1. How many grams of NaCl (FW = 58.44) are needed to make 125 ml of a 1 mM NaCl solution?

This is a molarity problem solved by VOL x CONC x FW, so make sure to express all units fully. A 1 mM solution is $10^{-3}$ moles/L; 125 ml is 0.125 L; the FW of NaCl is 58.44 g/mole. So,

$$0.125 \text{ L} \times 10^{-3} \text{ moles/L} \times 58.44 \text{ g/mole} = 7.3 \times 10^{-4} \text{ g}$$

2. You perform a serial dilution of steps $10^{-1}$, $10^{-3}$, and $10^{-2}$ for a bacterial sample from a local swimming beach. After plating 200 ul from each dilution on LB plates and incubating overnight at 37 C, you obtain the following colony counts:

<table>
<thead>
<tr>
<th>Total Dilution?</th>
<th>Red colonies</th>
<th>Cream-color colonies</th>
<th>Brown colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>TNTC</td>
<td>257</td>
<td>TNTC</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>148</td>
<td>1</td>
<td>TNTC</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>2</td>
<td>0</td>
<td>314</td>
</tr>
</tbody>
</table>

1. TNTC = “too numerous to count”

(a) What is the total dilution for each step (fill in table)?

(b) What is the estimated concentration (cells/ml) of bacteria of each type in the original sample?

Use the plate counts for each bacterium that is in the hundreds. Solution is # colonies x conversion factor to account for volume plated x dilution factor. Remember that each colony arises from 1 cell.

- For red colonies: $148 \text{ colonies} \times (5 \times 0.2 \text{ ml}) \times 10^4 = 7.4 \times 10^6 \text{ cells/ml}$
- For Cream-color: $257 \text{ colonies} \times (5 \times 0.2 \text{ ml}) \times 10^1 = 1.285 \times 10^4 \text{ cells/ml}$
- For brown colonies: $314 \text{ colonies} \times (5 \times 0.2 \text{ ml}) \times 10^6 = 1.57 \times 10^9 \text{ cells/ml}$
3. You have 5.5 ml of 128 mg/ml streptomycin stock solution. What is the maximum volume of 65 ug/ml streptomycin solution that you can make if you use all of the stock?

   This is a $V_1C_1 = V_2C_2$ problem in which you are solving for $V_2$. So $(V_1C_1)/C_2 = V_2$

   $1 \text{ mg} = 1000 \text{ or } 10^3 \text{ ug}; \text{ convert mass units to } \text{ ug}$.

   $$(5.5 \text{ ml} \times 128 \times 10^3 \text{ ug/ml}) / (65 \text{ ug/ml}) = 10.831 \text{ ml or 10.831 L…A LOT!!}$$

4. A bacterial disinfectant stock requires a 3:37 dilution to make the working strength solution. You have 100 ml of stock left after an arduous semester in the lab.

   - What is the maximum volume of working strength disinfectant you can make if you use all of the stock?

     This is a “unit volume” approach to the solution. The dilution is 3 unit volumes brought up to a total of 37 unit volumes $\Rightarrow$ 3 unit vol stock + 34 unit vol solvent.

     $100 \text{ ml of stock divided by 3} = 33.3 \text{ ml per unit volume}$

     $33.3 \text{ ml x 34 unit volumes solvent} = 1132.2 \text{ ml solvent}$

     $1132.2 \text{ ml solvent} + 100 \text{ ml disinfectant stock} = 1232.2 \text{ ml working strength disinfectant}$

   - How much stock do you start with to make up 100 ml of working strength solution?

     In this scenario, 100 ml = 37 unit volumes, so $100/37 = 1 \text{ unit volume} = 2.7 \text{ ml}$

     $2.7 \text{ ml} \times 3 = 8.1 \text{ ml stock}$

5. Starting with 95% ethanol, how would you make 500 ml of 70% ethanol in deionized water?

   This $V_1C_1 = V_2C_2$ again, solve for $V_1$, then make up the difference in volume with water.

   $$V_1 = (500 \text{ ml x 70%})/95\% = 368.42 \text{ ml 95% ethanol} + 131.58 \text{ ml water}$$

6. You are culturing the bacterium *Proteus vulgaris* in tryptic soy broth (TSB) to produce a growth curve. You inoculate 9.5 ml of TSB with 500 ul of fresh overnight bacterial culture that also contains vitamin A at a concentration of 80 ug/ml. What is the concentration of vitamin A in the newly inoculated culture?

