Basic Laboratory Techniques

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Outline

- Stoichiometry
- Volume Measurements
- Mass Measurements
- Statistically-Derived Detection Limits
- Laboratory Safety
- Laboratory Record Keeping

Stoichiometry

- Relationship between reactants and products
  - Quantitative, based on conservation of mass
- Units are important
- Atoms, moles, mass

Factor	Prefix	Symbol	Examples
10^{9}	giga	G	1 Gm = 1 gigameter = 10^{9} m 1 Gb = 1 gigabyte = 10^{9} bytes
10^{6}	mega	M	1 Mm = 1 megameter = 10^{6} m 1 Mb = 1 megabyte = 10^{6} bytes
10^{3}	kilo	K	1 Km = 1 kilometer = 10^{3} m 1 Kg = 1 kilogram = 10^{3} g
10^{-1}	deci	d	1 dm = 1 decimeter = 0.1 m
10^{-2}	centi	c	1 cm = 1 centimeter = 0.01 m
10^{-3}	milli	m	1 mg = 1 milligram = 0.001 g 1 ms = 1 millisecond = 0.001 s
10^{-6}	micro	μ	1 μm =1 micrometer = 10^{-6} m 1μs = 1 microsecond = 10^{-6} s
10^{-9}	nano	n	1 ns = 1 nanosecond= 10^{-9} s
10^{-12}	pico	p	1 pg = 1 picogram = 10^{-12} g

http://www.webassign.net/question_assets/tccgenchem2l1/measurements/images/figure2.png

Stoichiometry

- Mole
  - Representation of the number of atoms or molecules of an element or compound
  - 6.022×10^{23} atoms/molecules
  - Often abbreviated mol
- Molar mass = average mass of one mole of a element or compound
  - Takes into account different isotopes of an element
  - Ex. Sodium (Na) = 22.99 Da (g/mol)
  - Ex. Ammonium chloride (NH_4Cl) = 53.50 Da

Stoichiometry

- Solute = Minor component of a solution
- Solvent = Major component of a solution
- Molarity = moles of solute/total volume, in liters of solution
  - Ex. 15M Sodium hydroxide (NaOH) = 15 mol/L NaOH = 600 g/L NaOH
- Normality = mole equivalents of the component of interest
  - Ex. 1M H_2SO_4 = 2N H_2SO_4 where H^+ is the component of interest

Example

- How much solid Sodium nitrate is required to prepare 500 mL of a 2M solution of Sodium nitrate?
  - Sodium nitrate = $NaNO_3$
    - Molar mass = 22.99 Da (Na) + 14.01 Da (N) + 16.00 Da (O) x 3 = 85 Da = 85 g/mol
  - 2 mol NaNO3 = 170 g NaNO3
  - 500 mL / 1000 mL/L = 0.5 L
  - $Mass\ Required\ (g) = \frac{2\ mol}{1\ L} \times \frac{85\ g}{mol} \times \frac{0.5\ L}{1} = 85\ g\ NaNO_3$

