Appendix B

APPENDIX B

Dilution Techniques and Calculations*

Bacteria, under good growing conditions, will multiply into such large populations that it is often necessary to dilute them to isolate single colonies or to obtain estimates of their numbers. This requires mixing a small, accurately measured sample with a large volume of sterile water or saline, which is called the **diluent** or **dilution blank.** Accurate dilutions of a sample are obtained through the use of pipettes. For convenience, dilutions are usually made in multiples of 10.

A single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total (volume of the sample + the diluent)}}$$

For example, the dilution of 1 ml into 9 ml equals

$$\frac{1}{1+9}, \text{ which is } \frac{1}{10} \text{ and is written 1:10}$$

The same formula applies for all dilutions, regardless of the volumes. A dilution of 0.1 ml into 0.9 ml equals

$$\frac{0.1}{0.1+0.9}, \text{ which is } \frac{0.1}{1} = \frac{1}{10} = 1{:}10$$

A dilution of 0.5 ml into 4.5 ml equals

$$\frac{0.5}{0.5+4.5}, \text{ which is } \frac{0.5}{5.0} = \frac{1}{10} = 1{:}10$$

Experience has shown that better accuracy is obtained with very large dilutions if the total dilution is made out of a series of smaller dilutions rather than one large dilution. This series is called a **serial dilution,** and the total dilution is the product of each dilution in the series. For example, if 1 ml is diluted with 9 ml, and then 1 ml of that dilution is put into a second 9-ml diluent, the final dilution will be

$$\frac{1}{10} \times \frac{1}{10} = \frac{1}{100} \text{ or } 1{:}100$$

To facilitate calculations, the dilution is written in exponential notation. In the example above, the final dilution 1:100 would be written 10^{-2}. Remember,

$$1{:}100 = \frac{1}{100} = 0.01 = 10^{-2}$$

(See the section Exponents, Exponential Notation, and Logarithms, on page 418.) A serial dilution is illustrated in Figure B.1.

*Adapted from C. W. Brady. "Dilutions and Dilution Calculations." Unpublished paper. Whitewater, WI: University of Wisconsin, n.d.

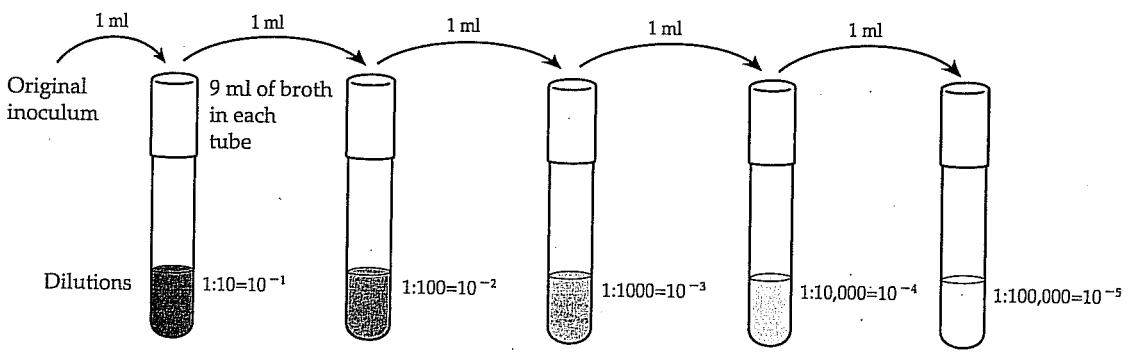


Figure B.1

A 1-ml sample from the first tube will contain 1/10 the number of cells present in 1 ml of the original sample. A 1-ml sample from the last tube will contain 1/100,000 the number of cells present in 1 ml of the original sample.

Twofold dilutions are commonly used to dilute patient's serum to measure antibodies. The same formula applies: a dilution of 100 µl of sample into 100 µl of saline equals

$$\frac{100}{100 + 100}, \text{ which is } \frac{100}{200} = \frac{1}{2} = 1{:}2$$

If 100 µl of this 1:2 dilution is put in 100 µl of saline, the final dilution is

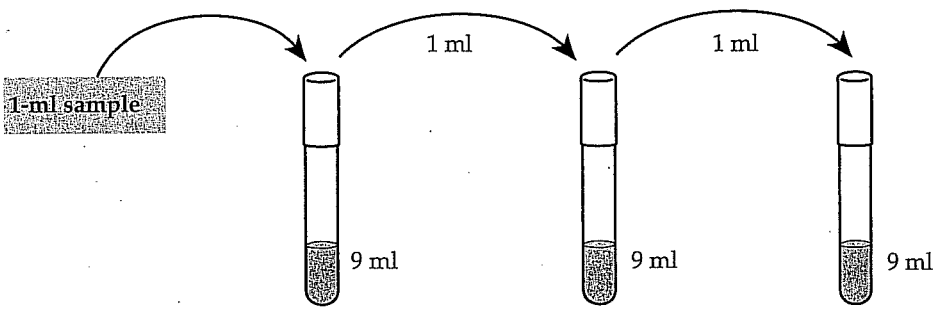
$$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$$

