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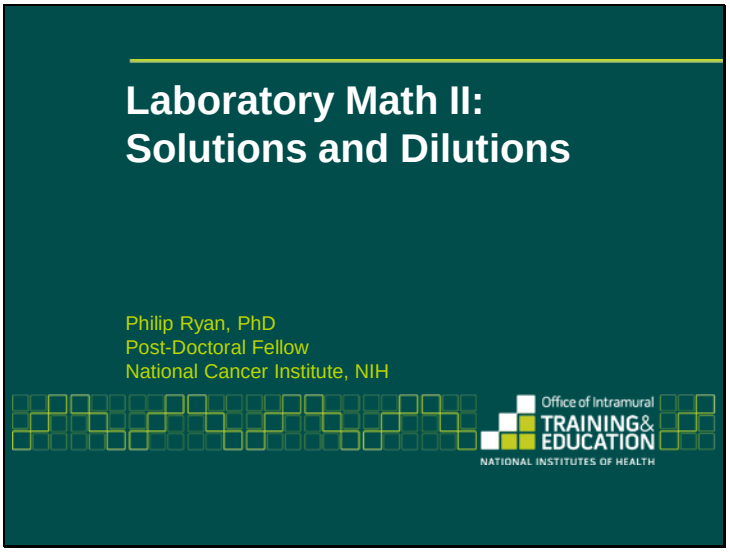

Welcome to the National Institutes of Health, Office of Intramural Training & Education's Webinar on Laboratory Math II: Solutions and Dilutions. This Webinar is intended to give a brief introduction into the mathematics of making solutions commonly used in a research setting. While you may already make solutions in the lab by following recipes, we hope this Webinar will help you understand the concepts involved so that you can calculate how to make any solution.

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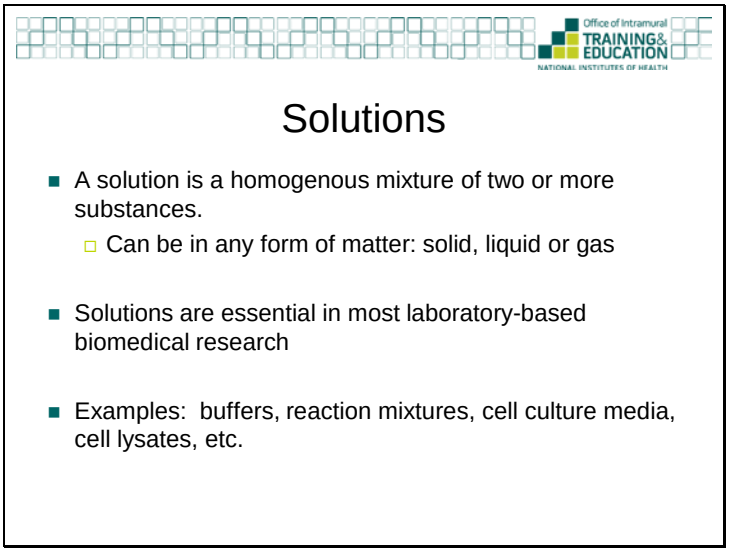

A textbook definition of a solution is a homogenous mixture of two or more substances. These mixtures can be in any form of matter, however for this Webinar we are going to focus exclusively on liquid solutions.

In a laboratory setting, solutions are an essential part of research. There is a reason that bench research is often referred to as “wet” research. Most biochemical reactions occur in liquid solutions. This should help explain why dehydration is such a life threatening ailment! Without water, the body is unable to perform many of the biochemical functions necessary to survive.

In the laboratory, solutions are everywhere. Buffers, reaction mixtures, cell culture media, cell lysates, liquid acids and bases are all examples of solutions commonly used in the lab.

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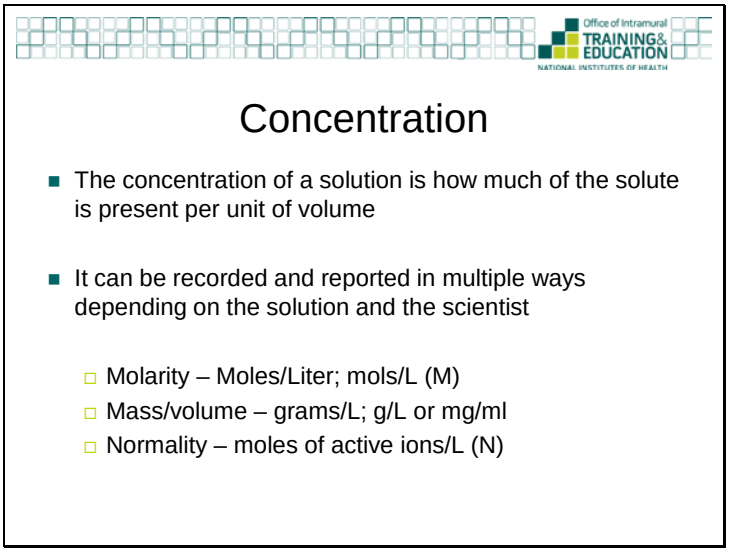

We talk about solutions in terms of concentrations, how much of each substance, or solute, the solution contains.

Concentration can be recorded and reported in many different ways depending on the solution, the scientist and what the solution is being used for. Let's review a few of the common ways of reporting concentration.

Molarity is used to report molecules of a substance per unit volume. Specifically, molarity is the number of moles of a substance per liter of the solution. It is reported in moles per liter or with a capital M for molar.

Mass per unit volume is often used to report the concentration of proteins and other complex substances with molarities that are not easily determined. So, the concentration of a complex solution of proteins often is reported as grams per liter.

Normality is like molarity, but is used for ionic solutions to more accurately represent their ionic strength. A single molecule of an ionic compound may (when in solution) separate into individual charged particles. For example: NaCl in solution consists of positive charged sodium ions and negatively charged chloride ions. What is relevant is solute particles" per unit volume, or ions per volume. So, normality is the number of moles of active ions per liter in a solution. Acid and base concentrations are often expressed in normality.

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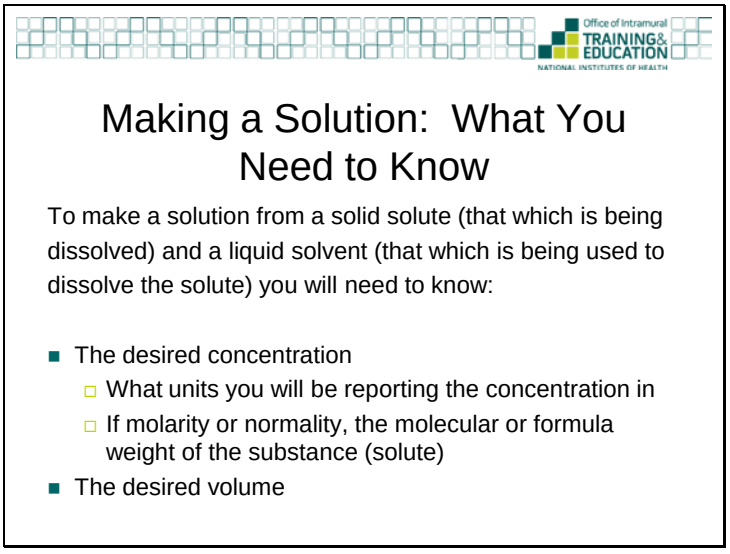

Before you can make a solution, you need to know a few things. In this case, we will be looking at how to make a solution from a solid substance or "solute" being dissolved in a liquid, or " solvent". To do this, you need to know: the desired concentration of the completed solution. That means, how much of your substance per unit volume do you want when you are finished making your solution? You will also need to know what units you will be reporting the concentration in. If you will be reporting in molarity or normality, you will need to know the molecular or formula weight of the substance. That is to say, you need to know how many grams of the substance are in a mole. You need this information because we cannot readily measure the number of moles of a substance, but we can measure the mass. So, we use the mass and the molecular weight to determine moles. The molecular weight is determined by the elemental composition of the compound and is usually listed on the container the solid solute is stored in.

