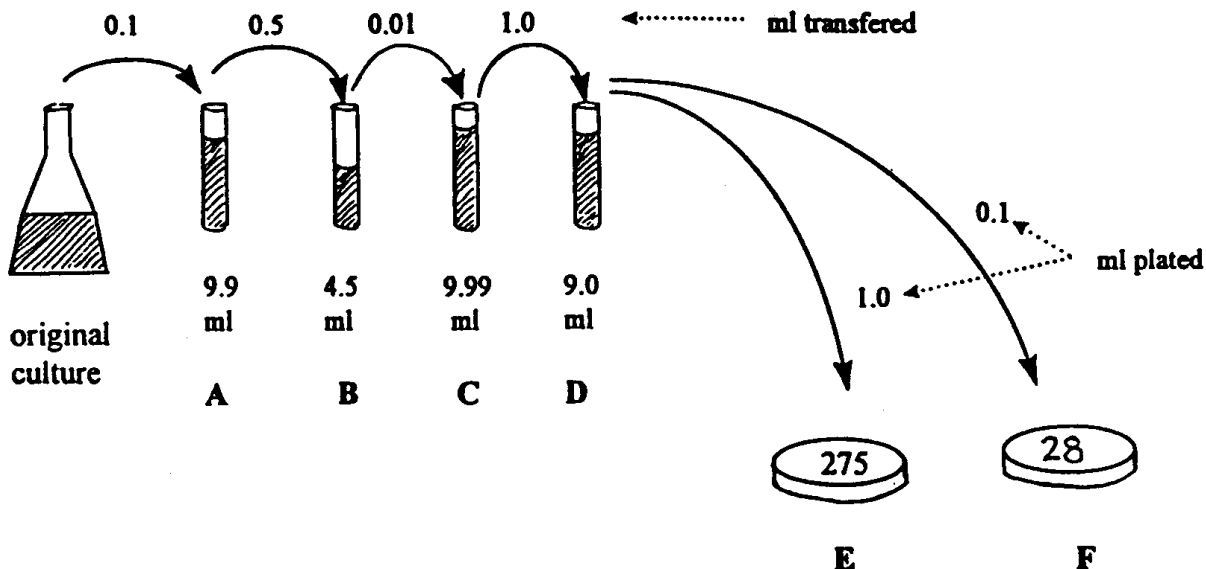


# BACTERIAL DILUTIONS and A FOOL-PROOF WAY TO FIGURE THEM OUT

Look at the dilution scheme below:



Most questions you will be asked to answer about serial dilutions are of two types:

The **FIRST TYPE** gives you the number of bacterial colonies found on a plate and asks for the number of bacteria per ml in the original culture.

The **SECOND TYPE** gives you the number of bacteria per ml in the original culture and asks you to devise a serial dilution scheme so that you will get plates with “countable” numbers (i.e., between 30 and 300 colonies) of colonies on them.

To solve **TYPE ONE** problems, first determine the individual dilution factor for each tube using the formula:

$$\text{INDIVIDUAL DILUTION FACTOR} = \frac{\text{AMOUNT TRANSFERRED}}{\text{AMOUNT TRANSFERRED} + \text{AMOUNT ALREADY IN TUBE}}$$

$$\text{For Tube A, the IDF} = \frac{0.1}{0.1 + 9.9} = \frac{0.1}{10.0} = 0.01 = 10^{-2}$$

$$\text{For Tube B, the IDF} = \frac{0.5}{0.5 + 4.5} = \frac{0.5}{5.0} = 0.1 = 10^{-1}$$

$$\text{For Tube C, the IDF} = \frac{0.01}{0.01 + 9.99} = \frac{0.01}{10.0} = 0.001 = 10^{-3}$$

$$\text{For Tube D, the IDF} = \frac{1.0}{1.0 + 9.0} = \frac{1.0}{10.0} = 0.1 = 10^{-1}$$

Next determine the total dilution factor for the entire dilution series using the formula:

$$\text{TOTAL DILUTION FACTOR} = (\text{IDF}_A)(\text{IDF}_B)(\text{IDF}_C)(\text{IDF}_D)$$

For the dilution series above, the TDF for tube A =  $10^{-2}$

The TDF for Tube B =  $(10^{-2})(10^{-1}) = 10^{-3}$

The TDF for Tube C =  $(10^{-2})(10^{-1})(10^{-3}) = 10^{-6}$

The TDF for Tube D =  $(10^{-2})(10^{-1})(10^{-1}) = 10^{-7}$

We can assume that each colony of bacteria arose from one living (or viable) cell immobilized on an agar plate. Thus each colony is a clone of cells. We can now determine the number of live bacteria (or Colony Forming Units [CFU]) per ml of original culture by using the formula:

$$\text{CFU/ml} = \frac{\text{number of colonies per ml plated}}{\text{Total dilution factor}}$$

As plate E has 275 colonies, in the original culture:

$$\text{The CFU/ml} = \frac{275 \text{ colonies/ml plated}}{10^{-7}} = 275 \times 10^7 = 2.8 \times 10^9 \text{ CFU/ml}$$

Plate F has 28 colonies, but only 0.1 ml was plated:

$$\text{The CFU/ml} = \frac{(28 \text{ colonies} / 0.1 \text{ ml plated})}{10^{-7}} = 280 \times 10^7 = 2.8 \times 10^9 \text{ CFU/ml}$$

\*\*\* If you use these two formulae, you can solve any serial dilution problem.

To solve **TYPE TWO** problems, simply rearrange the formula above to solve for the total dilution factor:

$$\text{TOTAL DILUTION FACTOR} = \frac{\text{NUMBER OF COLONIES/ML PLATED}}{\text{CFU/ML}}$$

For example, if you want to have a plate with approximately 30 colonies on it and the original culture contains  $2.8 \times 10^9$  CFU/ml, plug these values into the rearranged equation:

$$\text{TOTAL DILUTION FACTOR} = \frac{30}{2.8 \times 10^9} = 1 \times 10^{-8}$$

An easy way to set up dilution series like this would be to use 4 tubes, each having an IDF of  $10^{-2}$ , i.e., transfer 0.1 ml into a tube containing 9.9 ml four times. Spread 1.0 ml on a plate and incubate.

#### SELF TEST

- \*\* How many colonies would you expect if you plated our 0.1 ml from Tube C?
- \*\* How many colonies would you expect if you plated out 1.0 ml from Tube C?
- \*\* IF the IDF for Tube A was  $10^{-3}$  and the IDF for Tube B was  $10^{-2}$ , what would be the TDF for Tube D?
- \*\* Starting with a culture that contains  $3 \times 10^6$  CFU/ml, devise a serial dilution scheme that would yield a plate with 120 colonies.