DILUTIONS

Occasionally a solution is too concentrated to be used as is. For example, when one is performing manual blood counts, the blood contains too many cells to be counted as such. Or when performing a given test, it may be found that the concentration of the substance being measured is too high for measurement with a certain instrument. In both of the above cases, a dilution is necessary.

Laboratory procedures in which an amount of one substance is added to another to reduce the concentration of one of the substances are referred to as dilutions. A dilution usually means the volume of original substance in the total volume of final solution.

In dilution statements, the smaller number is the number of parts of the substance that is being diluted; the larger number refers to the total number of parts in the final solution unless explicitly stated otherwise. All of the following statements mean the same thing:

1. Make a 1 to 10 dilution of serum in saline.
2. Make a 1 in 10 dilution of serum in saline.
3. Make a 1 to 10 dilution of serum with saline.
4. Make a 1/10 dilution of serum with saline.
5. Dilute the serum 10 times with saline
6. Make a dilution of 1 part serum and 9 parts saline.
7. Make a dilution of 1 part serum to 9 parts saline.
8. Make a 1:9 dilution of serum and saline.

The first five statements are symbolically shown as "1/10" dilutions and the last three are shown as "1:9" dilutions. Terms such as "1/10", "1/100", "1/500", etc. are called dilution factors. In each of the preceding dilutions, the numerator, 1, refers to the number of parts of serum and the denominators, 10, 100, 500, refer to the number of total parts of each dilution. Saline is called “diluent” in the above statements.

Other variations can be made in the preceding statements, and the meaning is still retained. In all such statements, the directions can be followed by combining 1 ml of serum with 9 ml of saline to make 10 ml of the final solution, by adding 2 oz. of serum to 18 oz. saline, etc. In all cases, there is 1 part of the original substance in 10 parts of the final solution.

$$\frac{\text{1 part of the concentrated material}}{\text{+ 9 parts diluent}}$$
10 parts final solution

Example: 5 ml of serum is diluted to 25 ml with saline. What is the serum dilution?

Set up the problem as 5 ml serum + X ml saline = 25 ml of the final solution.

$$X = 25 - 5$$
$$X = 20 \text{ ml of saline}$$

The serum dilution is the amount of serum in the amount of total solution; hence, this is a 5/25 serum dilution which would equal a 1/5 dilution.

Titer

The titer is the smallest amount or concentration that will produce a particular effect or endpoint.

Example: A series of solutions of serum in saline is prepared in the following dilutions: 1/2, 1/4, 1/8, and 1/16. A test for the presence of an antibody was made on each solution. It was positive for the 1/2 and 1/4 dilutions but negative for the 1/8 and 1/16 dilutions. The titer of the antibody is said to be 1/4.

Series of Dilutions

Two important points should be considered in dilutions: how each dilution is made and what each dilution contains. The important consideration is the concentration of the materials in the dilution. However, it is first necessary to know how a dilution was made. A general rule to use in calculating the concentration of solutions in a series is to multiply the original concentration by the first dilution factor, this by the second dilution factor, this by the third dilution factor, and so on until the final concentration is known.

Example: A 5M solution of HCl is diluted 1/5. The resulting solution is diluted 1/10. Determine the concentration of each of the solutions.

In this example, one solution is made from a solution that was yet made from another. To calculate the concentration of any of the solutions in such a series, first express all dilutions as a fraction, then multiply the concentration of the beginning solution by the dilution factor used in each succeeding step. The following steps are used in solving this example.

1. The concentration of the first solution is given as 5M HCl. This then is the first answer.

2. The second solution was made by a 1/5 dilution of the first solution. The concentration of HCl in the second solution would be calculated by multiplying the concentration of the first solution by the dilution factor used to produce the second solution. Hence, the second solution has an HCl concentration of 1M.

$$5M\ HCl \times 1/5 = 5M/5 = 1M$$

3. To calculate the concentration of HCl in the third solution in the series, multiply the original concentration of HCl by the value of each succeeding dilution factor.

$$5M \times 1/5 \times 1/10 = 5M/50 = 1M/10$$

Therefore, the concentration of HCl in the last solution is 0.1M.

Notice that in each solution of this problem the concentration is expressed in molarity. This is because the concentration of the original solution was measured in molarity. In this type of problem the concentration of each dilution will be expressed in the same unit as that used in the original solution.

The calculations used to determine the concentration of each solution in a series may be used in reverse to produce a dilution series having prescribed concentrations at each step.