You will also need to know the desired volume of the finished solution.

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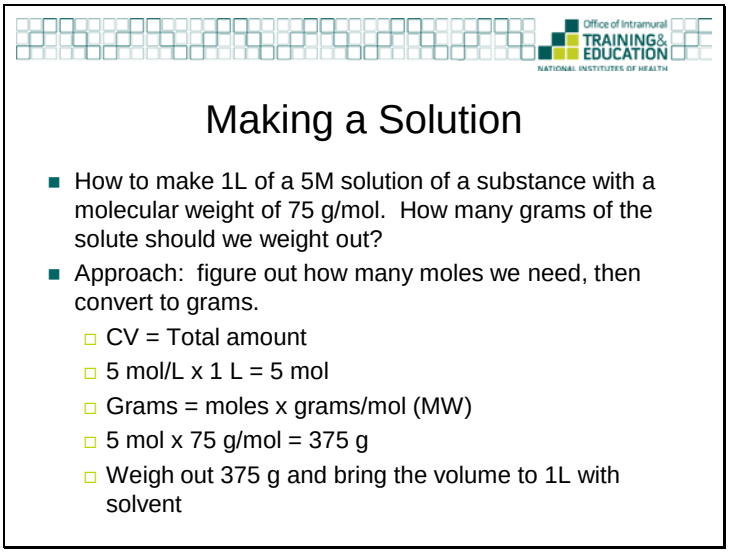

Let's take a look at a simple example. How would we make one liter of a five molar solution of a substance with a molecular weight of 75 grams per mole? The first thing to determine is: what don't we know? We know the volume, the molarity we want and the molecular weight. What we need to know is how many grams of the substance we need to measure out. So our approach is to first determine how many moles we need of the substance and then use the molecular weight to determine how many grams we need to weigh out.

It is important to note that concentration x volume (or CV) is equal to the total amount of substance. A five molar solution means we have five moles per liter. And we want a total of one liter. If we multiply five moles per liter times the total volume of one liter, we get our total number of moles needed for our solution, which is five.

To determine the number of grams we need, we multiply the number of moles by the molecular weight, which is grams per mole. We know that the molecular weight of our substance is 75 grams per mole and that we need 5 moles. So, we can multiply the five moles needed by 75 grams per mole and we can solve that we need 375 grams of the substance.

So, we can weigh out the 375 grams of the substance and then bring the volume to one 1 liter with solvent. It is important to note that you do NOT add one liter of solvent to the 375 grams of the substance. The dry substance has a volume as well, and if you add a liter of solvent, your total volume will be greater than one liter and your concentration will be wrong.

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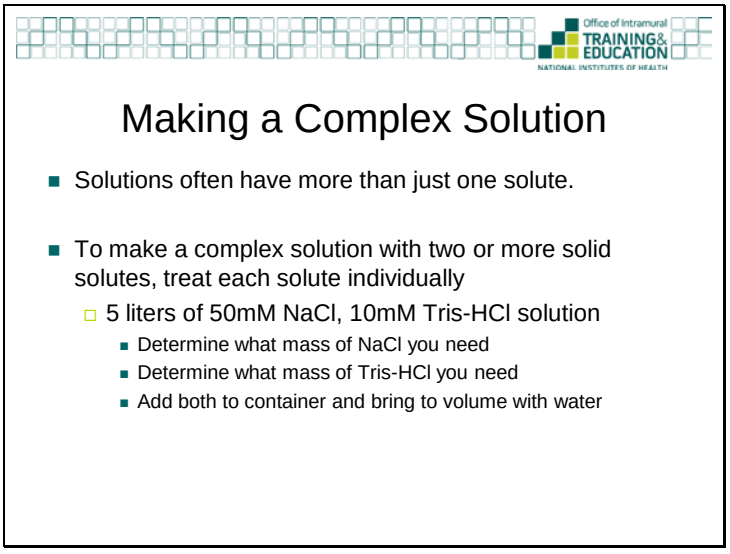

Solutions in a research setting often have more than just one solute component. Complex solutions are those that contain two or more chemical compounds in addition to the solvent. To make a complex solution with solid solutes, you treat each solute individually when determining the mass of that compound to add to the solution.

For example, to make a five liter solution of 50 millimolar NaCl and ten millimolar tris-HCl, you would first determine the mass of NaCl that you need. Then, you would determine the mass of Tris-HCl you need. You would weigh out both individually, add them to the desired container capable of holding 5 liters of liquid and then you would bring the solution to five liters of volume with water while mixing.

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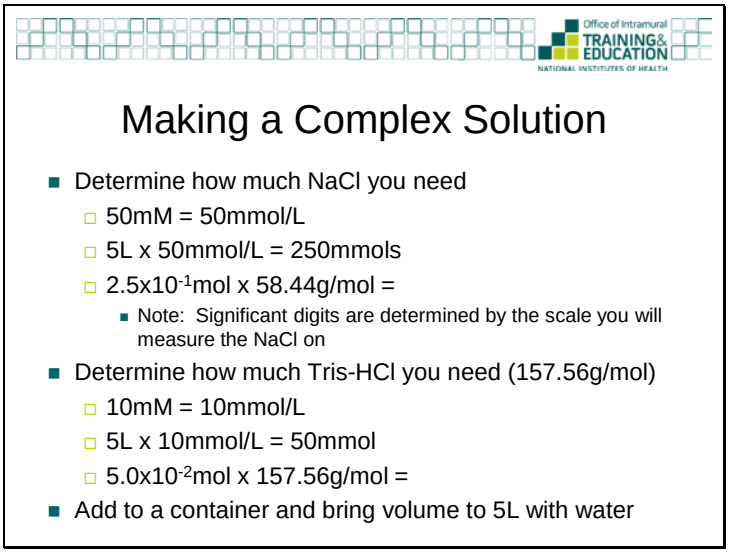


Let's go ahead and work out the arithmetic for this solution.

