Dilutions, Dilution Factors, proper use of $C_1V_1=C_2V_2$ and Serial Dilutions

Suppose we have a suspension of cells and the concentration is 6,000,000,000 cells/ml. That concentration of six billion cells/ml can be more conveniently written in scientific notation as 6×10^9 cells per ml. Lets designate this the *initial concentration* of cells in the *original* suspension.

Now lets take 1 ml of the original suspension, add it to 9 ml of water and mix thoroughly. **What is the total volume of this dilution after mixing?** We have 1 ml added to 9 ml for a total of 10 ml.

What is the *dilution* of the original suspension after mixing?
The *dilution* is the volume of the material being diluted (1 ml in this case) divided by the total volume after mixing (1 + 9 ml in this case).

1 ml of the original suspension is now in a total of 10 ml, so the dilution is 1/10. 1/10 can be written in decimal form as 0.1 or in exponential form as 10^{-1} .

What is the *dilution factor* for this dilution?
The dilution factor (DF) is the *reciprocal* of the dilution:

	Written as a Fraction	Written as a Decimal	Written in Exponential Notation
Dilution	1/10	0.1	10^{-1}
Dilution Factor	10	10	10^{1}

The dilution factor is 10 or 10^1 . We commonly say that for this step we have made a ten-fold dilution.

What is the new concentration of cells in the diluted sample?
Divide the initial concentration by the dilution factor (DF), which in this case is 10.

[Alternatively you can multiply the initial concentration by the dilution, which in this case is 1/10, but for our purposes we will stick with using the DF]

$$\frac{6 \times 10^9 \text{ cells per ml}}{\text{DF}} = \frac{6 \times 10^9 \text{ cells per ml}}{10} = 6 \times 10^8 \text{ cells per ml}$$

$$\frac{\textbf{Concentration before dilution}}{\textbf{DF}} = \textbf{Concentration after dilution}$$

Now lets make a 10-fold dilution of the previous dilution.

We take 1 ml of the previous dilution (6 X 10^8 cells per ml), add it to 9 ml of water and mix thoroughly.

What is the total volume of this dilution? We have 1 ml added to 9 ml for a total of 10 ml.

What is the dilution that has been made in this step?

$$\frac{1\ \text{ml}}{1\ \text{ml} + 9\ \text{ml}} = \frac{1}{10} = 10^{-1}$$

What is the dilution factor of just this dilution step?
The DF for this step is the reciprocal of the dilution for this step (1/10) so the DF for this step is 10.

What is the total dilution of the original suspension now that we have done a series of two 10-fold dilutions?

First Dilution	X	Second Dilution	=	Total Dilution	
1/10	X	1/10	=	1/100	written in fractional notation
10^{-1}	X	10^{-1}	=	10^{-2}	written in exponential notation

What is the total dilution factor for both steps?
The DF is the reciprocal of the total dilution (1/100 or 10^{-2}) so the total DF for both steps is 100 or 10^{2}.

We have made a series of two 10-fold dilutions. If the process was repeated a few more times with each new dilution being 1/10 of the previous dilution (the DF of each step was 10), we would have made what is termed a “Ten-Fold Dilution Series”.

Lets figure the dilutions and DF for a ten-fold dilution series when 3 dilution steps are made:

Ten-Fold Dilution Series

Step 1 (First Dilution)		Step 2 (Second Dilution)		Step3 (Third Dilution)		**Total Dilution**	**Total DF**
1/10	X	1/10	X	1/10	=	1/1000	1000
10^{-1}	X	10^{-1}	X	10^{-1}	=	10^{-3}	10^{3}

After a few 10-fold dilutions the use of exponential notation is much more convenient and more legible.

The initial concentration of the original suspension was 6×10^9 cells per ml. After the first dilution, the concentration of cells in the dilution tube was 6×10^8 cells per ml. We obtained this value by dividing the initial concentration of the original suspension by the DF of 10.

What would be the concentration of cells in the dilution tube after the third 10-fold dilution step? The total DF after the third dilution step is 10^3 . So the concentration of cells after the second dilution would be obtained by dividing the initial concentration of the original suspension by the total DF of 10^3 :

$$\frac{6 \times 10^9 \text{ cells per ml}}{\text{total DF}} = \frac{6 \times 10^9 \text{ cells per ml}}{1000} = 6 \times 10^6 \text{ cells per ml}$$

-or-

$$\frac{6 \times 10^9 \text{ cells per ml}}{\text{total DF}} = \frac{6 \times 10^9 \text{ cells per ml}}{10^3} = 6 \times 10^6 \text{ cells per ml}$$

$$\frac{\textbf{Concentration before dilution}}{\textbf{total DF}} = \textbf{Concentration after dilution}$$

[A quick review of exponential math is appropriate here: When multiplying numbers written in exponential form, add the exponents. When dividing numbers written in exponential form, subtract exponent of the denominator from the exponent of the numerator → as long as the same base (e.g. 10) is used.

When adding or subtracting exponential numbers make sure that all numbers must first be written so that they have the same exponent.

For example ($7.2 \times 10^{8)}$ + (4.3×10^5) should be rewritten as ($7.2 \times 10^{8)}$ + (0.0043×10^8) [same exponents] and the result would be 7.2043×10^8 .

-or-

(7.2×10^{8}) + (4.3×10^{5}) could be rewritten as (7200×10^{5}) + (4.3×10^{5}) [same exponents] and the result would be 7204.3×10^{5} which in correct scientific notation would be written as 7.2043×10^{8} .]

