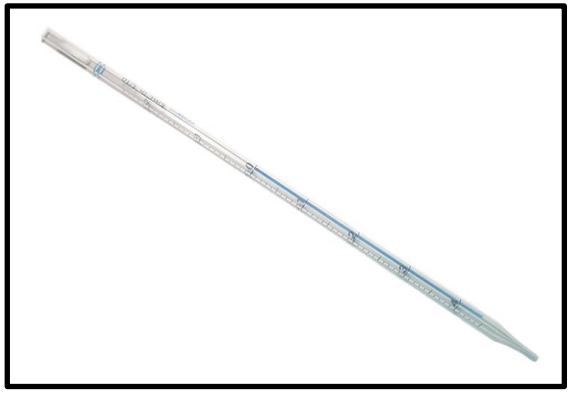




Lab Intro, Pipetting and Calculations

LAB SAFETY

- Please read over (Wk1) Lab Safety
- BSL-1, however..
- Always wear gloves

- COVID-19 Stuff
  - No more than 10 students in AH408
  - Disinfect your workstation-Before and After
  - Mask Policy
  - Physical Distance

LAB SAFETY-Waste Disposal

- Our materials are biohazardous and go in CELL BIO Trash Bins.
- Biohazard-Non Sharp
- Biohazard- Sharp
- Biohazard- Glass Sharp



PIPETTING
the cell biologist's silverware







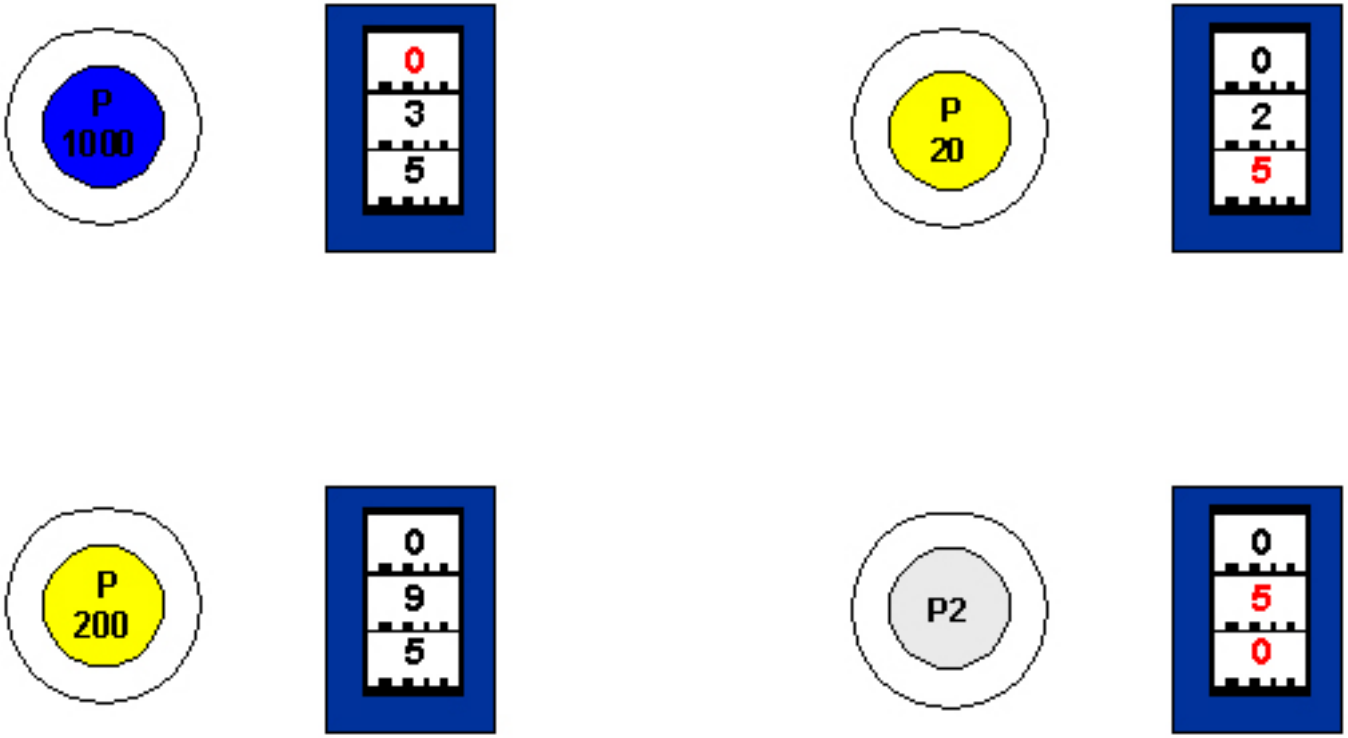








PIPETTING
the cell biologist's silverware



CALCULATIONS

Quiz week 1 will be primarily calculations, very similar to the practice sheet I am providing in lab. Answers are also posted on Canvas.