First, we need to determine the mass of NaCl that we will need. We know we need the final concentration to be fifty millimolar, which means we will need fifty millimoles of NaCl per liter of solution. Remember that CV = total amount. So, we multiply five liters by fifty millimole per liter. The liters cancel out and we now know that we need 250 millimoles of NaCl.

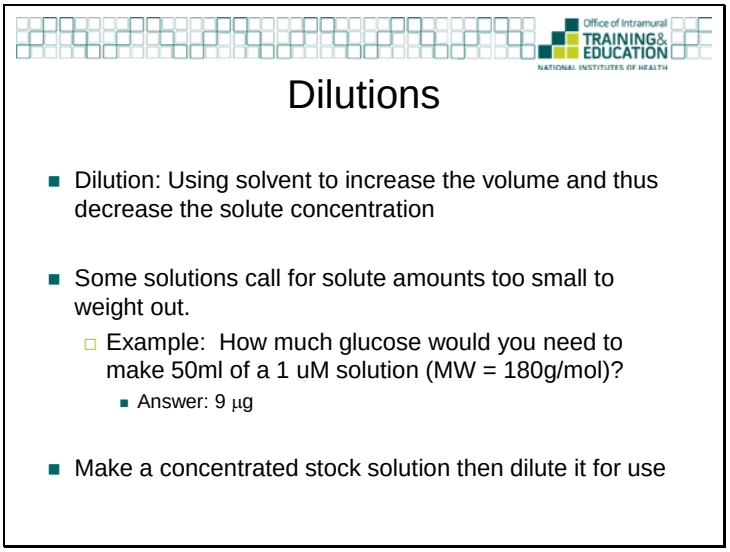
We also know that we can convert 250 millimoles into moles by dividing by ten to the negative three. This lets us know that we need two point five times ten to the negative one mols of NaCl. We can then multiply this by the molecular weight of NaCl, which is 58.44 grams per mole. The moles cancel out and we get 146.1 times ten to the negative one grams or 14.61 grams of NaCl.

Note that the significant digits will be determined by the scale you will use to weigh out the compounds.

Next you determine how much Tris-HCl you need. The molecular weight of Tris-HCl is 157.56 grams per mole. Can you work out how many grams we will need for five liters of a ten millimolar solution?

Again determine how much mass you need to weigh out by multiplying concentration by volume. In this case five liters times ten millimoles per liter. This will give you fifty millimoles or five times ten to the negative two moles. Then multiply that by the molecular weight of 157.56 grams per mole and you get seven point eight eight grams. Add that to a container and bring the volume to five liters with water.

Slide 8



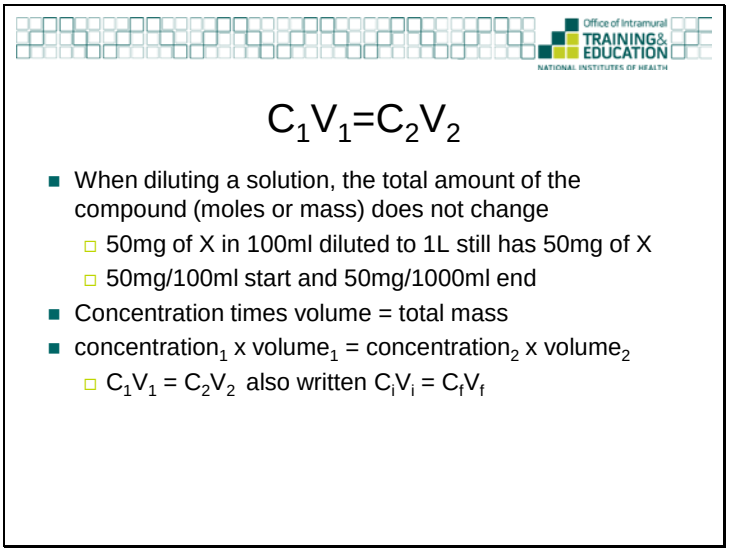
One of the benefits of working with liquid solutions is the ability to dilute them. Dilution is a technique that uses a solvent to increase the volume of a solution and thus decrease the concentration of that solution. It is a concept used in everyday life as well. If your coffee is too strong, you add water to dilute it and make it more palatable. Many people do this with their juice or other beverages. It can also happen inadvertently when your ice melts and makes your favorite carbonated soft drink taste less sugary.

In science diluting solutions has practical applications as well. Often times you will need a small volume of a low concentration solution. When you do the math you find that you need to weigh out microgram or nanogram amounts of the compound. For example, how much glucose would you need to make 50 milliliters of a one micromolar solution? The molecular weight of glucose is 180 grams per mole. Take a minute to work it out? (Pause) Did you come up with nine micrograms? Lab balances are not up to the task. So, you make a higher concentration solution, and then dilute it to the concentration you need.

The more concentrated solution is called a stock solution. You can then dilute the stock solution to the concentration you need, which is often referred to as a working concentration or final concentration.

Examples of stock solutions are a five molar solution of NaCl or two molar solution of Tris-HCl.

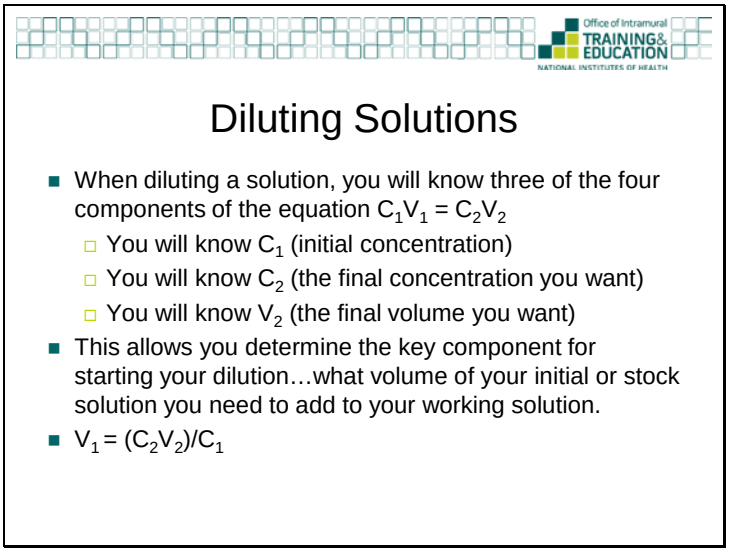
Slide 9



It is important to understand that when you are diluting a solution, you are not removing any of the solute. The total amount, or mass, of the solute does not change. If you have 50 milligrams of compound X in 100 milliliters and you dilute the whole thing to one liter, you now have a concentration that is one tenth what it was before. You still have 50 milligrams of compound X. It is just in a larger volume.

As you have worked out in a few previous examples, the concentration multiplied by the volume equals the total amount of the compound. Since the total amount doesn't change, it means that the initial concentration multiplied by the initial volume will equal the final concentration multiplied by the final volume. This gives rise to the equation, C_1V_1 equals C_2V_2. You might also see this written $C_i V_i$ equals C_fV_f, where I indicates initial and f stands for final.