   The critical information here is that the 500 ul of culture contains 40 ug of vitamin A. The total volume of the culture becomes 10 ml, so $40 \text{ ug} / 10 \text{ ml} = 4 \text{ ug/ml}$
7. In a fit of completely morbid curiosity, your lab group decides to test the LabOgre’s beard hair for antimicrobial activity. You collect a 5 g sample of what little non-gray beard hair he has left, grind it with 13.725764 ml of broth, coarse filter through cheesecloth, then centrifuge at 500x gravity for 12.34832 minutes to pellet the remaining debris. Remarkably, through masterful technique, you are able to recover all 13.725764 ml of the supernatant.

What is the extract concentration in mg/ml?

\[
\frac{5 \text{ g} = 5000 \text{ mg}}{13.725764 \text{ ml}} = 364.28 \text{ mg/ml}
\]

If you then set up a broth culture using 0.5 ml of extract in a total of 7 ml, what is the concentration of extract in the culture?

\[
0.5 \text{ ml contains } \frac{364.28}{2} = 182.14 \text{ mg beard hair extract}
\]

\[
\frac{182.14 \text{ mg}}{7 \text{ ml}} = 26.02 \text{ mg/ml}
\]

8. Briefly explain what is happening when you properly zero a spec 20 prior to taking a reading.

With an empty sample chamber, you set the left end of the scale (with left knob) to infinite absorbance. This calibrates the spec to a condition of no light passing through the sample, i.e., ALL light being absorbed. Then the BLANK is inserted and using the right hand knob, the full scale is set to 100 % transmittance, or 0 absorbance. The BLANK is formulated such that the light passing through it is the maximum amount of light that could be detected when none is absorbed. Overall, then, this sets the range of possible absorbance measurements for a given sample.

9. The table below shows the formulations for three cultures being used in a growth curve experiment to test the effect of the LabOgre’s beard hair on growth. The positive control uses antibiotic that absorbs the same wavelength as is being used to measure the bacterial growth (600 nm). In the space provided, write in the formulations for the BLANKS that are needed. The beard extract and antibiotic were prepared in broth.

A blank is required for each unique formulation UNLESS it can be shown that some components do not absorb or scatter light at the wavelength of interest. In this case, the + control must have a blank because the antibiotic absorbs at wavelength being used.

<table>
<thead>
<tr>
<th>Component</th>
<th>Treatment</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Treatment BLANK</th>
<th>(-) Control BLANK</th>
<th>(+) Control BLANK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth</td>
<td>6.0 ml</td>
<td>6.5 ml</td>
<td>6.0 ml</td>
<td>6.5 ml</td>
<td>7.0 ml</td>
<td>6.5 ml</td>
</tr>
<tr>
<td>Bacteria</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Beard extract</td>
<td>0.5 ml</td>
<td>----</td>
<td>----</td>
<td>0.5 ml</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>----</td>
<td>0.5 ml</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>
10. The LabOgre likes his coffee and drinks three mugs (1 mug = 150 ml or ~ 6 oz – oops, corrected) each morning. Each mug of java contains approximately 20 mg caffeine (FW = 194.2). 

- What is the molarity of the caffeine in one mug of coffee (150 ml)? In three mugs?

  Convert to standard units (g, L, g/L, g/mole).

  \[
  0.020 \text{ g caffeine} / 0.15 \text{ L coffee} = 0.1333 \text{ g/L}
  \]

  Now divide by the FW to molar concentration = (0.1333 g/L) / 194.2 g/mole

  \[
  = 6.866 \times 10^{-4} \text{ M per mug}
  \]

  **trick question….each mug has the same concentration.**

- If the L.O. cuts his coffee with 50 ml of hot, 1.5% milk, what is the molarity of the caffeine in his café au lait?

  If 50 ml is replaced by milk, this means the coffee is diluted to 2/3rds its original concentration (100/150 = 0.667 or 2/3), so,

  \[
  0.667 \times 6.866 \times 10^{-4} \text{ M} = 4.579 \times 10^{-4} \text{ M}
  \]