Scientific Calculations
Preparing Solutions

- One of the most common tasks in laboratory
- One of the greatest sources of error and variance
- Need to get concentrations right if we want to draw conclusions
- 1 Variable to be measured....
Solution Problems

- 10 % Serum in tissue culture media
- 10 ng/ml X to cells
- Prepare 20% w/v solution of X
- Dilution of stock solution
- Prepare 0.5M solution of X
Some Definitions

- **Solution**
  - Homogenious mixture of a solute and a solvent

- **Solvent**
  - What solute is dissolved in
    - E.g Water, DMSO, Alcohol etc

- **Solute**
  - What is added to the solvent
    - E.g chemical, cytokine etc.
Types of Solution

- Dilute solution
  - Weak solution
  - Relatively small amount of solute
  - Easily dissolved

- Concentrated solution
  - Strong solution
  - More difficult to prepare
The Concentration of a Solution

- Amount of solute per quantity of solvent
- Expressed in many ways
  - Mass/ Volume Ratio
  - Mass / Mass
  - Volume / Volume Ratio
  - Parts per million
  - Mole Fraction
  - Molarity
  - Normality
Mass / Volume Ratio

- Mass of solute relative to total volume of solvent

- Example
  - 10 ng / ml TNF
  - 10 ng of TNF in each ml of solvent
    » See later
Volume / Volume Ratio

- Volume of A relative to volume of B

- Example
- 10% FCS in Media
- 10% V / V solution

- I.e 10% of final volume is FCS
  » See later
Molarity

Molarity is probably the most commonly used unit of concentration.

It is the number of moles of solute per litre of solution.
Molarity

- FW/L = 1M

- Eg; Amphetamine
  - C9H13N

Molecular Mass / Weight (MW) / Functional Weight (FW)

: 135.2084 g/L = 1M
General Approach to Preparing a Solution

• determine the mass (g) of solute required to make up the desired volume (L) and concentration (mol/L) of solution. Use formula and show calculations.

• measure out and dissolve the solute in approximately 1/2 the total volume of solvent.

• raise the volume of solution to the desired total volume by adding more solvent.
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Tissue Culture Medium

• For routine culture of cell line A, we need to have a 10% FCS in Ham’s F12 Solution

• Notes
  – Basal media generally comes in 500ml volume
  – Need to figure out what volume of FCS needs to be added
  – This is a volume/volume Ratio problem
The Common Methodology

- Vol of Media ➔ 500ml

- 10% of this ➔ 50ml

- Add 50 ml of FCS !!!!

- Total Volume is ➔ 550ml
- Volume of FCS ➔ 50ml
- Volume / Volume Ratio ➔ 9.1 % !!!!!!
The Correct Approach

• Vol of Media ➞ 500ml

• 10% of this ➞ 50ml

• Remove 50 ml of basal
  – (Retain for serum free exposures)

• Add 50 ml of FCS !!!!

• Total Volume is ➞ 500ml
• Volume of FCS ➞ 50ml
• Volume / Volume Ratio ➞ 10 %
Key Message

- When considering volume / volume solutions
  - Final volume is the important one !!
Calculation

- Need 20 % volume / volume Serum in 200 ml of basal media

- What quantity of serum is needed?
Solution

- Vol of Media ➔ 200ml
- 20% of this ➔ 40ml
- Remove 40 ml of basal
  - (Retain for serum free exposures)
- Add 40 ml of FCS !!!!
- Total Volume is ➔ 200ml
- Volume of FCS ➔ 40ml
- Volume / Volume Ratio ➔ 20 %
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- Prepare 0.5M solution of
Cell Stimulation

- For our experiment we want to expose our cells to 10 ng/ml TNF-a
- This is a mass/volume calculation
- Questions
  - What is total volume of cell solution?
  - What is the concentration of the stock solution?
At the beginning....