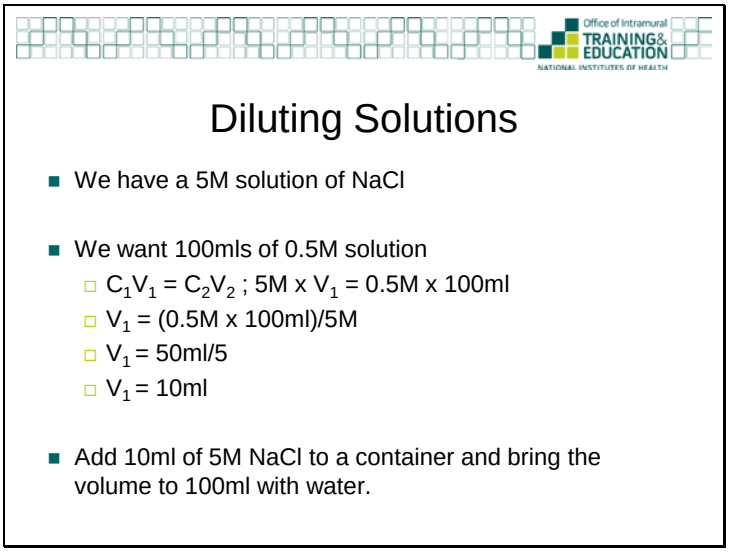
Slide 10



When making dilutions, you will know three of the four variables from the equation C_1V_1 equals C_2V_2. You will know your initial concentration, or the concentration of the stock solution you are about to dilute. You will know the final concentration of the solution that you will want to be working with and you will know how much of that final solution you will want to be making up. What you will need to solve for, is how much of your initial stock solution will you need to add to the final solution to get the desired concentration.

To do this, you can just divide both sides of the equation by your initial concentration (C_1). This yields the result that V_1 equals C_2 timesV_2 divided by C_1.

Slide 11



Let's work through an example of this together.

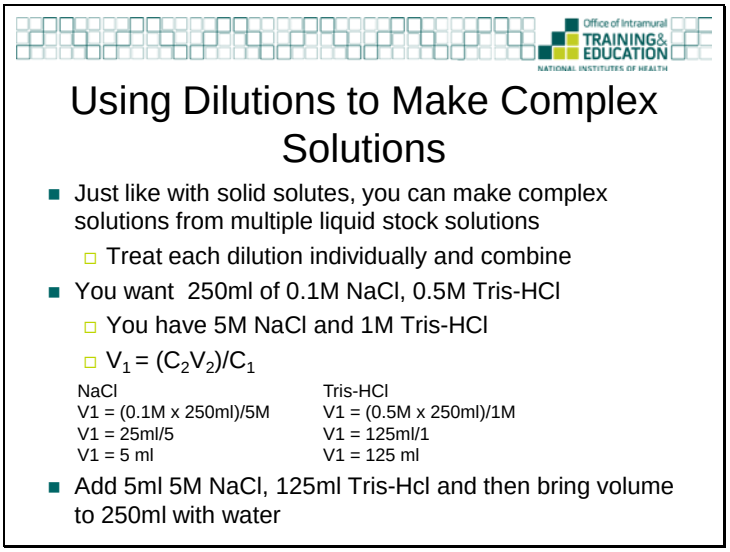
I mentioned in a previous slide that a common stock solution in a lab is 5 molar NaCl. This would be our initial concentration.

If we want 100 milliliters of 0.5 molar NaCl, 100 milliliters is our final volume and 0.5 molar is our final concentration. We can plug these values into the equation C_1V_1 equals C_2V_2, and solve for V_1 by dividing both sides by 5 molar.

The molar will cancel out and we will get 50milliliters divided by 5 which means our initial volume is 10 milliliters.

This means that you add 10 milliliters of the 5M NaCl stock solution to a container and then bring the volume to 100 milliliters with water.

Slide 12



Just like with solid solutes, we can make complex solutions from multiple liquid stock solutions for different compounds. To do this, we treat each dilution individually to determine the volume we need of each stock solution. Then we combine them all and bring the result to the appropriate final volume.

Let's look at an example. What we want is a working solution of 250 milliliters that is zero point one molar NaCl and zero point five molar Tris-HCl.

What we have is a five molar NaCl solution and a one molar Tris-HCl solution. We know that V_1 is equal to C_2 multiplied by V_2 and divided by C_1.

So, we start with NaCl and plug in our values to see that V_1 is equal to zero point one molar times 250 milliliters divided by five molar. The molars cancel out and we solve that we need five milliliters of our five molar stock NaCl solution.

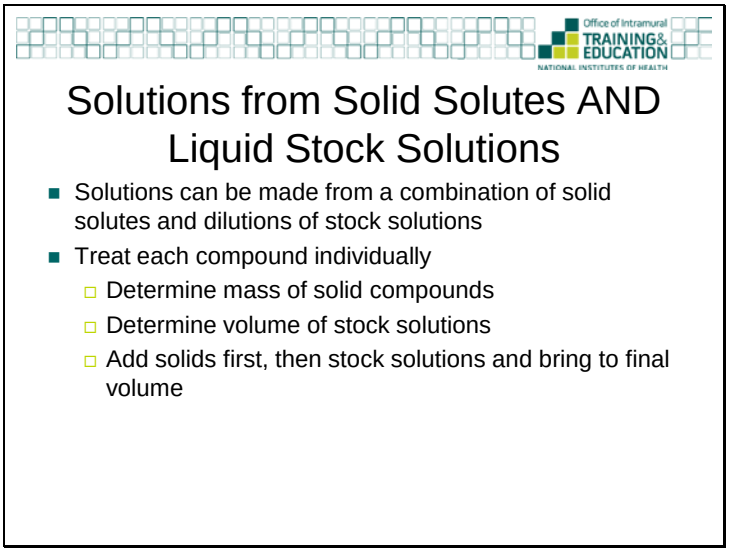
OK, now you work out how much Tris-HCl we need.

Again, we plug in the values for Tris Hcl that we know. Zero point five molar for C_2, 250 milliliter for V_2 and one molar for C_1. The molars cancel out and we solve that we need 125 ml of one molar Tris-HCl.

So, we add the five milliliters of NaCl and 125 milliliters of Tris-Hcl and then bring the volume up to 250 milliliters with water.

Note: IF we needed a much larger concentration of Tris or had to add aliquots of several other sotlutions, this might not work. We would need more concentrated stocks or to add some solutes as solids.

