Instrumentation Familiarity: Using Micropipetters and Serological Pipettes

Introduction: In molecular biology we sometimes need to measure volumes as small as one *millionth* of a liter (a liter is about 1 quart). Devices called micropipettes are used for these measurements. Using them requires attention and a steady hand. Sometimes we are making volumes 1000 times or more larger than microliters, or milliliters (similar to a teaspoon). For measurements of 1-25ml we usually use serological pipettes, which come in volumes of 1ml, 2ml, 5 ml, 10ml and 25 ml. Serological pipettes are usually wrapped in sterile packages. A pump must be attached to one end of the pipette, which pulls up the solution as you turn the wheel. Generally a green pump will fit the 2, 5 and 10 ml serological pipettes, a blue pump is needed for the 1 ml pipettes, and a red pump is needed for the 25 ml pipettes. We will demonstrate the use of these devices as well. Then you will practice a lot.

Your Objectives:

1. Recognize the units (microliters and milliliters) and determine which measurement device you should use.
2. For the micropipetters be able to set the volume correctly.
3. For both devices accurately pull up and then deliver the desired volume.
4. Know how to check that you are doing it well (accuracy and precision!) using a mass balance and water
5. Know how to record your results and determine how reproducible you are when using the devices.

Background and Instructions

Proper Device Selection – Remember you are generally most accurate using volumes in the middle of the device range, the top of the range (maximum volume you can use without damaging the device) is shown by the number on the label and the lowest volume you should use is 10% of that number.

- The 10ul micropipette should be used with a maximum setting of 10ul and a minimum setting of 0.5ul (this is 5% instead of 10% but it is OK for this one)
- A 20ul pipetter should never be set to MORE than 20ul, and you should not set it to LESS than 2ul (this is 10% of the total).
- A 200ul micropipetter should never be set to MORE than 200ul and it should never be set to LESS than 20ul – if you need volumes less than 20ul you would use the 20ul micropipetter.
- The 1000ul micropipetter should be used with a maximum setting of 1000ul and a minimum setting of 100ul.

Proper Micropipetter Use

- After setting the volume, seat a tip of the correct size firmly on the end of the barrel (tap the barrel of the micropipetter straight down on the tip *gently* a couple of times.

- Holding the micropipetter upright, depress the plunger just to the stop point (you can push past this, but if you do you will pull up to much of the solution).
    - You should pre-wet the inside of the tip by slowly pulling the solution up and down several times.
- Keeping the plunger down, put the end of the tip *just below* the surface of the solution you are pulling from.
- Slowly and smoothly release the pressure on the plunger – - if you do it too fast the solution will 'pop' and you will get droplets high up in the tip that you cannot push out again, so you will deliver too little of the solution (this is called aspiration).
- Keeping the micropipetter upright, smoothly pull up the tip out of the solution tube and move it to the delivery tube/container.
- Touch the end of the tip to the side of the delivery tube, just <u>above</u> the level of any liquid that is already in it, and slowly and smoothly <u>depress</u> the plunger to deliver all of the solution to the new tube.
    - If there is another solution in the tube, you can rinse the inside of the tip by slowly pipetting up and down several times.
    - If there is a droplet at the very bottom of the tip you can expel it by pushing past the first stop on the plunger (this is the only time you should depress it that far).
- With the plunger still depressed , pull the tip straight up out the tube and dispose of it (either remove it with your fingers or use the additional ejector button on the micropipetter that pushes off the tip).

Proper serological pipette use

- The serological pipette should only be used within the range of marks shown on the side – note that many have two sets of marks, one on eaech side of the horizontal marks. One set gives the volume you have <u>pulled up</u>, and the other, going down the other side shows you the volume you will <u>deliver</u> if you fill it to the top line and go down.
- If the pipette is individually wrapped, open the top of the package at the end where the pump will be attached – sometimes there is a cotton plug in the top of the pipette. This end is not round and does not taper from the barrel of the pipette. Leave the bottom of the pipette in the wrapper until ready to put in the solution, it will keep it from getting contaminated.
    - If the pipettes are not individually wrapped, be sure that the bottom (tapered) end does not touch anything until you are ready to pull up the target solution.
- Select the appropriate pump, and attach to the end of the pipette, pushing down firmly and twisting slightly.
- Bring the target solution and the delivery vessel to your bench.
- Remove the pipette from the wrapper, insert the tapered end into the target solution, below the meniscus and roll down on the pump wheel to pull up liquid
- Be sure you know which set of measurement lines you are using, and be sure to use the bottom of the meniscus at eye level to make the measurement.

- Move the pipette to the delivery vessel, touch the side just above any liquid that is already in it, and slowly push the wheel on the pump up. This will slowly deliver the solution so that none sticks to the side of the pipette.
    - When you have delivered the desired amount, carefully remove the tapered end of the pipette from the delivery vessel.
    - If there is solution remaining in the pipette, discard it in a sink or waste container.
- Remove the pipette from the pump and dispose of it in the appropriate type of waste receptacle (regular waste for most of our material).
    - If you pulled solution up into the pump, rinse it with water and air dry it.