Example 2

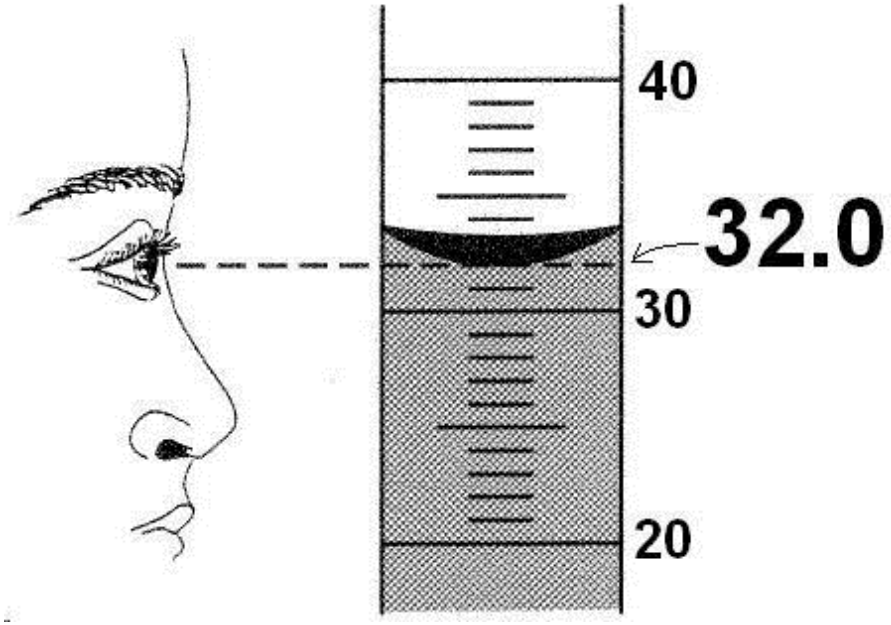
- How much 14.8 M Ammonium hydroxide (NH_4OH) is required to prepare 2 L of a 6 M Ammonium hydroxide (NH_4OH)?
- $M_1V_1 = M_2V_2$
  - Solution 1 is 14.8 M NH_4OH
  - Solution 2 is 6 M NH_4OH
- $V_1 = \frac{M_2V_2}{M_1} = \frac{6M\ NH_4OH \times 2L}{14.8M\ NH_4OH} = 0.810\ L\ of\ 14.8M\ NH_4OH$

Volume Measurements

- Three main devices
  - Pipets
  - Volumetric Flasks
  - Graduated Cylinders
- Different grades of glassware
  - Generally see Classes A and B
  - Class A glassware is meets tighter accuracy tolerances than class B
  - Methods often specify acceptable classes of glassware
- To Contain vs To Deliver
  - TC vs TD or Blowout
- Do not bake volumetric glassware!

Volumetric Measurements

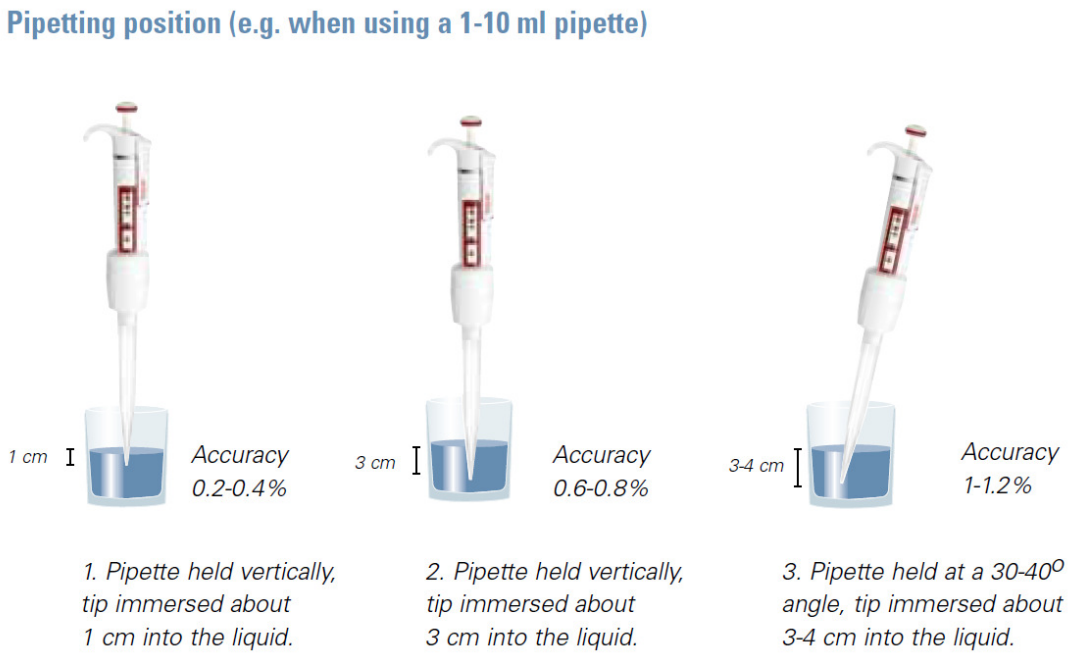
- Reading a meniscus
  - Glassware is designed to be read at the minimum of the curve
  - Meniscus needs to be level with the eye
  - If the meniscus is in between markings, interpolation is encouraged