Slide 13



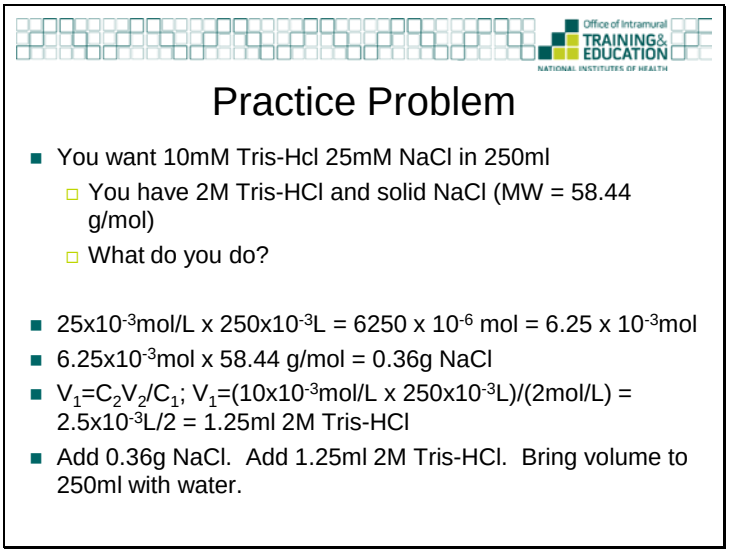
It should come as no surprise that you can even make complex solutions from a combination of solid solutes and dilutions of liquid stock solutions.

To do it, you again will treat each compound or stock solution individually. It is best to do things in a particular order.

Determine the mass of the solid compound or compounds that you will be dissolving. Then, determine the volume of the stock solution or solutions you will be diluting. Add the solids to the container, then the stock solutions and finally bring the whole solution up to your desired final volume.

On the next couple of slides, we will be going through some practice problems in making complex solutions from a combination of solids and liquids.

Slide 14



So let's start by working with some familiar compounds. What we want for a final solution is 10 millimolar Tris-HCl, 25 millimolar NaCl in a total volume of 250 milliliters. You are starting with a two molar stock solution of Tris-HCl and solid NaCl with a molecular weight of 58.44 grams per mole. So…how much of what are you going to use to make this solution?

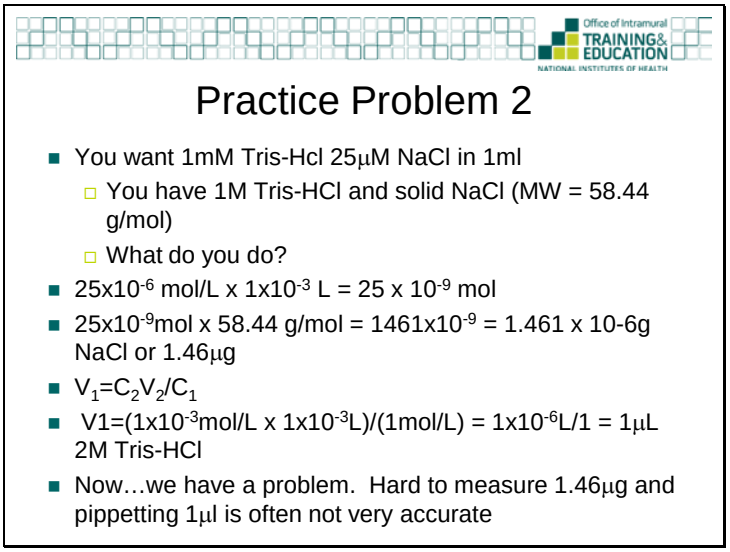
Start by finding the mass of the NaCl you will weigh out and add.
We know we want 25 times ten to the negative three moles per liter and a total volume of 250 times ten to the negative three liters. We multiply these together to get the total number of moles of NaCl we will need in the solution. In this case, 6.25 times ten to the negative three moles. We then multiple that number by the molecular weight of 58.44 grams per mole to get the total mass of NaCl we will need. This equals zero point three six grams of NaCl, which can be measured on most laboratory scales.

Next we use the equation C_1V_1 equal C_2V_2 rearranged to read V_1 equals C_2V_2 divided by C_1. We plug in our known numbers or 10 times10 to the negative third moles per liter final concentration, 250 times ten to the negative three liters for final volume and divide that by two moles per liter initial concentration. The moles per liter will cancel out and we will get two point five times ten to the negative three liters divided by two, or one point two five milliliters initial volume of our two molar Tris-HCl solution.

So, we add the zero point three six grams of NaCl to our container. Then we add the one point two five milliliters of two molar Tris-HCl to the container and finally we bring the volume to 250 milliliters with water.

So, how did you do? You can replay this slide or any previous slide if you need to review any of the concepts. How about one more example?

Slide 15



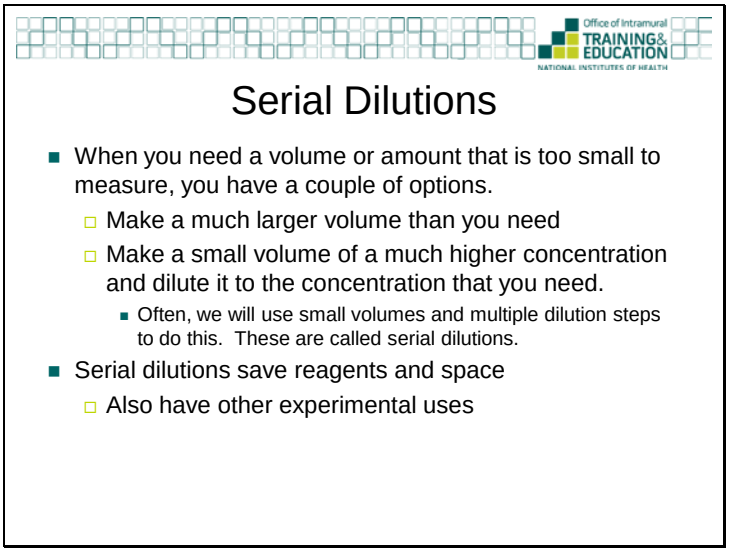
OK, same compounds, but we are going to change the concentrations of each and the total volume. What we want for a final solution is one millimolar Tris-HCl, 25 micromolar NaCl in a total volume of one milliliter. What you have to start with is a one molar stock solution of Tris-HCl and solid NaCl with a molecular weight of 58.44 grams per mole. So…work the numbers to find out how much of each compound you will need to make the solution that you want.

Again, we start by finding the mass of the NaCl you will weigh out and add.
This time we have 25 times ten to the negative six moles per liter and a total volume of one times ten to the negative three liters. We multiply these together to get the total number of moles of NaCl we will need in the solution. In this case, 25 times ten to the negative nine moles. We then multiply that number by the molecular weight of 58.44 grams per mole to get the total mass of NaCl we will need. This equals 1,461 times ten to the negative nine grams, or 1.46 micrograms of NaCl. That is a very small amount to measure…but let's move on to our volume of Tris-HCl.

Again, we use the equation C_1V_1 equals C_2V_2 rearranged to read V_1 equals C_2V_2/C_1. We plug in our known numbers. One times ten to the negative three moles per liter final concentration, one times ten to the negative three liters for final volume and divide that by 1 mole per liter initial concentration. The moles per liter will cancel out and we will get one times ten to the negative six liters divided by one, or one microliter initial volume of our two molar Tris-HCl solution.

Now we face a problem. There are very few scales that can measure that small of a mass of a compound. And while some micropipettes can accurately transfer one microliter, it is such a small volume that there is a high rate of error. So, what do we do? We perform serial dilutions.

