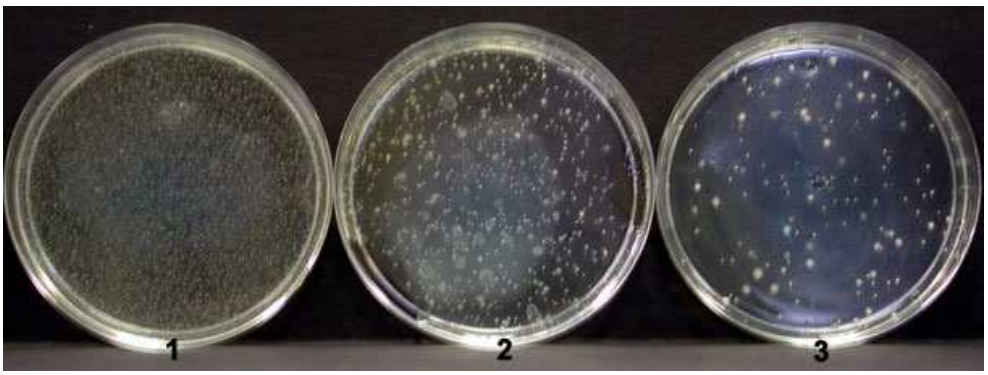
HOW TO SOLVE PRACTICAL ASPECTS OF MICROBIOLOGY

1. DILUTIONS AND CONCENTRATIONS. LIQUID AND SOLID SAMPLES



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1. DILUTIONS AND CONCENTRATIONS. LIQUID AND SOLID SAMPLES

In most environments, the microbial density is usually too high or too low to obtain good results in the enumeration of microorganisms by a direct culture of the sample. This situation requires the dilution or concentration of the sample prior to carry out any study. Additionally, solid samples must be diluted for an easier handling, in this way; they can be treated as liquid samples.

In most cases, we work with decimal dilutions. The simplest case is the preparation of 10 ml of the 1:10 dilution of the sample. For this, 1 ml of sample is added to 9 ml of diluent; consequently, in 10 ml of this 1:10 dilution, 1 ml corresponds to the sample. Expressing by an equation:

$$\text{1:10 dilution} = \frac{\text{1 ml of sample}}{\text{1 ml of sample + 9 ml of diluent}} \Rightarrow \text{10 ml of 1:10 dilution (or } 10^{-1} \text{ dilution)}$$

If a higher dilution of the sample is required, successive dilutions can be prepared. For example, if the 1:100 dilution is needed (or the 10^{-2} dilution), it can be prepared adding 1 ml of 1:10 dilution to 9 ml of diluent, according to the following equation:

$$\text{1:100 dilution} = \frac{\text{1 ml of } 10^{-1} \text{ dilution}}{\text{1 ml of } 10^{-1} \text{ dilution + 9 ml of diluent}} \Rightarrow \text{10 ml of 1:100 dilution } (10^{-2})$$

Or directly, adding 1 ml of sample to 99 ml of diluent:

$$\text{1:100 dilution} = \frac{\text{1 ml of sample}}{\text{1 ml of sample + 99 ml of diluent}} \Rightarrow \text{100 ml of 1:100 dilution } (10^{-2})$$

Or adding 0.1 ml of sample to 9.9 ml of diluent:

$$\text{1:100 dilution} = \frac{\text{0.1 ml of sample}}{\text{0.1 ml of sample + 9.9 ml of diluent}} \Rightarrow \text{10 ml of 1:100 dilution } (10^{-2})$$

Note: The final volume obtained is given by the denominator of the equation.

With these notions, you must propose solutions to the following problems:

1.1. How would you prepare 250 ml of a 10^{-1} dilution from a water sample?

1.2. How would you prepare 10 ml of a 10^{-5} dilution in 3 steps?

What if the sample is not liquid, for example, a food sample? In this case, we can consider that 1 gram of sample is equal to 1 ml:

$$\text{1:10 dilution} = \frac{\text{1 g of sample}}{\text{1 g of sample + 9 ml of diluent}} \Longrightarrow \text{10 ml of 1:10 dilution } (10^{-1})$$

1.3. 1.5 grams of food were added to 13.5 ml of Ringer Solution (diluent) and homogenized. What is the resultant dilution?

As previously indicated, sometimes, due to the low density of microorganisms, it is necessary to **concentrate** the sample by filtration or centrifugation. This fact requires for subsequent handling, the resuspension of the microbes retained in the filters or accumulated in the pellets in a given volume of diluent. In this case, it is necessary to determine the **concentration factor**. For example:

$$\text{Concentration factor} = \frac{\text{10 ml of filtered sample}}{\text{1 ml of diluent}} \Longrightarrow \text{1 ml 10X concentrate}$$

1.4. 100 ml of a water sample were filtered, and then the filter was suspended in 10 ml of saline solution and shaked vigorously. What is the concentration factor?

1.5. 100 ml of a dense microbial suspension were centrifuged at 5,000 rpm. The supernatant was removed and the pellet was resuspended after adding 2.5 ml of diluent. What is the concentration factor?

We must know not only how to dilute or concentrate a sample; we must also understand how to properly apply the **dilution or concentration factors** to determine the microbial density of a sample. Here are some problems based on the enumeration of microorganisms by the method of counting colony forming units (CFU).

$$\text{CFU/ml} = \frac{\text{A colonies}}{\text{B volume plated (ml)}} \text{ X Dilution Factor}$$

$$\text{CFU/ml} = \frac{\text{A colonies}}{\text{B volume plated (ml)}} \text{ X } \frac{1}{\text{Concentration Factor}}$$

1.6. We have prepared the followings dilutions from different sample: 1:10, 1:5, 1:4 and 1:2. Then, 0.1 ml of each dilution is plated. After incubation, in all cases 27 colonies grew in the plates, which are the microbial densities of the samples?

