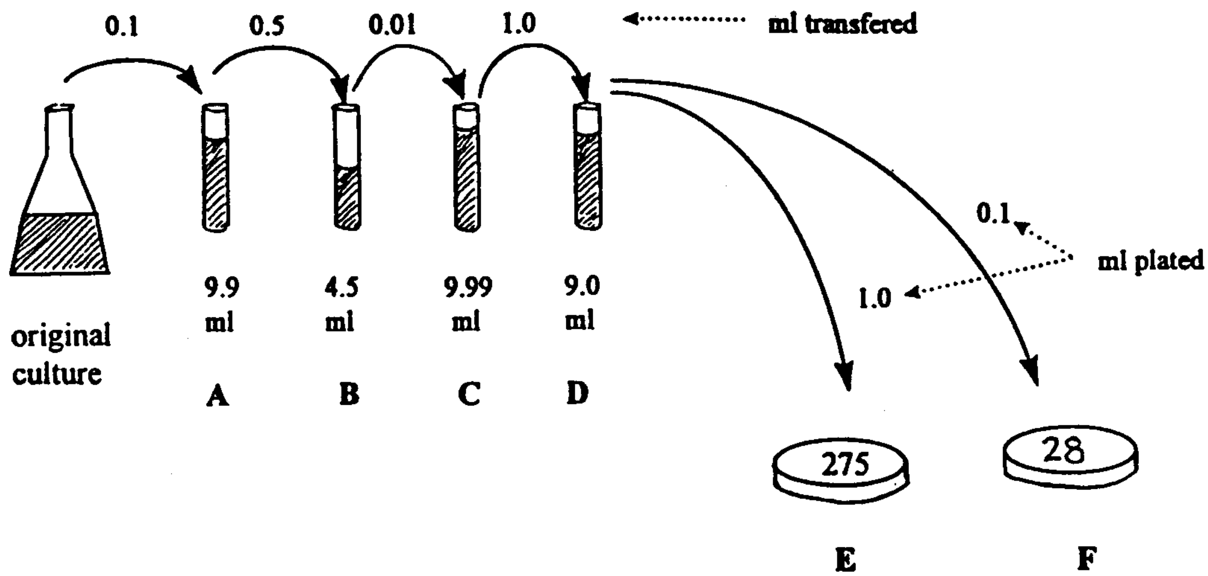
BACTERIAL DILUTIONS and A FOOL-PROOF WAY TO FIGURE THEM OUT

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:

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

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

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

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

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

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

$$\textbf{TOTAL DILUTION FACTOR} = (IDF_A)(IDF_B)(IDF_C)(IDF_D)$$

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 form 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 be using the formula:

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

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

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

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

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

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

For example, if you want to have a plate with approximately 30 colonies on it and the original culture contains 2.8×10^{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 form 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 3×10^{6} CFU/ml, devise a serial dilution scheme that would yield a plate with 120 colonies.