

MLAB 2360 Clinical I

Activity 3 Part 3 Serial Dilutions

Many of the laboratory procedures involve the use of dilutions. It is important to understand the concept of dilutions, since they are a handy tool used throughout all areas of the clinical laboratory. These dilutions have to be considered as they make a quantitative difference in what is going on.

A *serial dilution* is any dilution where the concentration decreases by the **same quantity in each successive step**. Serial dilutions are multiplicative.

What does it mean?

If a solution has a 1/10 dilution the number represents 1 part of the patient sample added to 9 parts of diluent. So the volumes used would be 10 mL total - 1 mL patient = 9 mLs diluent. Or to say it another way, this represents 1 part patient sample added to 9 parts of diluent.

If a 1/8 dilution of the stock solution is made followed by a 1/6 dilution what is the final dilution.

The final dilution is:

$$1/8 \times 1/6 = 1/48$$

This type of dilution is trickier and not used very frequently in the clinical lab.

“Doubling dilutions” are very popular. This is a series of 1/2 dilutions. Each successive tube will 1/2 the amount of the original concentrated solution. You add 0.5 mL of saline to each of 6 tubes. You then add 0.5 mL of serum to tube 1 and carefully transfer 0.5mL of tube 1 to tube 2, then 0.5 mL of tube 2 to tube 3 and continue on in this manner through tube 6 you would end up with:

1. 0.5 mL of saline + 0.5 mL of serum = 1 mL total volume
2. Divided 1 mL by 0.5 to get the DILUTION FACTOR (DF).
3. $1\text{mL}/0.5\text{mL} = 2$ - this is the dilution factor you will multiply by.

$$1^{\text{st}} \text{ dilution} = 1/2$$

$$2^{\text{nd}} \text{ dilution} = 1/2 \times 1/2 = 1/4 \text{ dilution of tube 2}$$

$$3^{\text{rd}} \text{ dilution} = 1/4 \text{ (dilution of tube 2)} \times 1/2 \text{ (DF)} = 1/8$$

$$4^{\text{th}} \text{ dilution} = 1/8 \text{ (dilution of tube 3)} \times 1/2 \text{ (DF)} = 1/16$$

$$5^{\text{th}} \text{ dilution} = 1/16 \text{ (dilution of tube 4)} \times 1/2 \text{ (DF)} = 1/32$$

$$6^{\text{th}} \text{ dilution} = 1/32 \text{ (dilution of tube 5)} \times 1/2 \text{ (DF)} = 1/64$$

This results in a series of dilutions, each a doubling dilution of the previous one.

To calculate a dilution factor:

The dilution factor (DF) is represented by this formula: **total volume/aliquot volume.**

EXAMPLE: What is the dilution factor if you add 0.1 mL aliquot of a specimen to 9.9 mL of diluent?

1. The final volume is equal to the aliquot volume plus the diluent volume:
 $0.1 \text{ mL} + 9.9 \text{ mL} = 10 \text{ mL}$
2. The dilution factor is equal to the final volume divided by the aliquot volume:
 $10 \text{ mL}/0.1 \text{ mL} = 1:100 \text{ dilution}$

Problem: What is the dilution factor when 0.2 mL is added to 3.8 mL diluent?

Set up the problem: dilution factor = final volume/aliquot volume

$$0.2 + 3.8 = 4.0 \text{ total volume}$$
$$4.0/0.2 = 1:20 \text{ dilution factor}$$

Remember that **serial dilutions** are always made by taking a set quantity of the initial dilution and adding it successively to tubes with the same volume. So each successive dilution would be multiplied by the dilution factor.

So in the above problem all successive tubes would have 3.8 mLs of diluent. You would then transfer 0.2 of the initial diluted sample into the next tube, mix transfer 0.2, mix and so on. If you had 4 tubes what would be the final dilution of tube 4?

