Serial Dilutions and Standard Plate Count

(COVID-19 substitute exercise)

We are going to focus on how to make serial dilutions and the standard plate count experiment. We are NOT going to do the bacterial growth curve so don't worry about that.

Serial Dilutions

The reason why we perform serial dilutions is to dilute a bacterial broth culture enough to grow discrete, isolated colonies that we can count. We will use the number of colonies on a plate to estimate the number of cells/mL in the original broth culture. (Note: we can do the exact same thing with viruses and viral plaques to estimate the number of viruses/mL)

Dilution = part ÷ whole; part = volume added to tube, whole = total volume in the tube (original amount + what you added)

Example 1: The dilution tube contains 9mL of sterile water or broth and 1ml from my bacterial broth culture is added.

Part = 1ml, whole = 10 mL (9+1 = 10) 1÷10 = 0.1 or 1/10 or 10^{-1}

We want to represent the dilution exponentially. Technically, it is 1x 10^{-1} but since we understand that everything is 1x 10 to the exponent, we don't have to write 1x for everything, just 10^{-1}.

Example 2: The dilution tube contains 99mL of sterile water/broth and 1 mL from my bacterial culture is added.

Part = 1 mL, whole = 100 mL (99 + 1 = 100) 1÷100 = 0.01 or 1/100 or 10^{-2}

Dilutions are cumulative

If you performed a 10^{-1} first and then performed another 10^{-2} dilution on the tube that was just diluted, the total dilution = 10^{-3}.

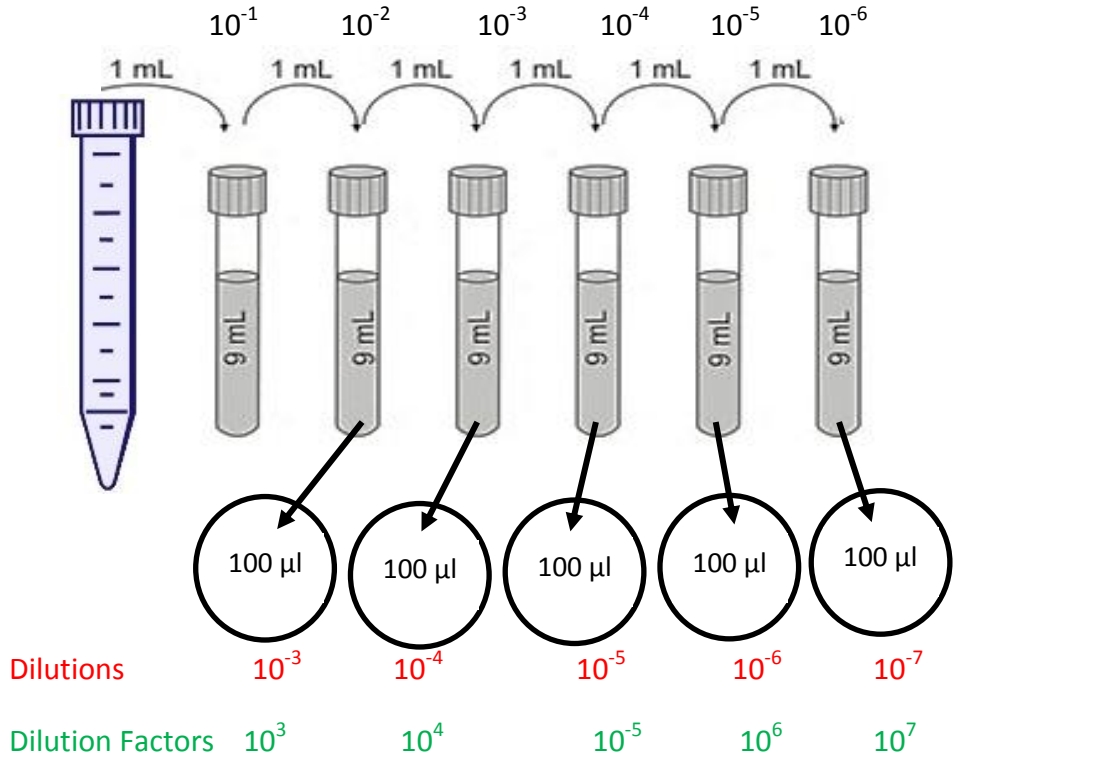
Another example of a serial dilution: $10^{-1} \times 10^{-1} \times 10^{-1} \times 10^{-1} \times 10^{-1} \times 10^{-1} \times 10^{-1} \times 10^{-1} = 10^{-8}$

Dilution Factor (DF)

DF is the number of times the original solution has been diluted. DF = 1 ÷ dilution

Example: if dilution is 10^{-3}, then the DF = 10^{3}. The exponent becomes positive (+).