Slide 16

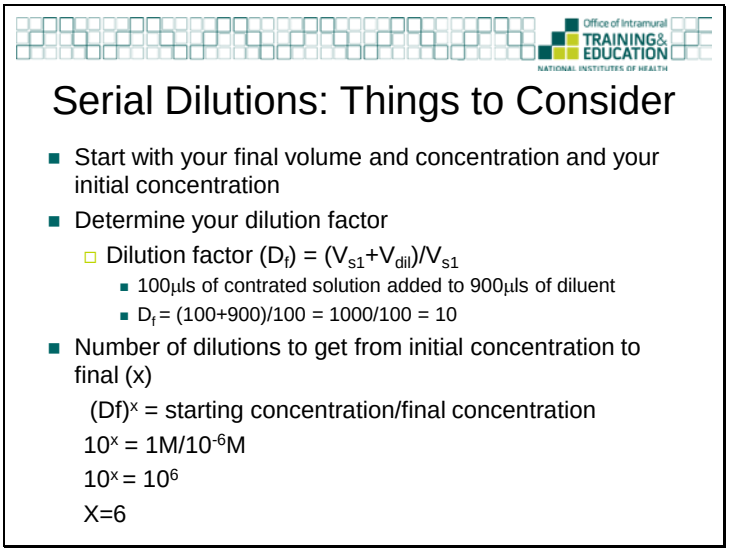


The previous slide demonstrates a common problem faced in a biomedical research lab. While using very little of a compound is always a financial benefit, it can be a practical hurdle. When you need a volume or an amount that is too small to accurately measure, you have a couple of options. You can make a much larger volume of the solution that you need at the proper concentration. For example we needed 1.5 micrograms of NaCl for a 1ml solution. We could make ten liters of the solution and weigh out 0.015 grams of NaCl . But, then will have nine point nine nine nine liters of our solution left over. A ten liter carboy is quite the space eater in a lab!

The other option is to make a small volume of a much higher concentration solution. Then you can dilute that solution down to the concentration that you need. Often times you will need to dilute the higher concentration solution a significant amount which would again require making a larger volume of the solution at the concentration you need. However, we can use a series of smaller dilutions into smaller volumes to get to the volume and concentration that we desire. This is known as using serial dilutions.

While serial dilutions are a great way to save on both reagents and space in the lab, they have other experimental uses as well. We won't be covering those uses in this Webinar, but it is likely you will come across their uses if you work in the lab long enough.

Slide 17



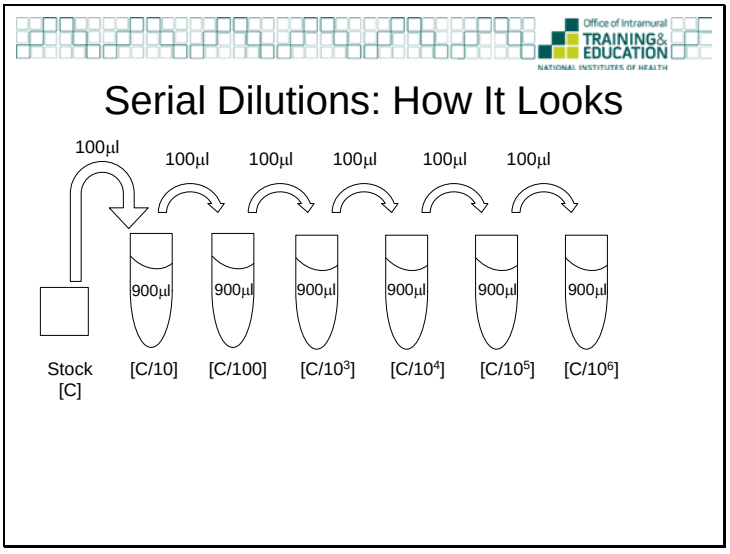
When preparing to perform serial dilutions, there are three main components to consider. Like many things in life, it is important to know where you are starting and where you hope to end up. In this case, you need to know the initial concentration you will start your dilutions with and you will need to know the final volume and concentration you will want.

You will also want to determine your dilution factor. The dilution factor is the factor by which you will dilute your sample at each step. Mathematically it is equal to the sum of the volume of your stock solution you are adding and the volume of the solvent you are making the dilution in, divided by the volume of your stock solution. So, if you are adding 100 microliters of your concentrated solution to 900 microliters of solvent your dilution factor would be the sum of 100 microliters plus 900 microliters divided by 100 microliters. This would mean your dilution factor is ten.

Finally you will need to know the number of dilutions you are going to use to get from your initial concentration to the final concentration at the volume that you want. Again, this can be determined mathematically. The number of dilutions is equal to the number of times the dilution factor will be multiplied by itself to equal the starting concentration divided by the final concentration.
So with a dilution factor of 10, 10 to the X power is equal to the starting concentration divided by the final concentration. In this case we will use 1 molar as our starting concentration and 1 micromolar as the the final concentration. So, 10 to the X power would be equal to ten to the sixth power and we would need to do six dilutions in this case to get to our final concentration. Please note that this example assumes all the dilution steps are the same; they need not be.

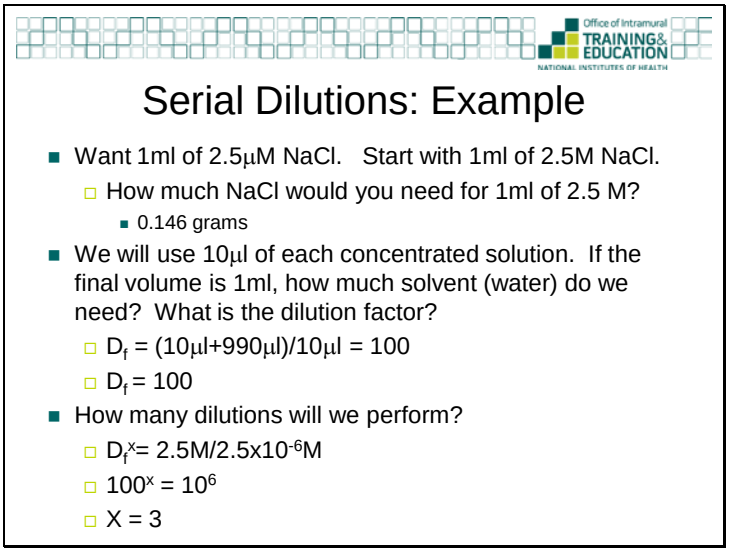
It is important to note the X may not be a whole number. In this case, you will either want to change your dilution factor, or use a combination of dilution factors. We will consider an example of that in a couple of slides.

Slide 18



Let's take a look at this particular serial dilution in cartoon form. We start with a stock solution with a high concentration. We determined we would perform dilutions of 100 microliters of the stock solution into 900 microliters of a solvent. This first dilution yields a new concentration that is one tenth that of the stock concentration. After assuring proper mixing, we will take 100 microliters from the first dilution and place it in 900 microliters of solvent in a new tube. This will again be a one to ten dilution, giving a concentration that is one one hundredth of the stock solution. This step is repeated four more times, assuring proper mixing at each dilution. Our final concentration then is equal to the stock concentration divided by our dilution factor of ten to the sixth power.