- TNF-a ordered from company

- 10 µg of lyophilised protein delivered

- Technical note with product recommends
  - Protein be reconstituted in Sterile PBS supplemented with 0.1% albumin
  - Stock solution of 10 µg / ml to be prepared
Stock Solution

• 10 µg / ml stock to be prepared first
• Solvent : PBS 0.1% BSA
• Have
  – 10 µg of solute
• Need
  – 1 ml of solvent
Solvent Preparation

- 0.1 % BSA in 1 ml
- 100 % BSA → 1g in 1ml
- 10 % BSA → 100mg in 1ml
- 1 % BSA → 10mg in 1 ml
- 0.1 % BSA → 1 mg in 1ml

- This weight is too small to be accurate!
Solvent Preparation

- Lets make more than 1 ml of the solvent
- E.g 100 ml of 0.1% BSA in PBS

- 100 % BSA → 100 g in 100 ml
- 10 % BSA → 10g in 100ml
- 1 % BSA → 1g in 100 ml
- 0.1 % BSA → 100 mg in 100ml

- This is easy to weigh and prepare
Solvent Preparation

- We now have 100mls of our solvent
- 100 mls of 0.1% BSA in PBS
- Use 1 ml of this to reconstitute our TNF-a
- 1 ml of solvent + 10 µg of solute
  - 10 µg / ml Solution of TNF-a
What do we want?

- Expose cells to 10 ng/ml TNF-a
- Volume of cell culture well is 2ml
- Therefore
  - 10 ng/ml for 2 mls
  - Need to add 20 ng to each well of cells
  - How do we get 20 ng
Getting Final Concentration

• Need
  – 20 ng of solution

• Have
  – 10 \( \mu \text{g} / \text{ml} \) Solution

  – Need to find the volume of our solution that contains 20 ng
Final Concentration

- 1 ml of solution ➞ 10 ug
- 1 ml of solution ➞ 10,000ng
- 100 µl of solution ➞ 1,000ng
- 10 µl of Solution ➞ 100 ng
- 1 µl of solution ➞ 10 ng
- 2 µl of solution ➞ 20 ng
The Problem

• We need to add 2 \( \mu l \) of our stock to each well to get the required concentration

• Problems
  – Volume is very small
    • Sticking to the pipette
    • Distribution throughout the well
  – Accuracy / Reproducibility
The Solution

- Our initial reconstitution solution is too concentrated
- Need to have bigger volumes
  - Need to make the stock more dilute
The Solution

- Stock solution is 10 $\mu$g / ml
- Lets dilute this 10 fold
  - 1 ml of this solution + 9 mls of solvent (PBS + 0.1% BSA)
    - Remember we prepared too much solvent!!!!
  - 1/10 Dilution
  - Now have 10 mls of
    - 1 $\mu$g / ml solution
Getting Final Concentration

- Need
  - 20 ng of solution
- Have
  - 1 mg / ml Solution
  - Need to find the volume of our solution that contains 20 ng
Final Concentration

- 1 ml of solution 1 mg
- 1 ml of solution 1,000ng
- 100 ul of solution 100ng
- 10 ul of Solution 10ng
- 20 ul of solution 20 ng

- Volume is 10 times greater and our measurement problems have dissolved......
Recap on Steps

- General Approach
- Reconstitute protein as per instructions
- This is your stock solution
- Dilute stock solution
  - Remember to use same solvent
- Calculate amount of this you need to add to each well
Note on Volume / Volume Ratio’s

• Recall from cell media example that final volume is important

• In this case we wanted to have
  – 20 ng in 2 ml of solution

• We ended up with
  – 20 ng in 2.02 ml of solution
  – Final Concentration is 9.99 ng/ml

  – As error is so low (1%) we can disregard the volume change
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Preparing a Molar Solution

- Prepare 1 litre of 0.5 M CaCl$_2$
- 1 Liter of solvent
- 0.5 Moles of CaCl$_2$
Preparing a Molar Solution

- I Mole of CaCl$_2$
  - Mol weight of CaCl$_2$ in I Litre of soln

- Mol weight (From reagent bottle)
  - Ca
  - Cl * 2
    - 110g

- 110 g CaCl$_2$ in I Litre = 1 Mole
Preparing a Molar Solution

- 1 Mole of CaCl$_2$ 110g in 1 litre
- 0.5 Moles 55g in 1 Litre
Recap

• Determine nature of the problem
• What type of solution are you preparing
  – Volume / volume
  – Moles
  – Mass / Volume

• Remember final concentration!!!
• Avoid small volumes