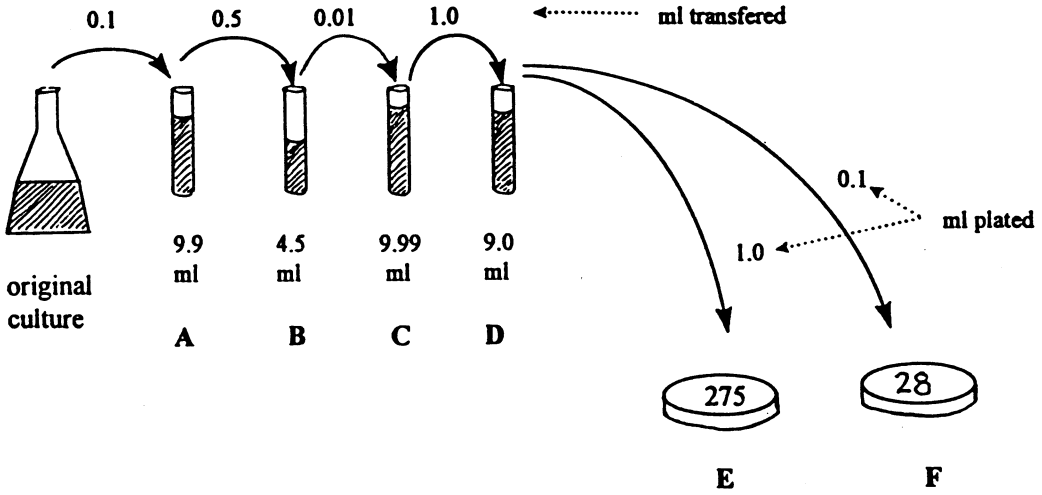
BACTERIAL DILUTIONS and A FOOL-PROOF WAY TO FIGURE THEM OUT

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 ORGINIAL 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:

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

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

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

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

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:

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

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** = $(10^{-2})(10^{-1})(10^{-3})(10^{-1}) = 10^{-7}$

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, the CFU/ml in the original culture = $\frac{275 \text{ colonies/ml plated}}{10^{-7}} = 275 \times 10^{7} = 2.8 \times 10^{9}$ CFU/ml

Plate **F** has 28 colonies, but only 0.1 ml was plated. The CFU/ml = $\frac{(28 \text{ colonies}/0.1 \text{ ml plated})(10)}{10^{-7}} = 280 \times 10^{7} = 2.8 \times 10^{9}$ 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 a 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 out 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 3×10^{6} CFU/ml, devise a serial dilution scheme that would yield a plate with 120 colonies.