Dilution (and Concentration) Theory

Dilution and concentration theory sounds ominous but you use dilutions and concentrations fairly frequently outside of your "scientific life". For example, when you are making orange juice from a frozen concentrate, you mix 1 can of concentrate with 3 cans of water. In other words, you are diluting the frozen concentrate by 1/4. The concentrate itself was a 4X concentrate of what you want to drink. So keeping in mind that what you are learning here is just an extension of your everyday life…let's look at dilutions and concentrations in science.

We use many techniques to quantitate items in natural systems. For example, we use colorimetric assays to determine such diverse chemicals as iron, protein, and DNA, chromatographic assays (e.g. HPLC) for low molecular weight organics such as hormones or toxins, and plating techniques for population studies of microbes. All of them have a valid assay range below which they do not detect the item accurately or at all and above which they can no longer distinguish between increasing concentrations or numbers of the item. Therefore, a very important part of many protocols for quantifying items includes either a dilution or concentration step to get the values of the item within the valid range of the assay. Dilution is used when the concentration of the item in the natural system is above the accurate detection range of the assay; concentration is used when the concentration of the item is below the detection range.

In both dilution and concentration steps, it is important to keep track of exactly what the dilution or concentration factor is in order to be able to mathematically compensate for it later when reporting the actual quantity of the item. So, the first rule of dilutions/concentrations is to keep good records.

The second "rule" of dilutions/concentrations is an obvious one: there is a limit to how much you can dilute or concentrate an item in one step. For example, if you want to accurately quantitate the amount of bacteria in a laboratory culture, you need to dilute the cells to be within the range of the assay: 30-300 bacteria per petri plate. If the starting population is 10^{10} bacteria/ml and you want to add 0.1 ml of culture to the petri plate, you would need to dilute the culture a minimum of $300/10^9$ or 3×10^{-7}. If you attempted to make this dilution in one step... and you had a micropipettor to use, you could pipette 1 μl of culture into diluent... but you would need over 30 liters of diluent! (Think about the volume of 15- 2L bottles of soda!) If you were doing 15 or 20 of these assays in a day (not at all unusual in a micro lab), you'd have a roomful of diluent. Instead, you can use serial dilutions which enable you to perform the same procedure using less than 10 ml of diluent!

Let's think through a practice dilution:

You will make several dilutions of a bacterial stock culture. For some dilutions, you will add 10μl of the more concentrated solution to 990μl of sterile diluent in a microfuge tube. For others, you will add 100μl of the more

concentrated solution to $900\mu l$ of sterile diluent. Following is a graphic representation of these dilutions:
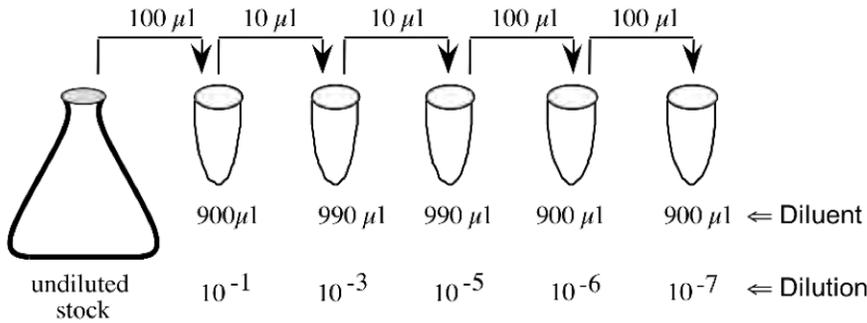
How did we get to those dilution values? Here is an example:

$10\mu l$ of sample put into $990\mu l$ of diluent gives:

$10\mu l$ divided by $(990 + 10)$ μl total volume $= 10/1000 = 1/100 = 10^{-2}$

You plate (put subsamples onto nutrient agar) the following dilutions:
(A) $10\mu l$ of the 10^{-3} dilution
(B) $100\mu l$ of the 10^{-5} dilution
(C) $100\mu l$ of the 10^{-6} dilution
(D) $100\mu l$ of the 10^{-7} dilution

You incubate the plates for 24 hours, after which you obtain the following results:

Plate	Colonies on plate
A	too many to count
B	685
C	52
D	4

What is the number of bacteria in the original culture?

To answer this question, you'll need to know a bit about bacteria and how they grow. Many, but certainly not all, bacteria reproduce by binary fission where one cell replicates its DNA, grows in size, then splits into two bacteria. If these progeny cells disconnect after cell division, the culture generally contains mostly single cells. If you serially dilute this culture, plate the dilutions, and count the colonies which form after incubation, most colonies represent a single cell. In many species of bacteria, the cells do not disconnect, but stay connected forming chains of cells or clusters or packets of cells. If you serially dilute these types of cultures, plate the dilutions, and count the colonies which form after incubation, most colonies represent more than one cell. Therefore, it is

common practice to report the final number of your dilution and plating protocol in Colony-Forming-Units per ml (CFU/ml).

There is both a lower limit and upper limit to the number of colonies that can be accurately counted on one plate. Below about 30 colonies per plate, random variation between samples becomes a large error in the results (this has been determined using statistical methods). In addition, if two cells (or groups of cells) land near each other, they might form a single colony or only one will grow, outcompeting the other for nutrients. This can happen even when small numbers of cells (or groups of cells) are added to a plate, however it becomes more likely as the number of cells gets large. Therefore, the upper limit of accuracy for counting colonies is 300 colonies per plate (determined experimentally and statistically).

Back to our example, what is the number of Colony-Forming-Units (CFU) per ml of original stock?

Only one of the plate counts (that of plate C) fits within the 30-300 range, so it is the only one to use. Now, what is the dilution of that plate and what do you do with it?

We will report concentration in CFU per milliliter. You had physically diluted the culture by 10^{-6} at this point in your dilution scheme. You then plated 100 μl (not a full ml) of that dilution, which is in effect an additional 10^{-1} dilution (100μl/1000μl). So you can calculate the **total effective dilution** as 10^{-7} (10^{-6} X 10^{-1} = 10^{-7}). So...

colony count x dilution factor (the inverse of the dilutions) = CFU/ml of original culture

$\qquad$ 52 CFU x 10^6 x 10^1 = 5.2 X 10^8 CFU/ml

Another way to look at this is

$$\frac{52\ \text{CFU}}{100\,\mu l} \times \frac{1000\,\mu l}{1\text{ml}} \times 10^6 = 5.2 \times 10^8 \text{CFU/ml}$$

The mathematical compensation for physical dilutions or concentrations is very straightforward and logical math. If you have any difficulties with the math, ask for help. After you perform enough of the calculations, the math will be easy.