https://calaski.files.wordpress.com/2015/09/meniscus.jpg

Mechanical Pipettes

- Two techniques
  - Forward pipetting
    - Collect solution from first stop, dispense to second stop
    - Standard technique for most solutions
  - Reverse pipetting
    - Collect solution from second stop, dispense to first stop
    - Alternate technique for viscous or foamy solutions



Mechanical Pipettes

- Should be checked for accuracy and precision quarterly
  - ISO 8655 Standard provides limits
  - Checks need to be documented
- Cleaning and maintenance as needed
- See Finnpipette Finntip Good Laboratory Pipetting Guide

https://www.labdepotinc.com/admin/uploads/scilogex-micropette-plus-single-channel-713111087.jpg

Mechanical Pipettes

- Pipette checks and calibrations can be done by a commercial vendor or your lab
- Can be tracked in excel
- Can be somewhat complicated

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	1st Quarter (January-March)																				
2	Date	User	Temp. (°C)	Pressure (mmHg)	ρ_{H2O} (g/mL)		Comments														
3	3/2/2018	tirado	23.0	758	0.998																
4																					
5	Pipette #	Pipette Range	Volume (µL)	1st Mass (g)	2nd Mass (g)	3rd Mass (g)	4th Mass (g)	5th Mass (g)	6th Mass (g)	7th Mass (g)	8th Mass (g)	9th Mass (g)	10th Mass (g)	Mean Mass (g)	Corrected Mean Volume (µL)	Mean Absolute Variance (µL)	Standard Deviation (µL)	Maximum Permissible Systematic Error (µL)	Maximum Permissible Random Error (µL)	Evaluation	Comments
6	1	20-200 µL	20	0.0199	0.0192	0.0191	0.0200	0.0201	0.0184	0.0191	0.0193	0.0201	0.0197	0.0195	19.5373	0.4627	0.5620	1.600	0.600	Acceptable	
7	1	20-200 µL	200	0.2007	0.2009	0.2010	0.2000	0.2000	0.2004	0.2004	0.2001	0.1998	0.1996	0.2003	200.7762	0.7762	0.4712	1.600	0.600	Acceptable	
8	2	100-1000 µL	100	0.1015	0.1015	0.1021	0.1014	0.1023	0.1017	0.1021	0.1019	0.1019	0.1022	0.1019	102.1073	2.1073	0.3212	8.000	3.000	Acceptable	
9	2	100-1000 µL	1000	1.0033	1.0009	1.0026	1.0041	1.0017	0.9994	1.0003	1.0015	1.0019	0.9984	1.0014	1003.8408	3.8408	1.7410	8.000	3.000	Acceptable	
10	3	0.5-2.5 mL	500	0.5150	0.5159	0.5174	0.5161	0.5151	0.5166	0.5154	0.5156	0.5172	0.5152	0.5160	517.2024	17.2024	0.8665	20.000	7.500	Acceptable	
11	3	0.5-2.5 mL	2500	2.4995	2.4930	2.4995	2.5015	2.5015	2.4975	2.4976	2.4966	2.4904	2.4962	2.4973	2503.3920	3.3920	3.5501	20.000	7.500	Acceptable	

Mass Measurements

- Certified weights should be used for balance checks
- Balances should be calibrated at least yearly
- Balances should be checked everyday prior to use.
  - This should be done in the normal weighing ranges for which the balance will be used
    - i.e. If you are weighing 100 mL water samples, you want to check the balance at 100 g
- Balances should also be checked monthly across their range
- If a balance is moved or bumped, it needs to be re-checked