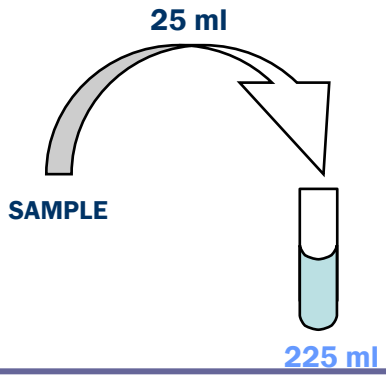
1.7. A food sample was processed following this protocol:

- 15 g of food were added to 135 ml of Ringer solution and homogenized.
- 2 ml of the suspension were mixed with 18 ml of diluent and homogenized.
- 0.2 ml were plated on nutrient agar and incubated.
- 100 colonies were counted in the plate.

From this information, how many microorganisms were present per gram of food?

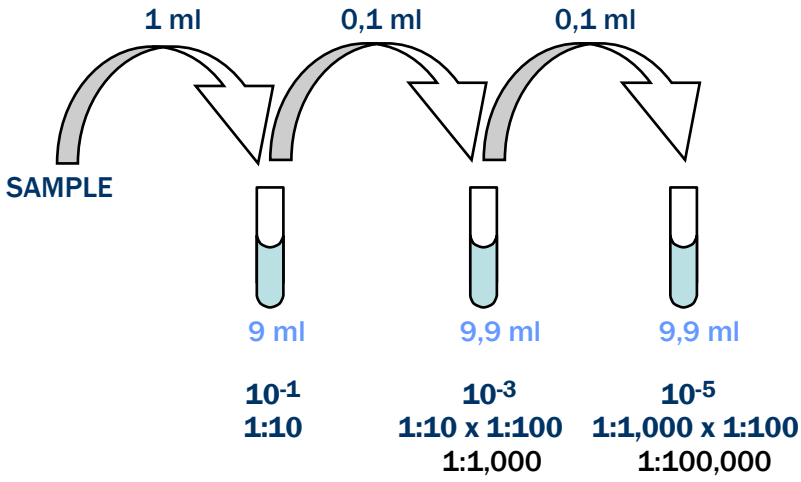
SOLUTIONS

1.1. 250 ml of a 10^{-1} dilution

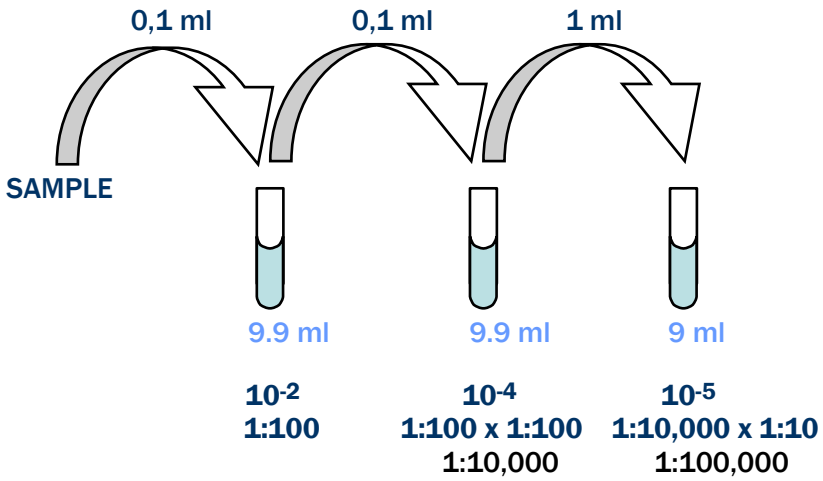


25 ml sample/(25 ml sample + 225 ml diluent)

1.2. 10 ml of a 10^{-5} dilution in 3 steps



There are other options, for example:



1.3. 1.5 g food + 13.5 ml diluent. Dilution?

1.5 g sample/(1.5 g sample + 13.5 ml diluent) = 1,5/15 = 1/10

1.4. 100 ml filtered and resuspended in 10 ml of diluent. What is the concentration factor?

100 ml sample/10 ml diluent = 10

1.5. 100 ml centrifugated and resuspended in 2.5 ml of diluent. Concentration factor?

100 ml sample/2.5 ml diluent = 40

1.6..1:10, 1:5, 1:4 and 1:2 dilutions. 1 plate/dilution. 0.1 ml/plate. 27 colonies/plate. CFU/ml?

$$\text{1:10 dilution: } \frac{27 \text{ colonies} \times 10^1}{0.1 \text{ ml plated}} = 2.7\ 10^3 \text{ CFU/ml}$$

$$\text{1:5 dilution: } \frac{27 \text{ colonies} \times 5}{0.1 \text{ ml plated}} = 1.35\ 10^3 \text{ CFU/ml}$$

$$\text{1:4 dilution: } \frac{27 \text{ colonies} \times 4}{0.1 \text{ ml plated}} = 1.08\ 10^3 \text{ CFU/ml}$$

$$\text{1:2 dilution: } \frac{27 \text{ colonies} \times 2}{0.1 \text{ ml plated}} = 5.4\ 10^2 \text{ CFU/ml}$$

1.7. 15 g sample + 135 ml diluent = 15:150 dilution = 1:10

2 ml 1:10 dilution + 18 ml diluent = 2 :20 dilution = 1 :10

Volume spread = 0.2 ml. Colonies/plate = 100 colonies. Microorganisms/g food?

$$\frac{100 \text{ colonies} \times 10 \times 10}{0.2 \text{ ml plated}} = 5\ 10^4 \text{ CFU/g}$$