Slide 19



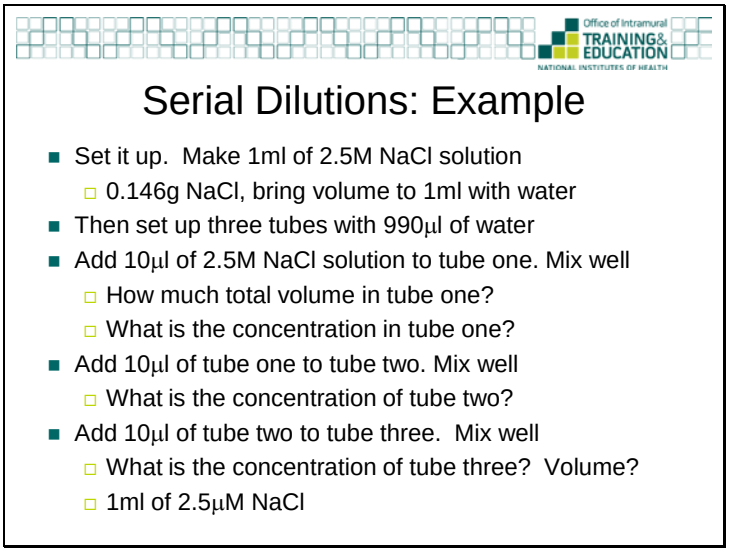
For our first example let's take a modified version of a previous example. We want one milliliter of two point five micromolar NaCl. To make this, we are going to start with one milliliter of two point five molar NaCl. For practice, how much NaCl will you need? (Pause) I hope you came up with zeri point one four six grams.

We will use 10 microliters of each concentrated solution. If we will have a final volume of one milliliter, how much diluent, in this case water, will we need in each tube? Knowing that, what is the dilution factor?

The volume of water is 990 microliters. Add this to the 10 microliters we will be adding and divide that sum by 10 microliters and we get a dilution factor of 100.

So, how many dilutions will we perform?
Again, the number of dilutions is equal to the number of times the dilution factor is multiplied by itself such that it is equal to the initial concentration divided by the final concentration. So 100 to the x will equal 10 to the six. 100 is also equal to ten to the two. Therefore, 100 to the X is equal to 10 to the 2 times X. If ten to the two X equals ten to the six, then X equals three. In this case, we will make three sequential or serial dilutions.

Slide 20



OK…now it is time to set it all up and work through the serial dilutions, step by step. We have already done the first step, which is to plan it out. The next step is to set it up. First, make up your solution with your starting concentration. In this example, we need to make up one milliliter of two point five molar NaCl solution. Last slide we determined that we need one point four six grams of NaCl. Weigh it out and place it in a tube and bring the volume to one milliliter with water.

Now, set up your tubes for a serial dilution. We know we will need three dilutions. This means we will need three tubes, each one with 990 microliters of water.

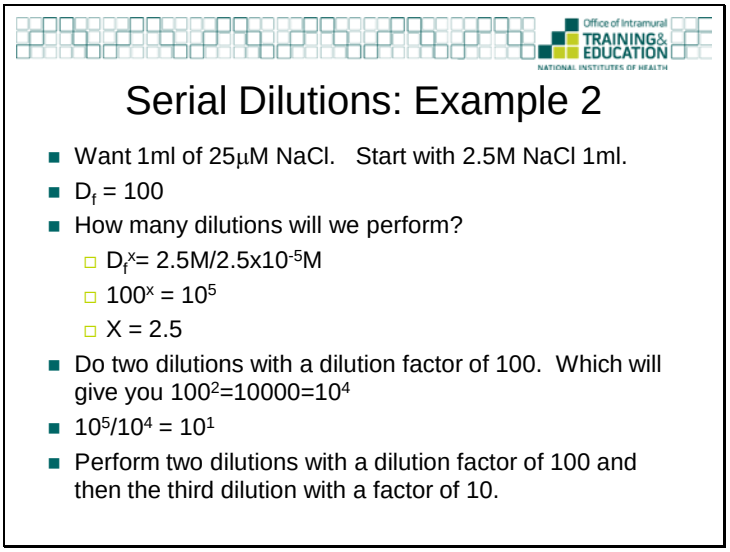
Add ten microliters of the two point five molar NaCl to tube one and mix well. Now, how much total volume is in tube one? One milliliter. And…what is the concentration of tube one? If the dilution factor is 100, we should now have a 25 millimolar NaCl solution.

Now, add 10 microliters from tube one to tube two and mix well. You will again have one milliliter total volume in tube two now, but what concentration do you have? 250 micromolar

Again, add 10 microliters from tube two to tube three and mix well. And the final concentration and volume of tube three is…

One milliliter of two point five micromolar NaCl.

Slide 21



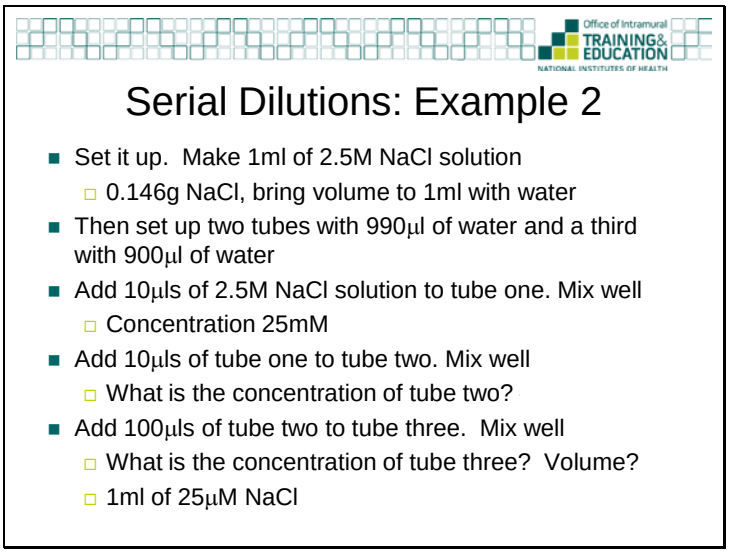
I mentioned earlier that sometimes x, the number of dilutions required, will not be a whole number. To look at a situation like that, we will make a small modification to the last example. This time, we want one milliliter of twenty five micromolar NaCl, again starting with one milliliter of two point five molar NaCl. We will again use a dilution factor of 100.

So, how many dilutions will we perform?
Again, if the number of dilutions is equal to the number of times the dilution factor is multiplied by itself to equal the initial concentration by the final concentration, then 100 to the x will equal 10 to the fifth. In this case, we will need to make two and a half dilutions. So, what happens now?

The easiest thing to do is to start with the integer to the left of the decimal point, which in this case is two, and make two dilutions with a dilution factor of 100. If you plug that back in, you get 100 squared which is ten thousand, or ten to the four. If you divide the 10 to the five you needed to dilute your sample in total by the ten to the four that you will do with your first dilutions that leaves you with a factor of ten. So, you can dilute your second dilution by ten to get the final concentration that you need. So you can perform two dilutions with a dilution factor of 100, and then do the third dilution with a factor of ten.

