Using Micropipetters and Serological Pipettes

Introduction: Micropipetters are used to measure volumes as small as one millionth of a liter (a liter is about 1 quart). Using them requires attention and a steady hand. We will demonstrate how to set the volume.

Remember

If you are using a 20ul pipetter you should never set it to MORE than 20ul, and you should not set it to less than 2u (this is 10% of the total).

Similarly, 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.

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 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). 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).

Try not to touch any of the edges of the solution container since you can cross-contaminate your reagents that way. Keeping the micropipetter upright, smoothly pull up the tip from the solution and move it to the tube you want to deliver the solution to.

Touch the end of the tip to the side of the delivery tube, just above the level of any liquid that is already in it, and slowly and smoothly depress the plunger to deliver all of the solution to the new tube. If there is another solution in the tube, you can rinse 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).

When there is no liquid left in the tip, pull the tip straight up out the tube and dispose of it (either remove it with your fingers or use the additional button on the micropipetter that pushes off the tip).

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.

A microgram is one-millionth of a gram. We may also use the Greek symbol gamma, γ for microgram.

Practice using micropipetters.

1. Pour water into a small beaker – 20-30 ml will be plenty.

2. You will measure 18ul, 80 ul, 180ul and 480 ul. For this you will need a 20ul micropipetter, a 100ul micropipetter, a 200ul micropipetter and a 1000ul micropipetter. Make sure you have the correct tips for each – the boxes are usually labeled.

3. For each volume required, set the correct micropipetter to the desired value.

4. Seat a tip on the micropipetter barrel.

5. Take the paper cover off a piece of Parafilm (it has the printing on it). Take it, with your micropipetter and beaker of water to an analytical balance.

    a. Make sure the balance is turned on, that the units say ‘gm; and that the balance is reading zero.

    b. Put the piece of Parafilm on the pan of the balance and tare it (zero it).

6. 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 (it is waxy so the water beads up on the surface).

    b. Shut the window of the balance so air movement does not affect the measurement.

7. Record the mass delivered (since water weighs about 1gm/ml it gives you a fairly accurate idea about how 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.

8. Remove the Parafilm from the balance pan and gently blot up the liquid with a Kimwipe.

9. Place the Parafilm back on the balance pan and re-tare it.

9. Repeat the same volume delivery (you can keep the same tip for this since it is just water) twice more, recording the masses each time (or until you can reliably deliver the same volume that is close to the target volume).

10. Repeat for each of the other 3 micropipetters and target volumes.

11. Take the average and standard deviation of your best three measurements.

12. What conclusions can you draw about your reproducibility and accuracy using the micropipetters. Is there another possible explanation for inaccuracy?

Using serological pipettes: 1ml, 2ml, 5 ml, 10ml and 25 ml.

Serological pipettes are usually wrapped in sterile packages and measure thousandths of a liter instead of millionths.

A pump must be attached to the end, 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.

Collect a 2,5 and 10 ml serological pipette, the green pipette pump and a beaker of water (tap water will do) and a weigh boat. 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-pointed end of the 2ml serological pipette.
3. Pull up 1.5 ml of water from your 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 in your notebook.
5. Remove the weigh boat from the balance and empty the weigh boat back into your beaker, dry the weigh boat with a lab tissue, 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).