Procedure

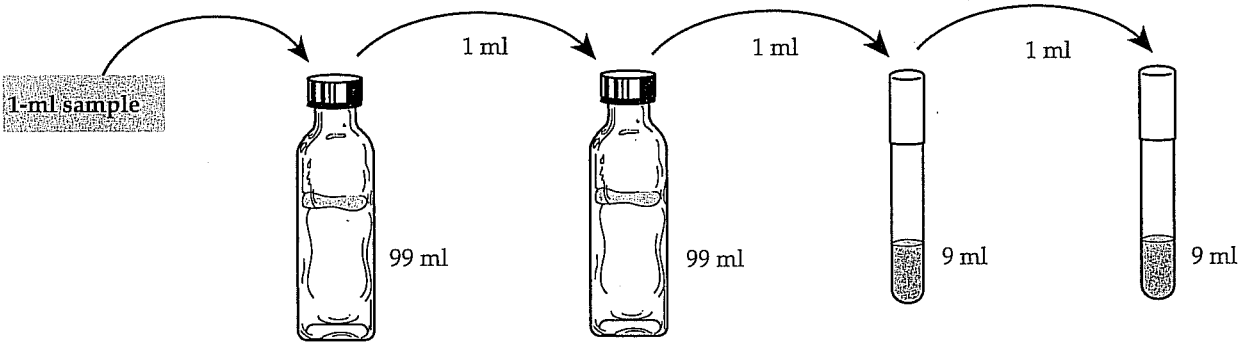
1. Aseptically pipette 1 ml of sample into a dilution blank.
   a. If the dilution is into a tube, mix the contents on a vortex mixer or by rolling the tube back and forth between your hands.
   b. If the dilution is into a 99-ml blank, hold the cap in place with your index finger and shake the bottle up and down through a 35-cm arc (see Figure 54.2).
2. It is necessary to use a fresh pipette for each dilution in a series, but it is permissible to use the same pipette to remove several samples from the same bottle, as when plating out samples from a series of dilutions.

Problems

Practice calculating serial dilutions using the following problems.

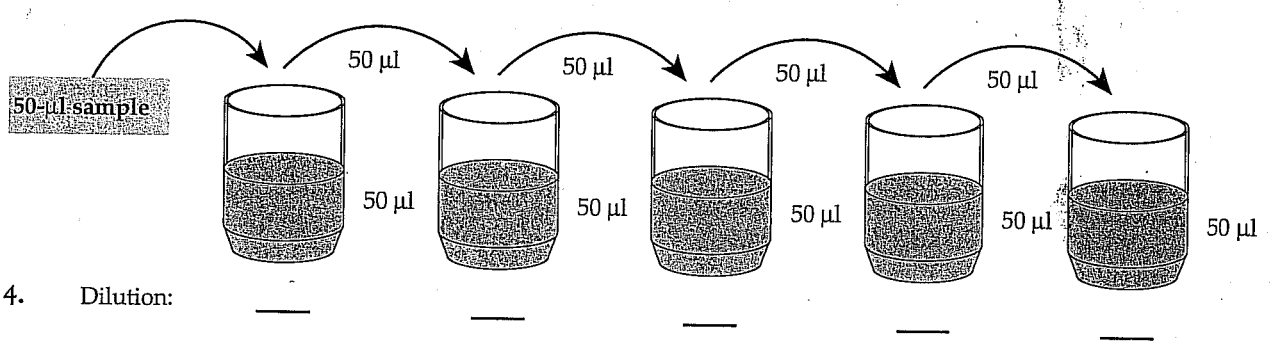


1. Dilution: ____ ____ ____

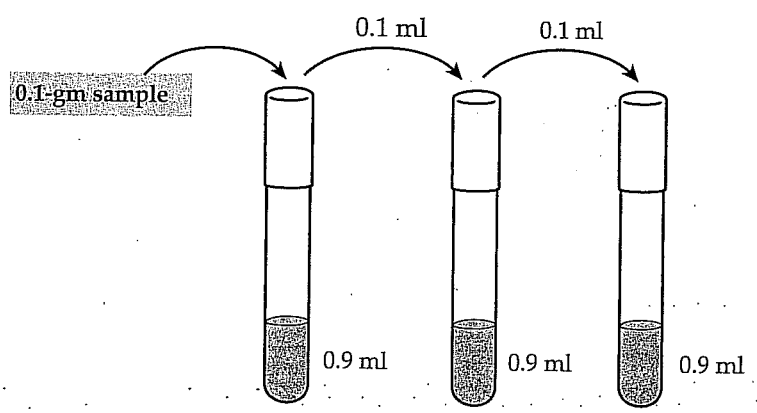


2. Dilution: ____ ____ ____ ____

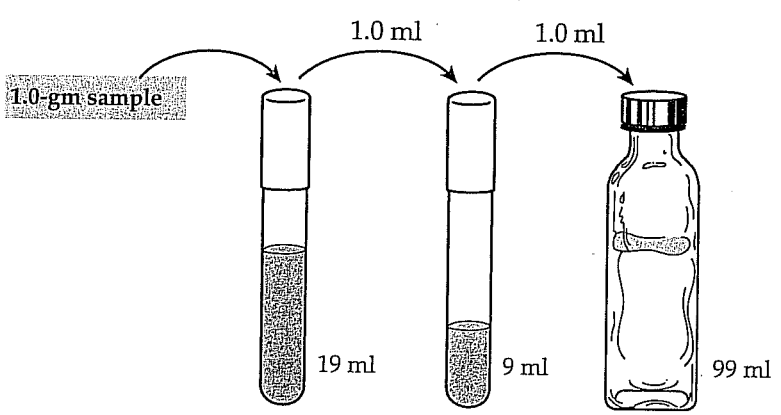
3. Design a serial dilution to achieve a final dilution of 10^{-8}.



4. Dilution: ___ ___ ___ ___ ___



5. Dilution: ___ ___ ___



6. Dilution: ___ ___ ___