Let’s take a look at this in practice.

Slide 22



Again, let's set this serial dilution up step by step. First, make up your solution with your starting concentration. Weigh out zero point one four six grams of NaCl and place it in a tube and bring the volume to one milliliter with water.

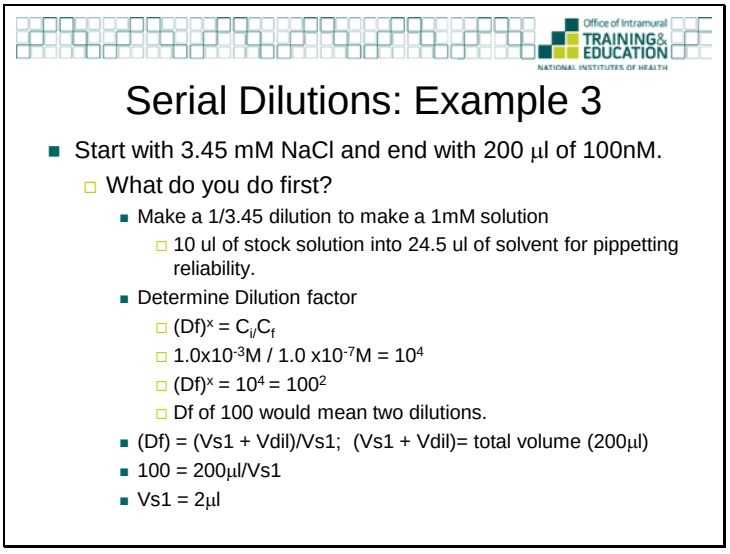
Now, set up your tubes for a serial dilution. We know we will need three dilutions; two dilutions with 10 microliters going into 990 microliters and one dilution with 100 microliters going into 900 microliters. This means we will need three tubes, two with 990 microliters water and one with 900 microliters of water.

Just like before add ten microliters of the two point five molar NaCl to tube one and mix well. The concentration is now 25 millimolar. Then, add ten microliters from tube one to tube two and mix well. What concentration do you have? 250 micromolar. This is an important step to double check and make sure your plan will work. Will diluting 250 micromolar by ten give you the desired concentration of 25 micromolar? Yes, it will!

Now you can add 100 microliters from tube two to tube three and mix well. And the final concentration and volume of tube three is…

One milliliter of twenty five micromolar NaCl.

Slide 23



So, lets look at something a little different. Often times you start with concentrations of your stock solution that are not factors of ten away from your desired final concentration. Take for example having a 3.45 millimolar stock solution of NaCl and wanting 200 microliters of 100 nanomolar NaCl.

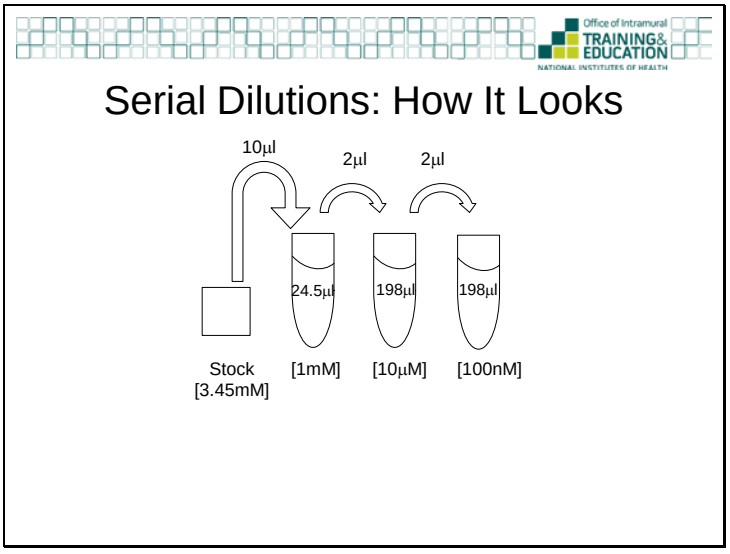
What would you do first? I would recommend making a dilution that makes the serial dilutions more simple by creating a concentration that IS factors of ten away from the desired final concentation. In this case, I would make a one to 3.45 dilution to give us a one millimolar solution. Note: I recommend that you do not use one microliter of your stock solution into two point four five microliters of solvent. That small of a volume can lead to pippetting errors. Use 10 microliters of your stock solution and 24.5 microliters of solvent.

Then, determine your dilution factor. You can either do this by setting the dilutions you want to do, or by looking at the equation $(Df)^x = C_i/C_f$ and then deciding on a strategy from there. To do this, simply divide the initial concentration by the final concentration. In this case 1mM (our new starting point) divided by 100 nanomolar to get ten to the power of four, or 100 squared. A dilution factor of 100 would mean two dilutions.

Go back to the equation (Df) = (Vs1 + Vdil)/ (Vs1) and realize that (Vs1+Vdil) needs to equal 200. You can then solve for Vs1 by plugging in the numbers and solve that Vs1 equals two and thus Vdil equals 198.

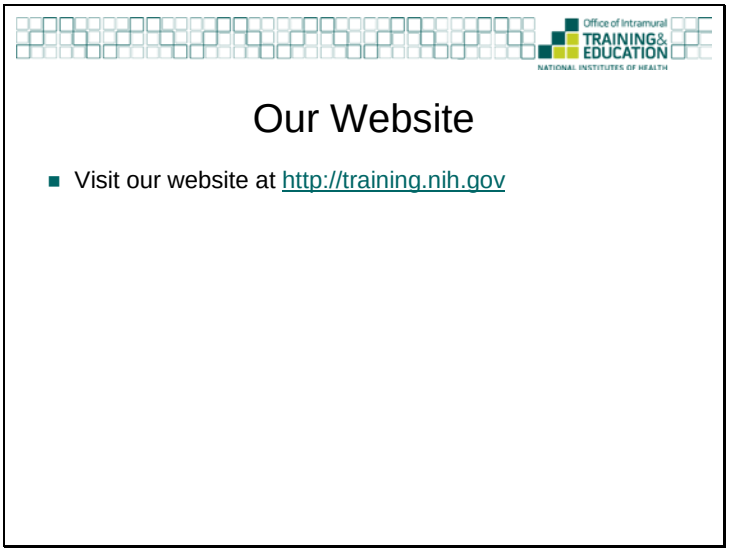
Now we just have to set it up from step one.

Slide 24



Our first step is to make our one to three point four five dilution. To do this, we add 10 microliters of our stock solution to 24.5 microliters of solvent. This give us a new concentration of 1 millimolar. After proper mixing, we would then add two microliters of this dilution to 198 microliters of solvent to give us a concentration of ten micromolar. Again, after proper mixing would transfer two microliters into 198 microliters of solvent and we would have 200 microliters of 100 nanomolar NaCl.

Slide 25



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