Tube	1	2	3	4
aliquot	0.2	0.2	0.2	0.2
diluent	3.8	3.8	3.8	3.8
Math	$4.0/0.2=2.0/0.1$	$1/20 \times 1/20$	$1/400 \times 1/20$	$1/8000 \times 1/20$
dilution	1:20	1:400	1:8000	1:160,000

$$1/20 \times 1/20 \times 1/20 \times 1/20 = 1:160,000 = \text{dilution of tube \#4}$$

In the cold agglutinin titer a volume of indicator, red blood cells, was added to each tube. The dilution factor this causes must be calculated so that the final dilution can be determined. The chart below has the original dilution listed on the second row. The dilution factor this results in is calculated and this is then multiplied times each of the original dilutions to calculate the final dilution. **REMEMBER:** In a serial dilution to which some other reagents are added it is **CRITICAL** to remove an aliquot from the last tube so that ALL volumes are the same and the dilution factor stays unchanged.

Tube	1	2	3	4
Dilution	1:20	1:400	1:8000	1:160,000
RBCs	0.2	0.2	0.2	0.2
Volume Left	3.8	3.8	3.8	3.8
Dilution factor				
Math	20x20	20x400	20x8000	20x160,000
Final	1:400	1:8000	1:160,000	1:320,000

When performing serial dilutions in the clinical laboratory one must know the dilution of each tube so a chart is always created to indicate the dilution of each tube. If you know the dilution factor you multiply each successive tube by it and can easily determine the concentration of each tube.

TITERS are reported out as the reciprocal of the last tube giving a positive reaction. So if tube 2 was the endpoint, the **DILUTION** is 1:400, the **TITER** is reported out as $400/1 = 400$

PROBLEM SETS

For each of the following calculate the dilution of each tube. You must show your work.

1. You add 1.5 mLs of diluent to 5 tubes. You add 0.5 mLs of serum to tube one. You transfer 0.5 mLs sequentially to each of the next 5 tubes.

Tube	1	2	3	4	5
Aliquot	0.5	0.5	0.5	0.5	0.5
Diluent	1.5	1.5	1.5	1.5	1.5
Dilution Factor					
Dilution					

2. You add 2 mLs of diluent to 5 tubes. One mL of serum is added to tube one. You transfer 1.0 mLs sequentially to each of the next 5 tubes.

Tube	1	2	3	4	5
Aliquot	1	1	1	1	1
Diluent	2	2	2	2	2
Dilution Factor					
Dilution					

3. You add 1.8 mLs of diluent to 5 tubes. You add 0.2 mLs of serum to tube one. You transfer 0.2 mLs sequentially to each of the next 4 tubes.

Tube	1	2	3	4	5
Aliquot	0.2	0.2	0.2	0.2	0.2
Diluent	1.8	1.8	1.8	1.8	1.8
Dilution Factor					
Dilution					

4. You add 1.9 mLs of diluent to 5 tubes. You add 0.1 mLs of serum to tube one. You transfer 0.1 mLs sequentially to each of the next 5 tubes.

Tube	1	2	3	4	5
Aliquot	0.1				
Diluent	1.9				
Dilution Factor					
Dilution					

5. You add 0.5 mLs of diluent to 5 tubes. You add 0.5 mLs of serum to tube one. You transfer 0.5 mLs sequentially to each of the next 4 tubes. YOU THEN ADD 0.5 mLs of RBCs to each tube. What is the final dilution of each tube?

Tube	1	2	3	4	5
Aliquot	0.5				
Diluent	0.5				
Dilution Factor					
Dilution					
Volume Left					
RBCs	0.5	0.5	0.5	0.5	0.5
Dilution Factor					
Dilution					

6. You are running a chemistry test. You get a result of 950 mg/dL on an ALT. The linearity of the machine is 750 mg/dL.
- State what you will do to resolve this problem.
 - You dilute the sample 1:10 and get a result of 80 mg/dL. Show your work to calculate the true value of the patient's ALT.