Dilutions

Concentration = Particles/Volume

or, $\mathbf{C = P/V}$

For example, if 0.1 ml of culture is used for a spread plate and 150 colonies are counted:

C = 150 CFU/0.1 ml =1500 CFU/ml

Where: C = the concentration of colony forming units (CFU) in the culture plated
P = the number of CFU on the plate
V = the volume plated

However, what if the aliquot plated was from a 1/10 dilution of the culture? It seems obvious that the concentration of CFU in the original culture would be 10X higher than the value calculated above, or 15,000 CFU/ml. How can this type of relationship be expressed mathematically, in order to simplify making dilutions and performing calculations later?

If a suspension is diluted, the number of particles doesn't change:

$$\mathbf{P_1 = P_2}$$

but, since the volume increases, the concentration (P/V) decreases. Based on the equation for concentration above:

$$\mathbf{P = CV}$$

and,

$$\mathbf{C_1V_1 = C_2V_2}$$

That is, since the number of particles is the same in each sample, the concentration in each sample (expressed, for example as CFU/ml) times the volume (ml) would be the same.

To calculate dilutions:

$$Dilution = \frac{Volume\ added}{Total\ volume} = \frac{Volume\ added}{Volume\ of\ blank + Volume\ added}$$

For example, if a culture contains 1 x 10^9 cells/ml and 0.1 ml is added to 9.9 ml of a blank,

***Dilution* = 0.1ml/(9.9 ml + 0.1 ml) = 0.1 ml/10 ml = 0.01 or 1/100**

The **Dilution Factor (DF)** is the reciprocal of the dilution, or **1/dilution**.

The **Total Dilution** is the product of all dilutions:

$$D_{total} = D_1 \times D_2 \times D_3 \ldots$$

What does all this mean for spread plates and pour plates? The initial concentration, $\mathbf{C_1}$ (that is, the concentration of cells in the undiluted culture) is equal to $\mathbf{C_2}$ (the number of colonies/volume plated, or $\mathbf{P_2/V_2}$) x **DF**, or:

$$C_1 = C_2DF = (\frac{P_2}{V_2})DF$$

where C_1 = initial concentration (or concentration in the stock culture)
C_2 = CFU/ml (or the concentration of CFU on the plate)
P_2 = CFU on the plate
V_2= Volume plated (ml)
DF = Dilution Factor

Dilution Problems

1. If there are 2×10^{12} bacteria in 200 ml, what is the cell concentration?

2. If you have 175 ml of a crystal violet solution of 5 μg/ml, how much crystal violet do you have?

3. If you add 2 ml of a bacterial suspension with a concentration of 2×10^{9} cells/ml to 13 ml of water, what is the new cell concentration?

4. Diagram a scheme to make a 1:10,000 dilution

5. Diagram a scheme to make a 1:7.2 dilution

6. You perform the following series of dilutions: 1:10, 1:3, 1:2. What is your total dilution? What is the DF?

7. After diluting your culture 1:5000, you have a cell concentration of 2.3×10^{2} cells/ml. What was the initial concentration?

8. After diluting your culture 1:2500, you plate 0.1 ml and get 154 colonies. What was the initial concentration?

Dilution Problems (solutions)

1. If there are 2 x 10^{12} bacteria in 200 ml, what is the cell concentration?

$$C = \frac{P}{V} = \frac{(2 \times 10^{12}\ cells)}{(200ml)} = 1 \times 10^{10}\ cells\ /ml$$

2. If you have 175 ml of a crystal violet solution of 5 µg/ml, how much crystal violet do you have?

$$P = CV = (5\ \mu g\ /ml\ )(175ml\ ) = 875\ \mu g\ crystal\ violet$$

3. If you add 2 ml of a bacterial suspension with a concentration of 2 x 10^9 cells/ml to 13 ml of water, what is the new cell concentration?