Mass Measurements

- Record of checks can be used to create tolerances
  - Standard deviation (σ) of previous year's checks
  - Mean ± 3σ to establish a working tolerance
  - Easiest to do in excel

Day of Use Balance Checks

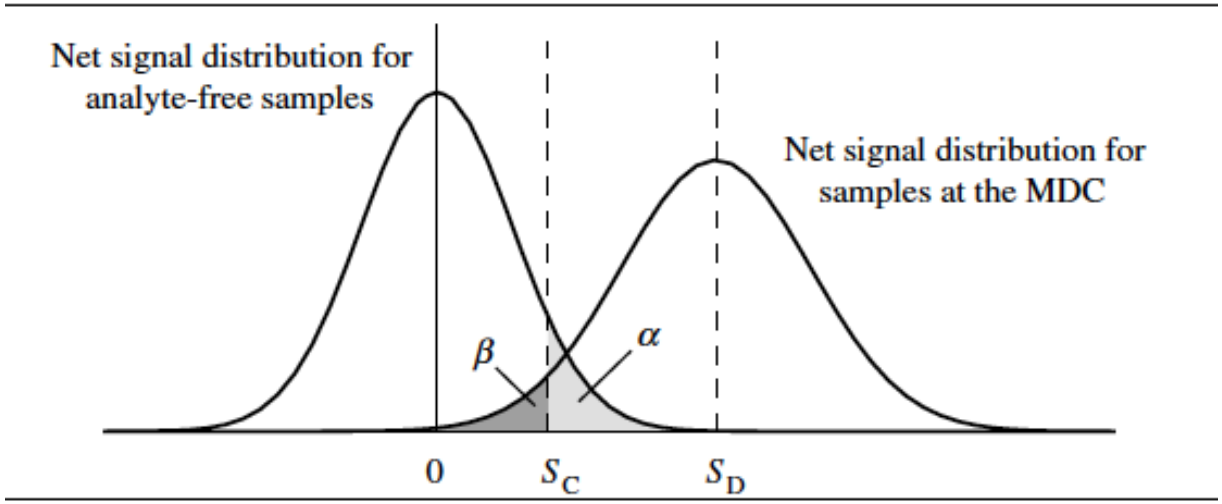
Year:	2018			
Balance Information		Weight Set Information		Comments
Model:	Mettler Toledo ML204	Model:	Troemner Set, Student Set	
Serial Number:	B506580417	Serial Number:	02288, Rad1	
Placed into Service:	2015-04-09	Calibration Date:	2015-06-16, Not calibrated	
Calibration Date:	2018-01-09	Recalibration Due:	2016-06, Not Applicable	
Recalibration Due:	2019-01			
Tolerance (g):	0.0003			

The mass and tolerance of the 10 g check weight is calculated using the average and standard deviation, adjusted to 3σ, from the previous year. The previous year's table can be found in M:\Radchem\QAQC\balance checks\history files. To be acceptable, the measurement must fall within the stated tolerance.

Date	Initials	Internal Calibration Performed?	10 g Weight	Comments
Average Mass (g)			10.0013	
7/9/2018	acarl	y	10.0010	okay
7/10/2018	acarl	y	10.0013	okay
7/12/2018	acarl	y	10.0011	okay
7/13/2018	dmay	y	10.0012	okay
7/16/2018	acarl	y	10.0011	okay
7/17/2018	dmay	y	10.0013	okay
7/18/2018	acarl	y	10.0012	okay
7/19/2018	acarl	y	10.0013	okay
7/20/2018	acarl	y	10.0012	okay
7/23/2018	dmay	y	10.0010	okay
7/24/2018	dmay	y	10.0012	okay
7/31/2018	acarl	y	10.0012	okay
8/1/2018	dmay	y	10.0012	okay
8/6/2018	acarl	y	10.0013	okay
8/7/2018	acarl	y	10.0012	okay
8/9/2018	acarl	y	10.0011	okay
8/10/2018	acarl	y	10.0012	okay
8/13/2018	acarl	y	10.0012	okay
8/15/2018	acarl	y	10.0012	okay
8/16/2018	dmay	y	10.0012	okay
8/17/2018	acarl	y	10.0011	okay
8/20/2018	acarl	y	10.0012	okay
8/21/2018	acarl	y	10.0013	okay
8/23/2018	dmay	y	10.0014	okay
8/28/2018	acarl	y	10.0013	okay
8/30/2018	acarl	y	10.0013	okay
8/31/2018	acarl	y	10.0013	okay
9/4/2018	acarl	y	10.0010	okay

