

Dilutions and Conversions:

Not only are dilutions used in enumerating microorganisms, they are also used in determining appropriate volumes of stock solutions to use. Both are essential skills in for industry, the government and academia. Remember: It is often not accurate to measure a very small mass or a very small volume. In some cases you will be more accurate by preparing a more concentrated stock solution and then using a dilution of the stock solution to generate the final solution at the desired concentration. Knowing how to figure dilutions and use them in calculations is an essential skill that you should practice and use frequently. Not only are dilutions used in determining appropriate volumes of stock solutions to use, they are also used in enumerating microorganisms. Both are essential skills in industry and academia. You need to also be adept at scientific notation (exponential notation) and conversions between various metric units of measure (kg, g, mg, μg , ng; Liter, ml, μl , nl; or mole, mmole, μmole , nmole, etc.)

Example Problems. You should know how to answer the following types of problems and conversions including showing your work and understanding how the calculations were made.

1. A medium formulation calls for adding 25 mls of a sterile 10 mM FeSO_4 stock solution to 1 liter (final volume) of sterile buffer. What will be the final concentration of FeSO_4 in the medium?

*Ans. $(25 \text{ ml} / 1000 \text{ ml}) * 10 \text{ mM} = 0.25 \text{ mM} = 250 \mu\text{M}$
In this case 1000 ml was the final volume, not 1025 ml.*

2. A common buffer used in molecular biology is "TE", pH 8.0 that is 10 mM Tris and 1 mM EDTA with the pH set to 8.0. Starting with 1 M Tris stock solution and 0.5 M EDTA solution, describe how you would prepare 100 ml of TE at pH 8.0.

*Ans. 1.0 ml of the 1 M Tris stock solution
0.2 ml of the 0.5 M EDTA stock solution
98.8 ml water*

(You would actually use about 90 ml of water, check the pH and adjust to pH 8.0 if necessary, then bring the final volume to 100 ml)

3. You are asked to make 3 control suspensions of *Salmonella* for a *Salmonella* ELISA (enzyme-linked immunosorbant assay) test kit. Using plate count data on a *Salmonella* detection agar you have determined that 6.9×10^9 *Salmonella* cells are present in one ml of medium. Describe how you would prepare 3 control *Salmonella* suspensions in phosphate-buffered saline so that the suspensions contained 3, 30 or 300 *Salmonella* cells per ml.

Ans. One way: $6.9 \times 10^9 = 690 \times 10^7$ cells per ml. Using a 10-fold dilution series and phosphate-buffered saline with seven tubes, reduce the concentration to 690 cells per ml. Do you know how to do this?

Then take 100 mls of the 690 cells/ml solution and add it to 130 ml of phosphate buffered saline.

$$\begin{aligned} 690 \text{ cells/ml} * 100 \text{ mls} / (100 \text{ mls} + 130 \text{ mls}) &= 690 \text{ cells/ml} * (100 \text{ mls} / 230 \text{ mls}) \\ &= 300 \text{ cells per ml} \end{aligned}$$

Dilute the 300 cell/ml suspension 1/10 (e.g. 10 ml plus 90 ml) to give the 30 cell per ml suspension. Dilute the 30 cell per ml suspension another 1/10 (10 ml plus 90 ml) to give the 3 cell per ml suspension).

4. A mixture of bacteria was collected and then diluted as follows. 1 ml of the mixture was added to and mixed thoroughly with 99 ml sterile water. This was designated dilution tube # 1. Then 0.1 ml of dilution tube #1 was added to and thoroughly mixed with 9.9 mls of sterile water. This was designated dilution tube #2. Finally 1 ml of dilution tube #2 was added to and thoroughly mixed with 9.0 ml of sterile water. This was designated dilution tube # 3. Then 0.1 ml of dilution tube # 3 was plated (spread plate technique, with the glass spreader) onto 3 separate nutrient agar plates (0.1 ml onto each plate). After incubation for 48 hours, the colonies on the plates were counted giving the following results: Plate 1 = 97 colonies; plate 2 = 102 colonies; plate 3 = 111 colonies. How many bacteria were present per ml of the original mixture?

Ans. 1.03×10^8 bacteria per ml assuming each colony-forming unit (CFU) arose from 1 bacterial cell.

5. 0.637 ml is the same as _____ μ l *Ans. 637 μ l*
6. 28 μ g is the same as _____ mg *Ans. 0.028 mg*
7. 346 nmoles is the same as _____ mmoles *Ans. 0.000346*
8. A serial dilution was performed using five tubes. All five tubes contained 1.0 ml of diluent. All tubes were mixed thoroughly after addition of the sample. 0.25 ml sample was added to Tube One. Tube Two contained 0.25 ml from Tube One. Tube Three contained 0.25 ml from Tube Two. Tube Four contained 0.25 ml from Tube Three and Tube Five contained 0.25 ml from Tube Four.

What is the dilution made in Tube One? *Ans 1/5 or 0.20*

What is the dilution made in Tube Two? *Ans 1/25 or 0.04*

What is the dilution made in Tube Three? *Ans 1/125 or 0.008*

What is the dilution made in Tube Four? *Ans 1/625 or 0.0016*

What is the dilution made in Tube Five? *Ans 1/3125 or 0.00032*

If the concentration of the initial sample was 75 mg per ml what was the concentration of the sample in Tube Five?

Ans. $75 \text{ mg/ml} \times 1/3125 = 0.024 \text{ mg/ml}$

or

$75 \text{ mg/ml} \times 0.00032 = 0.024 \text{ mg/ml}$

9. You have stock solutions of 10 M KCl and 100 mM HEPES buffer, pH 7.5. You are asked to prepare a 100 mls of 100mM HEPES buffer containing 0.35 mM KCl. How would you make this buffer solution?

Ans. 3.5 μ l of the 10 M KCl stock solution added to 100 mls of 100 mM HEPES buffer