$$C_1V_1 = C_2V_2$$

$$(2 \times 10^9\ cells\ /ml)(2\ ml) = C_2(15\ ml)$$

$$C_2 = \frac{4 \times 10^9\ cells}{15\ ml} = 2.67 \times 10^8\ cells\ /ml$$

4. Diagram a scheme to make a 1:10,000 dilution

$$\frac{1\ ml}{1\ ml + 9\ ml} \rightarrow \frac{1}{1+9} \rightarrow \frac{1}{1+9} \rightarrow \frac{1}{1+9}$$

$$\textit{Total Dilution}: \quad \frac{1}{10} \qquad \frac{1}{100} \qquad \frac{1}{1000} \qquad \frac{1}{10{,}000}$$

5. Diagram a scheme to make a 1:7.2 dilution

$$\frac{1\ ml}{1\ ml + 6.2\ ml} = \frac{1}{7.2}$$

6. You perform the following series of dilutions: 1:10, 1:3, 1:2. What is your total dilution? What is the DF?

$$Dil_t = \frac{1}{10} \times \frac{1}{3} \times \frac{1}{2} = \frac{1}{60}$$

$$DF = \frac{1}{dilution} = \frac{1}{(\frac{1}{60})} = 60$$

7. After diluting your culture 1:5000, you have a cell concentration of 2.3×10^2 cells/ml. What was the initial concentration?

$$C_1 = C_2 DF = (2.3 \times 10^2 \ cells/ml)(5000) = 1.15 \times 10^6 \ cells/ml$$

8. After diluting your culture 1:2500, you plate 0.1 ml and get 154 colonies. What was the initial concentration?

$$C = (\frac{P}{V})DF = (\frac{154 \ CFU}{0.1 \ ml})(2500) = 3.85 \times 10^6 \ CFU/ml$$

Dilution Problems (Set #2)

1. Diagram a scheme to make a 1:3500 dilution. (Just draw the dilutions, not the tubes.)

2. You dilute a culture of *Saccharomyces cerevisiae* 1:100,000 and plate 0.2 ml. After incubation, you count 60 colonies. What was the concentration of cells in the original culture?

3. A spread plate inoculated with 0.2 ml from a 10^{-8} dilution contained 90 colonies. Calculate the cell concentration of the original culture.

4. Your spread plates contain 23, 191, and 643 CFU for your 10^{-6}, 10^{-7}, and 10^{-8} dilutions, respectively. What was the concentration of cells in the original culture?

5. Starting with a stock bacterial culture with a **total** cell count of 6.2×10^{10} cells/ml, outline an experiment for performing a viable count using pour plates. Assume that at least 10% of the culture is still viable. Provide:

    a. a dilution scheme for the stock culture, including the volumes of cells and diluent used
    b. the dilutions used and the volume plated
    c. the rationale for using the dilutions selected

6. You decide to assay a stock solution of bacteria that is supposed to have 3.7×10^{8} cells/ml. In order to perform the assay, show:

    a. how you would dilute the stock (volumes of stock and diluent)
    b. the dilutions you would plate, as well as the volume plated
    c. the number of cfu expected

Dilution Problems (Set #2) **SOLUTIONS**

1. Diagram a scheme to make a 1:3500 dilution. (Just draw the dilutions, not the tubes.)

dilution	1/10	1/10	1/10	1/3.5	1/10 = 1 ml culture + 9 ml diluent 1/3.5 = 1 ml culture + 2.5 ml diluent
total dilution	10^{-1}	10^{-2}	10^{-3}	2.86×10^{-4} (or 1/3500)	
DF	10	100	1000	3500	

2. You dilute a culture of *Saccharomyces cerevisiae* 1:100,000 and plate 0.2 ml. After incubation, you count 60 colonies. What was the concentration of cells in the original culture?

$C_1 = C_2 \times DF = (P/V) \times DF = (60 \text{ cfu}/0.2 \text{ ml}) \times 100{,}000 = 3.0 \times 10^7 \text{ cfu/ml}$

3. A spread plate inoculated with 0.1 ml from a 10^{-8} dilution contained 90 colonies. Calculate the cell concentration of the original culture.

$C_1 = C_2 \times DF = (P/V) \times DF = (90 \text{ cfu}/0.1 \text{ ml}) \times 10^8 = 9.0 \times 10^{10} \text{ cfu/ml}$

4. Your spread plates contain 23, 191, and 643 CFU for your 10^{-6}, 10^{-7}, and 10^{-8} dilutions, respectively. What was the concentration of cells in the original culture?