Notation details:

The abbreviation for microliter is ul. Because it is sometimes hard to read the difference between the abbreviation for milliliter (ml) and microliter (ul) when hand-written on a label, another abbreviation for a microliter is the Greek symbol lambda, λ. I also use the symbol gamma γ for micrograms (μg).

How do you know if you have properly used a liquid-measuring device? We are going to use the fact that water weighs almost exactly one gram per milliliter at sea level and 25°C and 1 milligram per microliter to let you find out immediately how good your liquid measuring skills are by weighing dispensed water on a sensitive microbalance. That means you will pull up your liquid sample, dispense it into a plastic container that has been pre-weighed ('tared') so the increase in mass will tell you how much liquid you deposited in the container. Once you get good at this you will practice measuring and delivering glycerol with is much 'stickier' and therefore harder to measure accurately.

Active Skills -

Part 1: Practice using micropipetters.

1. You will determine how accurately and precisely you can measure 80 ul, 180ul and 480 ul of water (or other settings – be sure to write them down) and subsequently the same volumes of 50% glycerol.
2. Take your micropipetters and tips to a microscale balance.
3. Make sure you identify next to each balance: a beaker of water, a 50-ml tube of 50% glycerol, a box of Kimwipes, some Parafilm squares and some small weigh boats.
4. For each volume required, set the correct micropipetter to the value indicated above.
5. Seat a tip on the micropipetter barrel.
6. Make sure the balance is turned on, that the units say 'gm' and that the balance is reading zero.
    a. Put the piece of Parafilm (paper side down, so you are delivering solution to the waxy side) on the pan of the balance and tare it (zero it).
7. Pull up water into the micropipetter tip, carefully move the tip over to the Parafilm on the balance pan.
    a. Gently depress the plunger to deliver the water to the Parafilm (the water beads up on the surface). Remove the pipette from the balance.
    b. Shut the window of the balance so air movement does not affect the measurement.
8. Record the mass delivered (again, for water, since it weighs about 1gm/ml, it gives you a fairly accurate idea about the volume of water you delivered)
    a. Record any observations about bubbles, droplets or other things that might have caused your delivery to be inaccurate.
9. If you re-tare the Parafilm with the liquid on it you can immediately make another measurement
    a. If too much water is on the balance it may start to run off the Parafilm, which will change the mass – in that case remove the Parafilm from the balance pan and gently blot up the liquid with a Kimwipe.
    b. For the larger volume measurements (0.5-1ml) you may want to use a weigh boat instead – tare that as you do the Parafilm.
10. Repeat each volume delivery at least twice (you can keep the same tip for this since it is just water), recording the masses each time.
    a. Continue until you can reliably deliver the same volume that is close to the target volume.
11. Repeat for each of the other micropipetters and target volumes.
12. Now repeat steps 4-11 using the solution labeled 50% glycerol (in a blue-capped 50 ml tube).
13. Take the average of all of your measurements and of your best 3 if you did more than 3.

14. Find the standard deviation of your measurements by subtracting the measurement you got from the average, taking the square of that number (so you don't have any negative values), adding up those squared values and finding the average of those values. Now take the square root of that mean and you have the standard deviation.
15. What percent of the average in step 13 is the number in 14?
16. What conclusions can you draw about your reproducibility and accuracy using the micropipetters. If your inaccuracy is high, is there another possible explanation for inaccuracy than your pipetting?

Part 2: Practice using serological pipettes and Pipette Pumps

Collect a 2, 5 and 10 ml serological pipette, and the green pipette pump. Take these and your notebook to an analytical balance.

1. Place the weigh boat on the analytical balance and zero it.
2. Attach the green pipette pump to the non-tapered end of the 2ml serological pipette.
3. Pull up 1.5 ml of water from the beaker.
    a. Determine if you think you have the meniscus lined up to give accurate measurement of the water (the bottom of the curve is at the measurement line, and your eyes are level with the surface of the liquid.
    b. Write down the volume you estimate you have measured.
4. Deliver the water to the weigh boat on the balance.
    a. Are any droplets left on the sides? In the tip?
    b. How can you remove them to deliver all of the liquid?
    c. Write down the mass delivered in your notebook.
5. Re-tare the weigh boat so the water in it is subtracted, then repeat the measurement process twice more, writing down all volume estimates and weights.
    a. How precise are you? (how close together are the measurements?)
    b. How accurate are you? (how close to the desired value did you come?)
6. Repeat with a 4.75ml measurement (take 3 independent measurements).
7. Repeat with an 8.8 ml measurement (take 3 independent measurements).
8. Repeat steps 1-7 using the solution labeled 50% glycerol.
9. Take the average and standard deviation of your best three measurements.
10. What conclusions can you draw about your reproducibility and accuracy using the serological pipettes. What can you say about the density of 50% glycerol? What can you say about the density of 100% glycerol?