Example: Make the following dilutions of serum in buffer: 1/10, 1/100, and 1/500.

Before attempting to solve such a problem, it should be mentioned that any one dilution could usually be made by several procedures depending on how large a volume of the final solution is needed and how much of the original solution (to be diluted) is available. For example, in the problem above, we may need only 10 ml of the 1/500 dilution, so if we take 1 ml of the original serum and add 499 ml of buffer, we will be left with a large volume of a solution, most of which is of no use to us. Additionally, storage space is a precious commodity to many research and health workers. Now back to solving our original problem.

1. To make the first dilution, place 1 part serum in a vessel and bring the total volume up to 10 parts total. This is a 1 to 10 dilution of serum in buffer. The "parts" can mean any multiple or fraction of any unit of measurement. For example, let 1 part equal 1 ml. This means that 1 ml of serum was brought up to 10 ml total with buffer. One could let 1 part equal 0.5 ml, 0.001 ml, 1 liter, etc., as long as all related calculations are based on the same thing.

2. To make the 1/100 serum in buffer dilution, use the first dilution as a starting point and repeat the procedure in step 1 above; i.e., place 1 part of the 1/10 diluted serum in a vessel and bring the volume up to 10 parts total.

If a 1/10 dilution was not prepared ahead of time, one could make the 1/100 dilution directly, provided sufficient buffer was available. In this case, 1 part of the undiluted serum would need to be mixed with 99 parts of the buffer in the vessel.

3. To make the 1/500 dilution of serum in buffer, the easiest way is to make a 1/5 dilution of the 1/100 dilution that was already prepared; i.e., bring 1 part of the 1/100 dilution of serum in buffer up to 5 parts total volume. Thus:

$$1/100 \times 1/5 = 1/500$$

Serial Dilutions

Many procedures call for a dilution series in which all dilutions after the first one are the same. This type of dilution series is referred to as a serial dilution. This method and calculations discussed here are used in producing a series of solutions having equal increments of dilution. Note that "serial dilution" is a special case of "series of dilutions".

Example: A serum sample is diluted twice with buffer. A series of five dilutions is made of this first dilution by diluting it 1/10, rediluting 1/10, and then three times more, each resulting solution then being a 1/10 dilution of the previous one in the series. The concentration of serum in each solution is as follows: 1/2, 1/20, 1/200, 1/2,000, 1/20,000, and 1/200,000.

Example: The instructions indicate that a 1/10,000 dilution of a stock solution is to be made in water. This means 1 part of stock solution and 9,999 parts of water. If you equate 1 part as 1 ml, this would mean that we would end up with 10,000 ml (= 10 liters) of the diluted solution which is quite a large volume. Most probably, you may not have enough shelf space to store this amount. Furthermore, you may actually need only a few mls of the diluted solution. So we have to use some other way of doing the dilution without ending up with such surplus. One quick, economical and efficient way is as follows:

$$1 \text{ ml stock}/10 \text{ ml water} \times 1/10 \times 1/10 \times 1/10 = 1/10{,}000 \text{ dilution}$$

This means taking only 1 ml of the original stock and diluting it 1/10 four times. This would produce 10 ml of a 1/10,000 dilution of stock in water.

Another way is to dilute the stock 1/10 twice and then perform a further 1/100 dilution:

1/10 x 1/10 x 1/100 = 1/10,000 dilution

This would yield 100 ml of a 1/10,000 dilution of stock in water.

Yet another way is to make a 1/100 dilution twice:

1/100 x 1/100 = 1/10,000 dilution

This would produce 100 ml of a 1/10,000 dilution of stock in water.

Any combination of dilutions that will yield a final concentration of 1/10,000 may be used. As mentioned earlier, the combination is determined in part by the glassware available and the volume needed.

Determining Volumes and Concentrations

To decide what dilution to use, one needs to know several things:

1. Original concentration of the substance being diluted.
2. Final volume desired.
3. Final concentration desired.
4. Number of dilutions to be made (at times).

Example: A 1/200 stock solution of boric acid is on hand. The procedure requires 50 ml of a 1/500 solution. How would the necessary amount be made without making excess?

Going from 1/200 to 1/500 produces a 2/5 ratio. This ratio is found by X below:

(1/200) X = (1/500)

X = (200/500) = 2/5

This means taking 2 parts of the original boric acid solution and bringing up the volume to 5 parts. So, if we need 50 parts (1 part = 1 ml), we should take 20 ml of the stock.