Minimum Detection Limit

- The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.
- Limit of Quantitation is ~3 times MDL. This should be less than you reporting limit.



RE 20.1 — The critical net signal, S_C, and minimum detectable net sign

Minimum Detection Limit

- Initially, analyze 7 low-level spiked samples & 7 method blank samples
  - Calculate MDL for spiked samples and blanks
  - Select the greater of the two
- After the initial analyze two level spikes per quarter in which samples are analyzed for this method
  - Calculated MDL every year based on these spikes and the method blanks run routinely

Minimum Detection Limit

$$MDL_s = t_{(n-1,\ 1-\alpha=0.99)} S_s$$

where:

MDL_s = the method detection limit based on spiked samples

$t_{(n-1,\ 1-\alpha = 0.99)}$ = the Student's *t*-value appropriate for a single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom. See Addendum Table 1.

S_s = sample standard deviation of the replicate spiked sample analyses.

$$MDL_b = \overline{X} + t_{(n-1,1-\alpha=0.99)} S_b$$

where:

MDL_b = the MDL based on method blanks

$\overline{X}$ = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1,\ 1-\alpha = 0.99)}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom. See Addendum Table 1.

S_b = sample standard deviation of the replicate method blank sample analyses.

Minimum Detection Limit

- See EPA Document on MDL studies for further information
- https://www.epa.gov/sites/production/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.pdf

Chemical Safety

- Organization
  - Segregation of chemicals
    - Separate Oxidizing acids (ex. Nitric acid) from Flammable acids (ex. Glacial acetic acid) from mineral acids (ex. Sulfuric acid)
    - Bases should be stored separately
    - Flammable should be stored in a flammables cabinet
    - Oxidizers (ex. Bleach & Hydrogen peroxide) should be stored away from flammables and reducing agents
  - Store chemicals at or below eye level
  - Secondary containment for liquid chemicals

Chemical Safety

- Safety Data Sheets
  - Required by OSHA to have SDSs for every chemical in the lab
  - Must be manufacturer specific
  - Can be digital or hard copy
- Mark all chemical containers with date received and date opened
- Mind expiration dates, if chemical has no marked expiration, 5 years is a good lab-assigned option

Chemical Safety

Accident Preparation

- Spill Kits
  - Spill kits for various types of accidents
    - Acid, base, formaldehyde
  - Silver shield gloves, do not breakdown overtime like latex or nitrile
- Safety Showers & Eyewash Stations
  - Should be checked at least monthly
  - Cannot be obstructed

Lab Dress

- Safety glasses or googles at all times in the lab
- Lab coats to prevent splashes
- Pants and non-permeable shoes
- Face shield and apron is there is a potential splash hazard
- Keep an extra change of clothes at work

Documentation

- IF YOU DON'T HAVE A RECORD, IT DIDN'T HAPPEN!
- Records of reagent preparation and use
  - Log when a reagent was prepared, what chemicals were used (Manufacturer, lot #, etc)
  - Log what reagents were used in a particular test
    - Troubleshooting issues later
    - Tracking down contaminants