Text	Symbol	Factor
kilo	k	10^{3}
none	none	1
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}

CALCULATIONS

atomic weight (amu or daltons)

Standard unit for mass on an atomic scale. One atomic mass unit is approximately the weight of one nucleon and is equivalent to 1 g/mol.

C = 12.01 amu = 12.01 g/mol
H = 1.01 amu = 1.01 g/mol
O = 15.99 amu = 15.99 g/mol

CALCULATIONS

Molecular weight (M.W.)

The mass of a single molecule, calculated as the sum of the atomic mass of the atoms that make up the molecule.

MW of ethanol (C_2H_6O) =

Carbon (C)	12.01 amu	x2	24.02 amu
Hydrogen (H)	1.01 amu	x6	6.06 amu
Oxygen (O)	15.99 amu	x1	15.99 amu
Ethanol			**46.07 amu**

CALCULATIONS

Molar mass (g/mol)
The mass of one mole of a substance.

M of ethanol (C_2H_6O) =

Carbon (C)	12.01 g/mol	x2	24.02 g/mol
Hydrogen (H)	1.01 g/mol	x6	6.06 g/mol
Oxygen (O)	15.99 g/mol	x1	15.99 g/mol
Ethanol			**46.07 g/mol**

CALCULATIONS

Molarity (mol/L)
A measure of concentration. 1mol/L = 1M

How many grams of ethanol are in a 1M solution?
Molar mass ethanol = 46.07 g/mol
*1M ethanol = **46.07 g/L***

CALCULATIONS

Performing dilutions:

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

You have a stock solution that is 1M (i.e. 1 mol/L) concentration. You want to make 10mL of a 100mM solution for your experiment. How much of the stock solution and how much deionized (purified) water should be added?

Remember: units MUST be the same across the equation

CALCULATIONS

Performing dilutions without concentration:

(Final Volume/Dilution Factor) = Solute Volume

DF of 10 means a 1:10 dilution, or 1 part solute + 9 parts diluent, for a total of 10 parts.

How can we make 300 µl of a 1:250 dilution ?

1.2 µl into 298.8

CALCULATIONS

Step dilutions:

Final DF = DF1 x DF2 x DF3

If the dilution factor is larger than the final volume needed, or the amount of stock is too small to be pipetted you may need to use step dilutions.

How can we make a 1:1,000,000 dilution ?

Text	Symbol	Factor
kilo	k	10^{3}
none	none	1
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}

CALCULATIONS

Step dilutions:

Final DF = DF1 x DF2 x DF3

Example: Make only 300 uL of a 1:1000 dilution, assuming the smallest volume you can pipette is 1 uL

300µl/1000 = 0.3 µl Way to small!!

Let's try 1:10 and then 1:100
10 x 100 = 1000

Dilution 1 gets 1µl of the solution into 9µl of water.
Dilution 2 gets 1µl of Dilution 1 into 999µl of water

You are given two primers. Primer (A) is 20mM. Primer (B) is 35mM. You want to make a 40µL solution that contains 5nM of each primer. How much of each primer will you need? How much sterile water?

mM to nM is a 1:1,000,000 total dilution

Lets make 2 1:1000 serial dilutions
By taking 1µl of A and adding it to 999 µl of water
Then taking 1µl of our dilution and adding to 999µl of water

We now have a solution or A that is 20nM, but we need 5nM

$20nM \times V1 = 5nM \times 40\mu l$
$V1 = 10\mu l$

CALCULATIONS

Other important concepts:

- **Normality (N) – a measure of the concentration of the reactive species in a solution (e.g. H^+ in HCl)**
- **% solutions**
- **Formula weight vs. molecular weight**
- **Working concentrations and fold dilutions**

TIPS FOR SUCCESS IN LAB

• Follow the safety guidelines and help to assure that those around you do the same.

• Come prepared, having read the protocol and familiarized yourself with the concepts. You can even write a lab introduction before class!

• ALWAYS bring your lab notebook. The more thorough, clear and complete your notes are, the easier your assignment will be to complete.

• Be a proactive team member. These earlier labs are designed to introduce you to very important basic concepts and skills that you will need for later more complicated experiments, including your final project.

PRACTICE CALCULATIONS

How many mL of isopropanol should be added to water to make 15ml of 70% isopropanol?

You are given two primers. Primer (A) is 20mM. Primer (B) is 35mM. You want to make a 40uL solution that contains 5nM of each primer. How much of each primer will you need? How much sterile water?

You are given a powder form of the antifungal Fluconazole. The bottle says the molecular weight is 30.27amu and the bottle contains 100mg. How much water should you add to the bottle to make a 1M solution?