Using this example, we are going to transfer 1 mL of stock bacteria (conical tube) into the first 9 mL dilution tube. We thoroughly mix that dilution tube and then transfer 1 mL from it into the next dilution tube and so on. Each dilution tube contains 9 mL of sterile broth/water and we are adding 1 mL from the previous tube. The dilution between each tube is a 10^{-1} dilution (1 ÷ 10). The dilution in each tube is cumulative. Starting with the first 9 mL dilution tube, the dilutions are as follows:



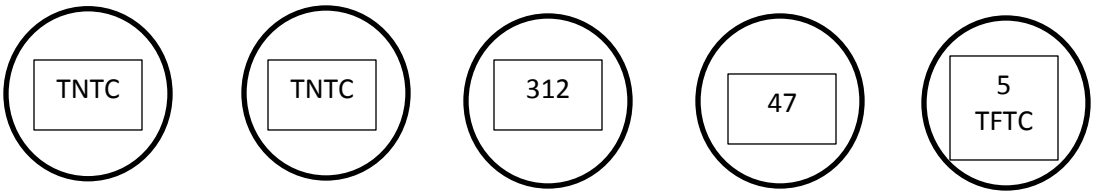
In this experiment we spread 100 uL samples from the last 5 tubes onto TSA plates. **In order to determine the "effective dilution" on the surface of the plate, you have to multiply the dilution in the tube times the amount you put on the agar plate.** We are using 100 uL. As you recall, 100 uL = 0.1 mL. We know that 0.1 = 10^{-1}. Therefore, multiply the dilution for each tube by 10^{-1}. The "effective dilution" on the surface of the agar plates is shown in red above.

We need to calculate the dilution factors for the plates. Remember the DF is simply the positive exponent of the dilution. They are shown in green. The DF represents the number of times the original stock culture has been diluted. Notice that by the end of the procedure, we have diluted the original sample by 10 million times!

Now we have almost everything we need. When the bacteria grow on the agar plates, they will form colonies. Some plates will have too many colonies (TNTC = too numerous to count) and some will have too few colonies to count (TFTC). The range of countable colonies on a plate is between 30-300. Below 30 colonies is generally considered statistically unreliable due to experimental error. Above 300 colonies, the colonies grow into each other and cannot be seen as single colonies.

The following results from the experiment shown above are hypothetical. Because pipetting involves error, the number of colonies from plate to plate is not exactly proportional. In the results shown below, if you pipetted perfectly you would expect colonies to be 312, 31, and 3. Each plate is 10 times more dilute than the previous plate so there should be 10 times fewer colonies on each subsequent plate. The results below are approximately proportional.

These are example colony counts.



DF 10^{3} 10^{4} 10^{-5} 10^{6} 10^{7}

The plate with 47 colonies is the only plate that falls between 30-300 colonies, so that is the only value we will use.

The formula to estimate cells/mL is: #colonies x DF = cfu/mL

CFU = colony forming unit. A single cell forms a colony; therefore, cfu/mL is the same as cells/mL.

Finally, 47 x 10^{6} cfu/mL

Not quite – we have to put the number into scientific notation: 4.7 x 10^{7} cfu/mL (We made 47 into 4.7 which is smaller by a factor of 10 so the exponent must get bigger.)

In this example, the original broth culture had a concentration of 4.7 x 10^{7} cells for every mL in that tube. That means 47,000,000 cells were in every milliliter of broth. Let's say the original tube contained 5 mL of broth. That means there were 235,000,000 bacteria in the original tube!

Take Home Lesson:
You should be able to calculate dilutions, cumulative dilutions, dilution factors, and cfu/mL.
A picture of some plates to show you what they might look like. These plates do not match the results shown above. They are just an example.