Dilutions

Dilutions can be divided into five categories:

1. To dilute a solution to an unspecified final volume in just one step.
2. To dilute a solution to a specified final volume in just one step.
3. To dilute a solution to an unspecified final volume in several steps.
4. To dilute a solution to a specified final volume in several steps.
5. Serial dilutions.

Diluting a Solution to an Unspecified Volume in Just One Step

One must first calculate how many times to dilute (this is called the dilution factor) the initial material (stock solution) to obtain the final concentration. To accomplish this type of dilution, use the following formula:

$$\frac{IC}{FC} = DF\ ,$$

Where IC is the initial concentration, FC is the final concentration, and DF is the dilution factor.

For example, if you want to dilute a solution with an initial concentration of solute of 5% down to 1%, using the above formula gives

$$\frac{IC}{FC} = DF \quad = \quad \frac{5\%}{1\%} = 5$$

Thus, in order to obtain a 1% solution from a 5% solution, the latter must be diluted 5 times. This can be accomplished by taking one volume (e.g., cc, ml, liter, gallon) of the initial concentration (5%) and adding 4 volumes (e.g., cc, ml, liter, gallon) of solvent for a total of five volumes. Stated another way, 1 ml of a 5% solution +4 ml of diluent will give a total of 5 ml, and each ml contains 1% instead of 5%.

Diluting a Solution to a Specffied Volume in Just One Step

First, calculate the number of times the initial concentration must be diluted by dividing the final concentration (FC) into the initial concentration (IC).

Second, divide the number of times the initial concentration must be diluted into the final volume specified to determine the aliquot (or portion) of the initial concentration to be diluted.

Third, dilute the aliquot of the initial concentration calculated in step 2 by the volume specified.

For example, you have a 10% solution and want a 2% solution. However, you need 100 ml of this 2% solution.

$$\frac{IC}{FC} = DF = \quad \frac{10\%}{2\%} = 5$$

Divide the number of times the 10% solution must be diluted (DF) into the final volume specified:

$$\frac{100ml}{5} = 20ml$$

Dilute the portion of 10 to the volume specified:

20 ml of a 10% solution + 80 ml of diluent = 100ml (each milliliter = 2%)

Another method for performing this type of dilution is to use the following formula:

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

Where:

C_1 = Initial concentration or standard concentration available

C_2 = Final concentration or standard concentration desired

V_1 = Initial volume or volume of C_1 required to make the new concentration

V_2 = Final (total) volume of desired concentration.

For example, if you want to prepare 100 ml of 10% ethyl from 95% ethyl alcohol, then

C_1 = 95%, C_2 = 10%

V_2 = 100 ml, V_1 = X

So,

$$C_1V_1 = C_2V_2 = (95)(X)=(10)(100) = 10.5 \text{ ml}$$

Thus, 10.5 ml of 95% ethyl alcohol + 89.5 ml of H_2O= 100 ml of a 10% ethyl alcohol solution.

Diluting a Solution to an Unspecified Volume in Several Steps

Frequently in the microbiology laboratory, large dilutions must be employed. They cannot be done in one step because they are too large. As a result, they must be done in several steps to conserve not only amounts of diluent to be used but also space. For example, a 0.5 g/ml solution diluted to 1 µg/ml is a 500,000-fold dilution.

$$0.5 \text{ g} = 0.5 \text{ g} \times 10^{\wedge 6} \text{ µg/g}$$

$$= 500{,}000 \text{ µg}$$

To obtain a solution containing 500,000 µg/ml in one step would require taking 1 ml of 0.5 gm/ml stock solution and adding 499,999 ml of diluent. As you can see, it would be almost impossible to work with such a large fluid volume.

A 500,000 times dilution can be easily performed in two steps by first taking 1 ml of the initial concentration and diluting it to 500 ml and second, by diluting 1 ml of the first dilution to 1,000 ml.

1 ml of 500,000 µg/ml + 499 ml of diluent = 1,000 µg/ml

1 ml of 1,000 µg/ml + 999 ml of diluent = 1 µg/ml

Thus, by this two-step procedure, we have cut down the volume of diluent used from 499,999 to 1,498 (499 ml + 999 ml).

Diluting a Solution to a Specified Volume in Several Steps

This type of dilution is identical to all previous dilutions with the exception that the specified final volume must be one factor of the total dilution ratio.

For example, you want a 1/10,000 dilution of whole serum (undiluted) and you need 50 ml.

Divide dilution needed by the volume:

$$\frac{10{,}000}{50} = 200$$

200 (1/200 dilution) = the first step in the dilution factor; the second is 1/50, obtained as follows:

1 ml of serum + 199 ml of diluent = 1/200 dilution.

1 ml of 1/200 dilution + 49 ml of diluent = 1/50.

To check: 50 x 200 = 10,000.

Serial Dilutions

The usefulness of dilutions becomes most apparent when small volumes of a material are required in serological procedures. For example, if 0.01 ml of serum were required in a certain test, instead of measuring out this small volume with a consequent sacrifice of accuracy, it would be more advantageous to dilute the serum 100 times. One ml of this 1/100 dilution would then contain 0.01 ml of the serum. Each ml of this dilution would be equivalent to the required 0.01 ml of undiluted serum.

Dilutions represent fractional amounts of a material and are generally expressed as the ratio of one volume of material to the final volume of the dilution. Thus, a 1/10 dilution of serum represents 1 volume of serum in 10 volumes of dilution (1 volume of serum + 9 volumes of diluent). Undiluted serum may be expressed as 1/1.

- 1 ml of serum + 1 ml of saline may be expressed as 1/2. Each milliliter of this dilution is equivalent to 0.5 ml of undiluted serum.
- 1 ml of serum + 2 ml of saline may be expressed as 1/3. Each milliliter of this dilution is equivalent to 0.33 ml of undiluted serum.
- 1 ml of serum + 99 ml of saline may be expressed as 1/100. Each milliliter of this dilution is equivalent to 0.01 ml of undiluted serum.

From the above, one can see that dilution expressions are fractions written as ratios where the numerator is unity and the denominator is the dilution value.

Division of the numerator by the denominator will give the amount of material per milliliter of the dilution. For example, in a 1/25 dilution, 1/25 = 0.04. Therefore, each milliliter of this dilution contains 1/25 or 0.04 ml of the original material (e.g., serum).

To convert a ratio into a dilution expression, divide both the numerator and denominator by the value of the numerator. For example, in a mixture consisting of 4 ml of serum and 6 ml of saline,

4 ml of serum + 6 ml of saline = 10 ml of serum dilution

Ratio of serum dilution = 4/10.

Dividing both numerator and denominator by the numerator value (4),

$\frac{4 \div 4}{10 \div 4} = 1/2.5$ = serum dilution.

In the preparation of dilutions, any multiple or submultiple of the constituent volumes may be used. For example,

1/30 dilution = 1 ml serum + 29 ml saline

= 0.5 ml serum + 14.5 ml saline

= 2 ml serum + 58ml saline

Serial dilutions indicate that an identical volume of material is being transferred from one vessel to another. The purpose of this procedure is to increase the dilutions of a substance by certain increments. For example, in a twofold dilution, the dilution factor is doubled each time (e.g., ½, ¼, etc.)