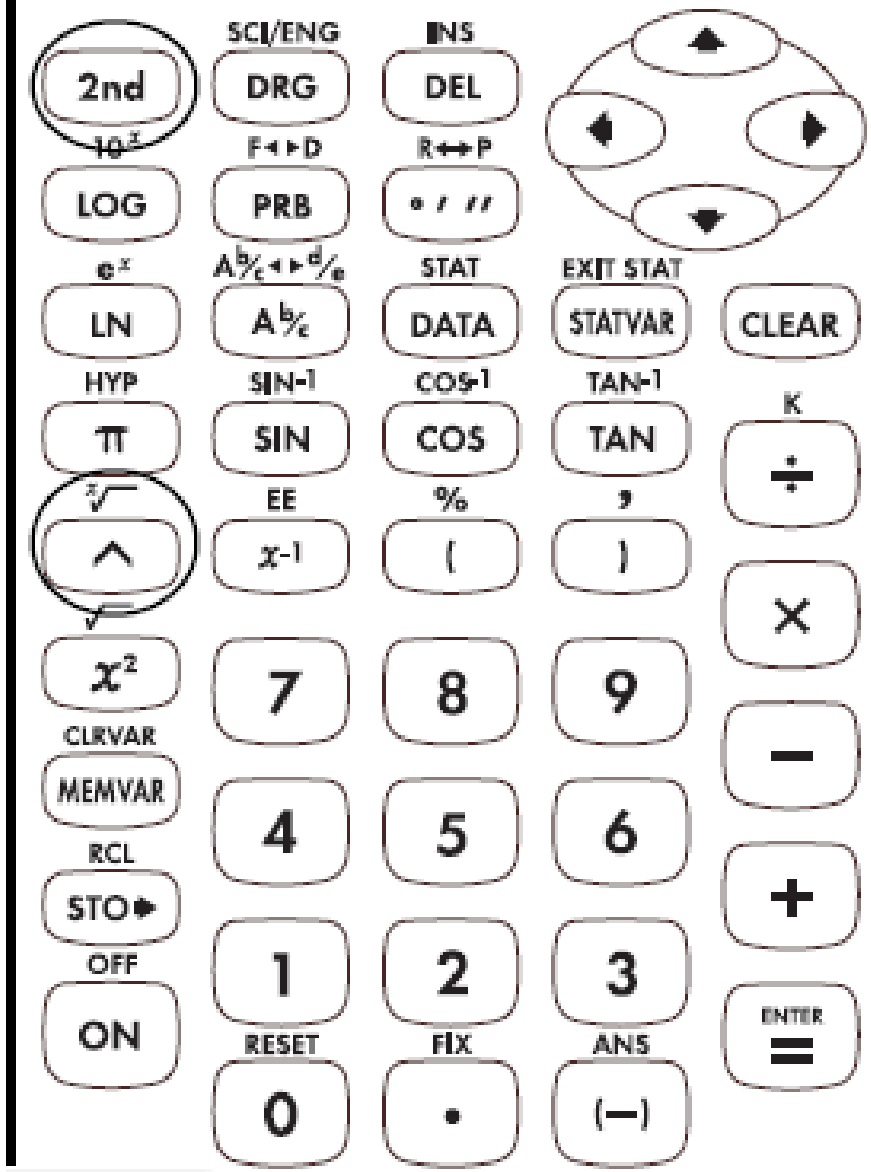


Abs
x^3
$x!$
x^{-1}
log
$\sqrt{\square}$
$\sqrt[3]{\square}$
$\sqrt[\square]{\square}$
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FACT
$\cos^{-1}$
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INTRO 6.1.9

Define Laboratory Control Standard (LCS)

Historical: 33% pass

"Laboratory Control Standard" or "LCS" means a sample of reagent water spiked with known amount of the analyte of interest. The purpose of an LCS is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

In many EPA methods, the term "lab-fortified blank" is substantially equivalent to a laboratory control sample.

INTRO 6.1.18 Define LOD and LOQ.

Historical: 33% pass.

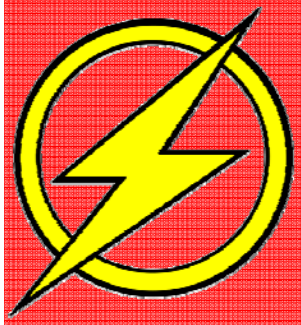
Limit of detection (LOD) means the lowest concentration or amount of analyte that can be identified, measured, and reported with confidence that the concentration is statistically different from a blank (or, not a false positive value). For department purposes, the LOD approximates the "method detection limit" (MDL, the term used by the EPA and in many methods). The LOD is determined using the procedure in 40 CFR Part 136 Appendix B, Revision 1.11.

Limit of quantitation or (LOQ) means the lowest concentration or amount of an analyte for which quantitative results can be obtained with a specified degree of confidence. The limit of quantitation is typically considered to be a value 10/3 or 3.333 times the limit of detection (LOD).

INTRO 6.1.18

Define LOD and LOQ.

33% pass



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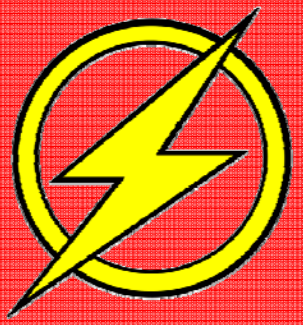

LOD= Limit of detection ≅ MDL

- =lowest concentration …statistically different from a blank
- Statistically not a false positive value.
- determined by 40 CFR Part 136 App. B, Rev. 1.11.

LOQ= Limit of QUANTITATION ≅ 3.333 x LOD

- = lowest concentration …QUANTITATIVE results at a specified degree of confidence.
- LOD is to be a value 10/3 or 3.333 times the limit of LOD.

INTRO 7.1.2 Define SOP (Standard Operating Procedure).

Historical:

- Standard operating procedures (SOP) are used to describe a procedure or set of procedures to perform a given task, analysis, or in response to a specific event or situation.
- SOPs often offer guidance where official methods are lacking, or are extremely broad.
- SOPs typically provide practical detail to the sometimes generic language provided in methods or to identify specific situations where choices are available. Every good quality system is based on its standard operating procedures (SOPs).
- SOPs may be documents written by laboratory personnel or may consist entirely of copies of published documents, manuals or procedures if the laboratory follows the chosen source exactly. SOPs must ndicate their dates of issue or revision.

INTRO 7.1.2 Define SOP (Standard Operating Procedure).

Historical:

- SOPs may partly consist of copies of published documents, manuals or procedures **if**:
  - - Modifications to the published source are described in writing in additional documents.
  - Clarifications, changes or choices are completely described in additional documents, when published sources offer multiple options, ambiguous directives or insufficient detail to perform or reproduce an analysis.