Reagent Name	*Prepared Date*	*Prepared By*	*Expiration Date*	*Reagents Used*	*Lot Number*	*Received Date*	*Storage Conditions*	*Manufacturer*	*Reagent Used Expiration Date*	*P.O. #*	Notes
Yttrium 3+ 18 mg/mL Carrier	2018-01-05	lfloryance	2023-01-05	Yttrium III Oxide	A0366013	2016-04-01	room temperature	Acros	N/A	1001581091	
*	*	*	*	16 N Nitric acid	174595	2017-11-15	room temperature	Fisher	N/A	1001867148	
0.1 N Sulfuric acid	2018-01-09	acarl	2023-01-09	36 N Sulfuric acid	53232	2015-08-27	room temperature	EMD	N/A	N/A	
2N Aluminum nitrate	2018-01-09	dmay	2023-01-09	Aluminum nitrate nonahydrate	151052	2016-05-16	room temperature	Fisher	N/A	1001603180	
*	*	*	*	*	161788	2017-11-03	room temperature	Fisher	N/A	1001860383	
1.5M Sulfamic acid	2018-01-10	dmay	2023-01-10	Sulfamic acid	A0335345	2014-04-03	room temperature	Acros	N/A	UNK; opened 2014-04-25	
1M Ascorbic acid	2018-01-10	dmay	2018-01-17	L-Ascorbic acid	144594	2015-02-13	room temperature	Fisher	N/A	UNK	
3M Sodium nitrite	2018-01-11	dmay	2018-01-11	Sodium nitrite	A0312016	UNK	room temperature	Acros	N/A	UNK	
5N Hydrochloric - 0.05M Oxalic acid	2018-01-11	dmay	2023-01-11	12N Hydrochloric Acid	0000134217	2017-11-09	room temperature	Macron	N/A	0000701107	
*	*	*	*	Oxalic acid	044274	2005-03-15	Room Temperature	Fisher	N/A	UNK	

Documentation

- Deviations from SOPs
- Lab accidents
- Unusual events that may affect testing
- Document instrument installation, maintenance, instrument functionality issues, decommissioning
- Log important environmental conditions
    - Time, temperature (ex. room temperature for BOD)

Documentation

Biochemical Oxygen Demand, Standard Methods 5210B

OpenELIS Worksheet #	Date/Time In	Worksheet Creation Analyst	Incubator Temp. In (°C)	Date/Time Out	Other Analyst(s)	Incubator Temp. Out (°C)
142866	2018-08-23 10:47	rderrig	20.31	2018-08-28 13:10	dmay	19.97

Reagent	Lot #/Prepared Date	Manufacturer/ Prepared By	Received Date	Expiration Date	Seed in Samples (mL)
Dilution Water	#3 -08/17/18 #4 -08/17/18	RAD RAD	N/A	N/A	N/A
Phosphate Buffer	2018-08-06	dmay	N/A	2023-08-06	N/A
Nitrification Inhibitor	A8166	Hach	2018-07-02	2023-06-08	N/A
Glucose/Glutamic Acid, 300 mg/L	A8159	Hach	2018-07-02	2023-03-07	N/A
Seeding Material	SHL SAMPLE	696706	N/A	N/A	2

Batch QC Results				Result Comparison Tool				
BOD Blank Results	CBOD Blank Results	Duplicate Results (RPD / Abs. Diff.)	G/GA Results	1st Result	2nd Result	RPD / Abs. Diff.	RPD UCL (>5 mg/L)	Abs. Diff. UCL (<5 mg/L)
0.005		4.6%	91%	60.20	45.77	27.2% / 14.43	30.0%	1
#N/A		#N/A	#N/A					
#N/A		#N/A	#N/A					
#N/A		#N/A	#N/A					

DO/pH Meter Information

DO Meter #1: YSI 5100, S/N 10E 101362. DO Probe #1: YSI 5905, S/N 10F 100339. DO Meter #2: YSI 5000, S/N 11B 100676. DO Probe #2: YSI 5905, S/N 05C1612. pH Meter: Oakton pH 2700, S/N 2151866. pH Electrode: Accumet 13-620-289, S/N VUW15118