A quick way to do problems of this sort is to use the famous formula:

$$C_1 V_1 = C_2 V_2$$

where C_1 and C2 are concentrations of solutions 1 and 2 and V_1 and V_2 are their respective volumes. If we fill the parts for the problem at hand, we will have:

$$(1/200) V_1 = (1/500) (50)$$

Solving the fractions for V_1, we get V_1 = 20 ml. Again this means that if we take 20 ml of the stock boric acid and add 30 ml water, we would have 50 ml of the desired solution.

Example: When a blood sample was diluted 500 times, 300 cells were counted in the diluted sample. How many cells could there be in the blood before the dilution was made?

If a 1/500 dilution of blood was made, the diluted sample would contain only 1/500 as many cells as the undiluted sample, or, stated another way, whole blood would contain 500 times the number of cells as the diluted specimen. Thus, if the number of cells counted in the diluted sample is multiplied by 500, one would have the number of cells present in the original specimen.

$$300 \times 500 = 150{,}000.$$

Note that we use the reciprocal of the dilution (dilution 1/500; reciprocal 500/1 = 500) in such problems.

Example: A test on a urine sample was ordered. The concentration of the substance in the urine was too high to be determined with our instruments. We made a 1/10 dilution and ran the test on the diluted solution. The answer obtained was 50 mg/ml. What should be reported as the concentration of the substance in the undiluted urine?

The 1/10 dilution of urine contained one-tenth the amount of the substance as the undiluted sample, or the undiluted sample contained 10 times the quantity of the substance as the diluted solution. Thus we should multiply the answer by the reciprocal of the dilution made (dilution = 1/10 and reciprocal = 10).

$$50 \times 10 = 500 \text{ mg/ml}$$

Some Final Notes

1. After doing your calculations, it is always a good idea to work backwards and see if you come up with the original data given. This is a good check as to the correctness of your calculations.

Example: In example 9 above, we could work backwards by assuming that a urine sample was measured for the concentration of a substance and found to contain 500 mg/ml of that substance. The question to ask is: If the urine is diluted 10 times, what would be the concentration of the substance per ml? It is obvious that the 1/10 diluted sample would contain 10 times less of the substance. In other words:

$$500 \div 10 = 50 \text{ mg/ml}$$

Note again that we used the reciprocal of the dilution factor.

2. When working with very large or very small numbers, it is customary to convert them to a number between 1 and 9, times a power of base 10. For example:

$32{,}000{,}000 = 3.2 \times 10^{7}$
$0.000047 = 4.7 \times 10^{-5}$

Note in the above examples that (a) the first number in the conversion is between 1 and 9 and (b) the power of base 10 can be positive or negative. A positive power means the number is large(r than 1) and a negative power means the number is small (er than 1).

Dilution factors are similarly converted to powers. For example:

$1/10 = 0.1 = 10^{-1}$ $1/100 = 0.01 = 10^{-2}$
$1/10{,}000 = 0.0001 = 10^{-4}$ $1/1{,}000{,}000 = 0.000001 = 10^{-6}$

Similarly, reciprocals of dilution factors can be converted to powers as well, e.g. $10{,}000 = 10^{4}$.

3. Units of measurements can be converted as follows:

1 Kg = 1000 g	1 g = 1000 mg
1 mg = 1000 μg	1 μg = 1000 ng
1 liter = 1000 ml	1 ml = 1000 μl

4. Although already mentioned at the beginning of this chapter, it is worth repeating that " the original solution is always more concentrated (think big numbers, possibly with positive powers) than the diluted samples (think small numbers, possibly with negative powers). Check these rules of thumb whenever you are confronted with a dilution problem. Can you verify these rules in the preceding examples?

5. The ratio of the numbers rather than the numbers themselves, is important. For example, 1 ml in 10 ml, 0.1 ml in 1.0 ml, 5 ml in 50 ml, etc. all give the same dilution (ratio). The only differences are the amounts of the individual constituents as well as the final product.

Now that we have covered the basics of dilution calculations, try to see if you can solve the "Beginner Questions". Leave the "Advanced Questions" for when you have had more practice in the lab exercises and have grasped more microbiological knowledge.

Simple Dilution Problems

1. Find the dilution factor:
- a. 30 mg methionine/ml diluted to 3 mg/ml
- b. 220 g aspartic acid/ml diluted to 2.2 g/ml
- c. 2 ml of a solution diluted to a total of 8 ml
- d. 5 ml a solution diluted to 12.5 ml.