First, select the proper plate to use. We want between 30 and 300 colonies/plate, so that would be the one with 191 colonies, which was inoculated from the 10^{-7} dilution. Since the volume plated wasn't given, we'll assume it was 0.1 ml, since that's most common with spread plates.

$C_1 = C_2 \times DF = (P/V) \times DF = (191 \text{ cfu}/0.1 \text{ ml}) \times 10^7 = 1.9 \times 10^{10} \text{ cfu/ml}$

5. Starting with a stock bacterial culture with a **total** cell count of 6.2×10^{10} cells/ml, outline an experiment for performing a viable count using pour plates. Assume that at least 10% of the culture is still viable. Provide:

   a. a dilution scheme for the stock culture, including the volumes of cells and diluent used
   b. the dilutions used and the volume plated
   c. the rationale for using the dilutions selected

We want 30-300 colonies/plate. With the total cell concentration given, 62 colonies is a good target number. If all of the cells are alive, the dilution that gives us 62 cfu in a 0.1 ml inoculum will have a concentration of 620 cfu/ml, since C = P/V = (62 cfu/0.1 ml).

C_1 (stock culture concentration) = C_2 (concentration in the dilution plated) x DF

DF = C_1/C_2 = $(6.2 \times 10^{10}$ cfu/ml)/(620 cfu/ml) = 1×10^8

Total dilution = 1/DF = 10^{-8}

a. A dilution scheme to give a total dilution of 10^{-8} could be 1:100 ➔ 1:100 ➔ 1:100 ➔ 1:10 ➔ 1:10.

b. Plate 0.1 ml (as used in the calculation above) from the 10^{-8} and 10^{-7} dilutions.

c. We use two dilutions as inocula because the cells may only be 10% viable. That is, if the cells are all alive, the 0.1 ml from the 10^{-8} dilution will contain 62 cfu. But, if it's only 10% viable, it will contain only 1/10 as many cfu, or only about 6 cfu. If that's the case, we protect ourselves (since we won't know how many cells are viable for a couple of days) by plating from the next lower dilution. Since that dilution is 10x more concentrated (or less dilute) it will have 10x more viable cells, or 62 cfu/0.1 ml.

6. You decide to assay a stock solution of bacteria that is supposed to have 3.7×10^8 cells/ml. In order to perform the assay, show:

   a. how you would dilute the stock (volumes of stock and diluent)
   b. the dilutions you would plate, as well as the volume plated
   c. the number of cfu expected

   We want 30-300 colonies/plate. We could try for 37, but that's too close to 30, so we'll double it and aim for 74 colonies/plate. We could adjust for that in our dilution scheme, but it's easier to just double our inoculum to the maximum permitted volume of 0.2 ml. That means C_2 = (74 cfu/0.2 ml) = 370 cfu/ml.

   DF = C_1/C_2 = $(3.7 \times 10^8$ cfu/ml)/$(3.7 \times 10^2$ cfu/ml) = 1×10^6 ; Total dilution = 10^{-6}

   a. For a total dilution of 10^{-6} we could do serial 1/10 dilutions = 1/10 ➔ 1/10 ➔ 1/10 ➔ 1/10 ➔1/10 ➔ 1/10

      We do 1/10 dilutions (instead of some 1/100) because we have no idea how many cells are viable and will need to plate from lots of dilutions in case viablility is very low. (Remember, with 3.7×10^8 cells/ml if even 0.0001% are alive, that's still 3.7×10^2 cells/ml.)

   b. As indicated above, since we don't know the concentration of viable cells, we plate from a number of dilutions in order to cover a wide range of possibilities. I would probably use every dilution, just to be safe. That would cover me from viable cell concentrations of 370 cfu/ml up through 3.7×10^8 cfu/ml.

   c. Since I would be plating 0.2 ml, I would expect 74 cfu from the 10^{-6} dilution if all cells were viable. As viability dropped, each lower dilution would compensate for decreases of 10%. As I stated above, by plating 0.2 ml from each dilution, I would be covered for 100% viability, down to 0.0001%.