OpenELIS Number	Sample Information	pH	BOD or CBOD?	Sample Type	Sample Volume (mL)	BOD Bottle Number	Initial D.O. (mg/L)	Final D.O. (mg/L)	D.O. Depletion (mg/L)	Dilution BOD (mg/L)	Use Dilution?	Dilution Result (mg/L)	Final Result (mg/L)	Final Result Override (mg/L)	Date/Time In Override	Comments
Blank1	N/A	N/A	BOD	Blank	N/A	2A	8.26	8.26	0.00	N/A	Y	0.00	0.01		2018-08-23 08:15	
Blank1	N/A	N/A	BOD	Blank	N/A	3	8.32	8.31	0.01	N/A	Y	0.01			2018-08-23 08:15	
Seed Control	N/A	N/A	BOD	Seed Control	4	4	8.29	7.18	1.11	83.25	Y	83.25	81.38		2018-08-23 08:15	
Seed Control	N/A	N/A	BOD	Seed Control	4	5	8.28	7.22	1.06	79.50	Y	79.50			2018-08-23 08:15	
Seed Control	N/A	N/A	CBOD	Seed Control	4	6	8.29	6.93	1.36	102.00	Y	102.00	100.50		2018-08-23 08:15	
Seed Control	N/A	N/A	CBOD	Seed Control	4	11A	8.24	6.92	1.32	99.00	Y	99.00			2018-08-23 08:15	
G/GA Standard	N/A	N/A	BOD	G/GA Std	3	18	7.86	3.71	3.61	361.00	Y	361.00	358.67		2018-08-23 08:45	
G/GA Standard	N/A	N/A	BOD	G/GA Std	3	21A	7.87	3.72	3.61	361.00	Y	361.00			2018-08-23 08:45	
G/GA Standard	N/A	N/A	BOD	G/GA Std	3	21	7.86	3.78	3.54	354.00	Y	354.00			2018-08-23 08:45	

Documentation

- Records of analysis
  - Who did what and when
  - Quality Control
  - Supporting information (ex. pH for BOD)
  - What was reviewed,
  - Lots of other information

Biochemical Oxygen Demand, Standard Methods 5210B

	Review & Release Checklist	Analyst	Reviewer
1	Check to ensure that the dates for set-up and final readings, analyst initials, temperature and air pressure are entered.	☑	☑
2	Ensure that DW Blanks have acceptable depletion (≤ ±0.20).	☑	☑
3	Ensure that the sample information is entered correctly.	☑	☑
4	Ensure that there is a depletion of 2.00 mg/L or more and a remaining DO of 1.00 mg/L or more. If more than one dilution meets this requirement, average the results. NOTE: If the depletion for a 250 mL dilution is less than 2.00 mg/L, report as <2.	☑	☑
5	Ensure that the Started Date entered into OpenELIS matches the In date in the workbook and both the In and Out analysts are listed as having completed the analyses.	☑	☑
6	Ensure an approximate pH has been recorded in the spreadsheet.	☑	☑
6	Ensure that all BOD results are correctly reported in OpenELIS (rounded to integers with no more than 2 significant figures). *Note: PT providers request 3 significant figures for reporting.14.3.* When more than two dilutions of a single sample are far apart is the relative percent difference (RPD) between the two results are greater than the Upper Control Limit (UCL) for the previous 20 duplicate samples? If so the higher of the two results is reported and the result is qualified.	☑	☑
7	Ensure that comments/QA Events are entered as appropriate (e.g. If a larger sample amount has a significant decrease in BOD, etc.). A list of BOD-specific QA Events and descriptions of when to use them is located at M:\IC_Nutrient_Demand\OpenELIS_BOD_QA_Events.xlsx	☑	☑
8	Final report was reviewed.		☑
9	Release the samples in OpenELIS, and initial and date the Review/Release Checklist.		☑
Comments	Worksheet Number	142866	
	User	dmay	acarl
	Date	8/28/2018	8/30/2018

Documentation

- Documentation is important
  - Can you remember exactly what happened? One week ago? One month? Five years
  - You need proof, generated at the time not retroactively
  - No one is going to just take you word for it.

Time for a Quiz!

Questions?

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