2. How would you dilute a 10 M antibiotic solution to a
- a. 0.1 M solution
- b. 1.0 M solution
- c. 0.5 M solution
- d. 0.85 M solution.

3. You have a 100 mg/ml stock solution of methionine. You dilute 0.1 ml of it in 9.9 ml water.
- a. What is the concentration of methionine in the diluted sample?
- b. If you now take 2 ml of the diluted sample and add 8 ml of water, what is the new methionine concentration?
- c. If you add 2 ml of the original stock to the diluted sample in step (b) above, what would be the methionine concentration?

4. Show a scheme for making a 10^{-6} dilution using only 3 tubes and starting with 0.1 ml of the original (stock) solution.

Advanced Dilution Problems

1. For the following 5 different experiments, you find the number of phage plaque forming units (pfus) seen on a plate using the volume of the dilutions given. In each case calculate the number of pfu/ml of the original solution.

	# plaques	dilution	volume plated
a)	75	1/1000	1 ml
b)	41	10^{-6}	0.1 ml
c)	30	10^{-4}	100 μl
d)	26	10^{-8}	50 μl
e)	30	1/5	1 ml

2. You have a set of 5 test tubes that can hold a maximum of 10 ml and your pipettes can measure volumes from 0.1 ml to 10 ml. Outline a method for making a 10^{-7} dilution from your original stock solution (assume you have at least 0.1 ml of stock). If you are then able to determine that your final dilution contains 20 bacteria in every 50 μl, what is the concentration of your stock (in bacteria/ml)?

3. You have a stock solution with 2×10^6 bacteria/ml and you need 500 ml of a solution with a concentration of 1.5×10^2 bacteria/ml to run an experiment. How do you make this dilution?

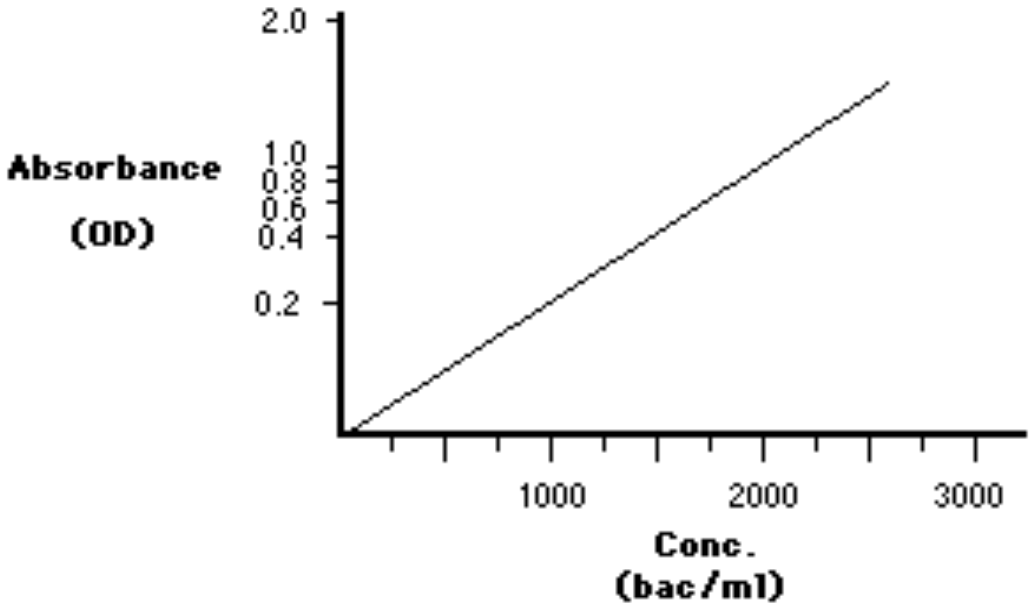
4. Let's say that on the average, 1000 bacteria weigh 5 ng (NOTE: this is not a factual number; 1000 ng = 1 μg; 1000 μg = 1 mg; 1000 mg = 1 g). You have 10 ml of a stock solution of bacteria. If you make a 10^{-6} dilution and find that you get 36 colonies when you plate 0.1 ml of it (the dilution), what is the total weight of the bacteria in the original solution in mg?

5. You plate out the following amounts of a phage/bacteria mixture at various incubation times. From the data, calculate the titer of the original solution, the relative titer, and the burst size.