[Quick review of negative number math: subtracting a negative number is the same as adding the positive number.]

What would be the concentration of cells in the dilution tube after the fourth 10-fold dilution? The total DF after the fourth dilution step is 10^{4} . So the concentration of cells after the second dilution would be obtained by dividing the initial concentration of the original suspension by the total DF of 10^{4} :

$$\frac{6 \times 10^{9} \text{ cells per ml}}{\text{total DF}} = \frac{6 \times 10^{9} \text{ cells per ml}}{10^{4}} = 6 \times 10^{5} \text{ cells per ml}$$

Note: if the initial concentration of a sample is either known or expected to be high based on past experience, larger dilutions may be made for the first few steps. For example adding 1 ml to 99 ml of diluent results in a 10^{-2} dilution. Taking 1 ml of the thoroughly mixed 10^{-2} dilution and adding it to another 99 ml of diluent results in a 10^{-4} dilution that has been made in just two steps, saving some time. Then a series of smaller dilutions (e.g. 10-fold) may be made. In another example, taking 0.1 ml of sample and adding it to 99.9 ml of diluent results in a 10^{-3} dilution. Depending on the sample and the types of pipettes and glassware available, several different volumes and diluents can be used for a dilution series. For example, 0.5 ml added to 4.5 ml (5 ml total volume) is also a 10^{-1} dilution; 0.1 ml added to 9.9 ml (10 ml total volume) results in a 10^{-2} dilution, etc. Once the dilution is known, the DF can be determined (DF is the reciprocal of the dilution).

The use of Dilution Factors is an important concept in dealing with concentrations. As we have seen the DF can be used to calculate the final concentration resulting from the dilution of an initial concentration. If the concentration of cells after making a series of dilution can be determined, the DF can be used to figure the initial concentration of the original undiluted material.

Concentration before dilution = Concentration after dilution X total DF

This calculation is often used in plate counting procedures where the concentration of cells after making a series of dilutions is determined by plating known volumes of some of the dilutions onto agar plates and counting the colonies (colony forming units) that develop on the plates. Once the concentration of colony-forming units (CFU) per ml is determined for a particular dilution, the DF can then be used to determine the initial concentration of CFU per ml in the original undiluted material.

The DF can be determined and then used to figure the amount of a concentrated chemical stock solution needed to come up with a particular volume of less concentrated material:

$$\frac{\textbf{Concentration before dilution}}{\textbf{Concentration after dilution}} = \textbf{DF}$$

-or-

$$\frac{\textbf{Concentration of chemical in stock solution before dilution}}{\textbf{Desired concentration of chemical after dilution}} = \textbf{DF}$$

You may have learned the same principle with the following equation:

$$C_1V_1 = C_2V_2 \quad \text{or} \quad \frac{V_1}{V_2} = \frac{C_2}{C_1}$$

where C_1 is the initial concentration of the undiluted material, V_1 is the volume of the undiluted material to be used in the dilution, C_2 is the desired final concentration and V_2 is the total volume after the dilution has been made..

The dilution to be made is V_1 / V_2 and the DF is the reciprocal of the dilution, so DF = V_2 / V_1 .

By simple algebra you can determine that the DF is also equal to C_1 / C_2

The above set of equations is to convince you that the use of dilution factors or the $C_1V_1 = C_2V_2$ system are the same *as long as the definitions of C_1, V_1, C_2, and V_2 are properly used*.

Many people find use of the DF to be easier, more intuitive and resulting in fewer calculation errors. If you use $C_1V_1 = C_2V_2$ keep in mind that V_2 is the ***total volume*** after the dilution is made – it does not directly give you the volume of diluent to use. For example, if 1 ml of material is added to 99 ml of diluent, V_2 is 100 ml, ***not*** 99 ml.

In addition to a ten-fold dilution series, a two-fold dilution series and a three-fold dilutions series are commonly used in biological analyses. Lets figure the dilutions and DF's for a two-fold dilution series and a three-fold dilutions series when 3 dilution steps are made:

Two-Fold Dilution Series

Step 1 (First Dilution)	X	Step 2 (Second Dilution)	X	Step3 (Third Dilution)	=	**Total Dilution**	**Total DF**
1/2	X	1/2	X	1/2	=	1/8	8

Three-Fold Dilution Series

<u>Step 1</u> (First Dilution)	X	<u>Step 2</u> (Second Dilution)	X	<u>Step3</u> (Third Dilution)	=	**Total Dilution**	**Total DF**
1/3	X	1/3	X	1/3	=	1/27	27

Summary of dilutions, dilution factors, total dilutions and total dilution factors for a number of steps in commonly used dilution series.

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8	Step 9
Ten-Fold Dilution Series									
Dilution of individual step	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}
DF of individual step	10^{1}	10^{1}	10^{1}	10^{1}	10^{1}	10^{1}	10^{1}	10^{1}	10^{1}
Total Dilution after step	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Total DF after step	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}	10^{6}	10^{7}	10^{8}	10^{9}
Two-Fold Dilution Series									
Dilution of individual step	½	½	½	½	½	½	½	½	½
DF of individual step	2	2	2	2	2	2	2	2	2
Total Dilution after step	½	¼	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Total DF after step	2	4	8	16	32	64	128	256	512
Three-Fold Dilution Series									
Dilution of individual step	1/3	1/3	1/3	1/3	1/3	1/3			
DF of individual step	3	3	3	3	3	3			
Total Dilution after step	1/3	1/9	1/27	1/81	1/243	1/729	… etc.		
Total DF after this step	3	9	27	81	243	729			