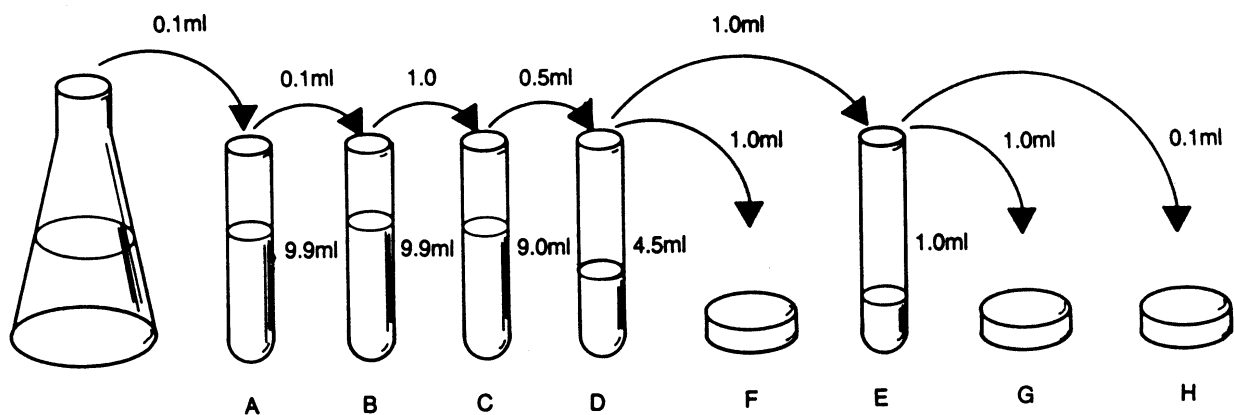


## DILUTION PROBLEMS

1. One ml of a sample was mixed with 99 ml of sterile diluent. One ml of this was transferred (using the pour plate method) to melted nutrient agar. Plates were poured and incubated at 37°C for 48 h. After incubation, 133 colonies were present on the plate. How many colony-forming units (CFUs) were present per ml in the original sample?
  
2. One ml of a sample was mixed with 99 ml of sterile diluent. One-tenth of a ml (0.1 ml) of this was plated (using the spread plate method) on nutrient agar. After incubation for 48 h, 69 colonies were present on the plate. How many colony-forming units (CFUs) were present per ml in the original sample?
  
3. Given the dilution series outlined below:



- A. Give the dilution used for each step (A-E).
  
- B. (i) What is the **total** dilution at each step (A-E)?  
 (ii) What is the resulting dilution found on each plate (F,G,H)?
  
- C. If the original broth culture contains  $3 \times 10^8$  cells  $\text{ml}^{-1}$ ,  
 (i) How many bacteria  $\text{ml}^{-1}$  are in each tube (A-E)?  
 (ii) How many colonies can be found on each plate (F,G,H)?  
 (iii) Which are the most reliable colony counts, those from plate F, G, or H?
  
- D. If tube C contains  $7 \times 10^4$  bacteria  $\text{ml}^{-1}$ , how many bacteria  $\text{ml}^{-1}$  were in the original culture **and** in each of the other tubes? How many colonies can be found on each plate?

4. An overnight culture of *Serratia marcescens* is used as a sample. One ml of the culture is added to a bottle containing 99 ml of culture media. This dilution is mixed well, and one ml of the diluted culture is mixed in 9 ml of culture media. This second dilution is diluted by three successive 1/10 dilutions. The last dilution is used for plating, and 0.1 ml is plated on nutrient agar. After incubation, 100 colonies are counted on the plate. How many colony-forming units (CFUs) were present per ml of *Serratia marcescens* culture?
5. Devise a scheme to prepare a  $10^{-6}$  dilution **on a plate** using the **least number** of sterile saline dilution tubes or bottles necessary. No scheme should use volumes greater than 100 ml or less than 0.1 ml.
6. You have been asked to generate the growth curve of a mutant *E. coli* strain. At the last time point on your growth curve, you plate 0.1 ml of a  $10^{-7}$  dilution. After incubation for 24 hours at 37°C, there are 38 colonies on your plate.
  - A. What was the number of CFUs  $\text{ml}^{-1}$  in the  $10^{-7}$  dilution tube?
  - B. What was the number of CFUs  $\text{ml}^{-1}$  in the original culture flask?