Time	Dilution Factor	Amount plated	# plaques on plate
1 min	10^{-4}	0.1 ml	20
5 min	10^{-4}	0.1 ml	21
10 min	10^{-4}	0.1 ml	25
20 min	10^{-4}	0.1 ml	45
30 min	10^{-4}	0.1 ml	65
40 min	10^{-5}	0.1 ml	8
50 min	10-5	0.1 ml	12
60 min	10^{-5}	0.1 ml	20
70 min	10^{-5}	0.1 ml	22
80 min	10^{-5}	0.1 ml	21

6. We want to prepare a liter of BHI+Amp broth. We know that we need to add 50 mg of ampicillin to 1 L of the medium. We already have made a stock solution of ampicillin by dissolving 1 g of ampicillin in 10 ml of water. We dissolved the components of the BHI broth in a flask of 1 L of water, autoclaved the flask and cooled it to 50°C. How much of the stock ampicillin solution do we need to add?

7. You plot the following standard curve with a series of serial dilutions. You find that 5 ml of a 10^{-3} dilution of your unknown has an absorbance of 0.5 OD. What is the concentration of your original solution?



8. We have a can of chicken broth soup that is spoiled due to the growth of undesirable bacteria. We take 2 ml of this soup and add 3 ml of water. After mixing well, we take 10 microliters of the mixture and bring it up to 1 ml with water. Finally we take 50 microliters of the last mixture and spread it on a BHI agar plate. We find 82 colonies growing on the plate the next day.
(a) Determine the concentration of undesirable bacteria in the spoiled soup.
(b) Based on above result, do you expect the soup to be clear or turbid (as a result of bacterial presence), assuming an uncontaminated soup is clear? Why?

9. Starting from a stock solution, how would you create a 10^{-7} dilution so that you always have a final volume of 10 ml?

10. If your original solution is 25 mg/ml and you add 5 ml of it to 495 ml of saline, what is the new concentration?

11. If the original concentration was 50 mg/ml and the new concentration is 5 micrograms/ml what was the dilution factor?

12. You have 1 ml of a bacterial sample. Describe how to make a 10^{-7} dilution of this sample using as little sample and diluent as possible.

13. A 0.1 ml sample of lake water was spread on an agar petri dish. After incubation, 450 colonies grew on the plate. What is the cfu count per ml of the water?

14. One should expect the same number of cfu in 5 ml of an undiluted sample as in _____ml of a 1/100 dilution of the same sample.

15. A bacterial sample was serially diluted through the following scheme: 1 ml culture + 4 ml diluent, 1 ml of that dilution + 9 ml of diluent, 1 ml of that dilution + 19 ml diluent. What is the total dilution factor?

16. Back in the "old days" doing a PCR reaction consisted of adding dNTPS, buffers, enzyme and salts to the reaction tube separately instead of using the ready-made material we use now. In those days we would make 2.5 mM stock solutions of dNTPS (N is any of the four letters of the DNA alphabet); i.e., a stock solution that is 2.5 mM of each dATP, dTTP, dCTP and dGTP. Commercial companies typically sell the 20 mM stock solutions of each of these bases. Describe how you would make 50 μl each of 2.5 mM dNTP stock solutions.

17. You wish to stain your cells with fluorescent brightener (FB) and examine them under a fluorescence microscope. You have decided to use serial dilutions to achieve a 2 μg/ml solution of FB. If you begin with a stock solution of 20 mg/ml of FB and two 1.5 ml Eppendorf tubes, describe what you would do.

18. Using indirect estimate of microbial biomass, we constructed a standard curve for the number of microorganisms (M.Os) in a specific pond. This standard curve shows that a million M.Os weigh 8 μg. We are given 10 liters of water from this pond and we are asked to enumerate the M.Os. After centrifuging the water and weighing the remains, we find that the M.Os weigh 2 μg. Give an estimate of the number of M.Os per ml of this pond water.

19. You have a microwell plate with 4 wells (wells 1, 2, 3 & 4) and you do a MIC test in the following way:
-You add 0.75 ml of TSB to all wells.
-You add 0.25 ml of an 8 mg/ml tetracycline to well 1 and mix the contents.
-You transfer 0.25 ml from well 1 to well 2 and mix the contents of well 2.
-You transfer 0.25 ml from well 2 to well 3 and mix the contents of well 3.
-You discard 0.25 ml from well 3.
-You add 0.25 ml of *E. coli* grown in a broth to all wells.

(a) Calculate the concentration of tetracycline in each well.
(b) If you get growth in wells 3 and 4, what is the MIC of tetracycline for *E. coli*?