

# Introduction to micropipettors and centrifuges



## A. Objectives

Become familiar with the functions and proper uses of micropipettors and centrifuges.

## B. Before coming to lab

Read this lab exercise. You do not need to prepare a protocol.

## C. During lab

1. Take notes during your instructor's demonstration of the different centrifuges.
2. With a partner, answer the review questions in Part E.
3. Carry out the exercise in Part H. Report your results in your notebook.

## D. Background Centrifugation

Particles suspended in a liquid will move at a rate which depends on:

1. the applied force (e.g. gravity);
2. the **density** difference between the particles and the liquid;
3. the size and shape of the particles;
4. the **viscosity** of the liquid.

If you want to speed up the process of sedimentation, you can increase the force by using a centrifuge. In a centrifuge, your sample is spun about a central axis with centrifugal forces that are multiples of earth's gravity. Particles of different size, shape or density will sediment at different rates, depending on the speed of rotation and their distances from the central axis. After slow (short) centrifugation, the **pellet** will be composed of the largest particles, while the **supernatant** contains the smaller particles. If you respin the supernatant, a new pellet will form, containing smaller particles.

We will be using three different types of centrifuges.

- (a) Low speed (clinical) centrifuges (Fig. 1) with a maximum speed of 3000 – 6000 rotations per minute (r.p.m). This centrifuge is used to harvest cells, larger organelles and coarse precipitates (e.g. antibody-antigen complexes).
- (b) Microcentrifuges (microfuges) with a maximum speed of 12000 r.p.m. They are used to sediment larger particles in small volumes over short periods of time.
- (c) High speed centrifuges with maximum speeds of 30000 r.p.m. They are used for smaller organelles and biological macromolecules.



**Figure 1:** Clinical centrifuge

### **Vocabulary**

Density: the mass of a substance per unit of volume ( $\text{kg m}^{-3}$ )

Viscosity: a measure of the resistance to flow that a fluid offers when subjected to shear stress

Pellet: settled particles that have precipitated out of a liquid

Supernatant: clear liquid remaining when a precipitate has settled

### **General precautions**

1. Place centrifuge at some distance from plug so that you can pull plug in case the centrifuge starts vibrating.
2. Choose appropriate tube size and materials for your application.
3. Fill containers to the appropriate level.
4. Never use tape to label tubes, remove writing with alcohol.
5. Balance each pair of sample tubes.
6. Make sure outside of tubes and sample chambers are dry.
7. Bring centrifuge up to operating speed by **gentle** acceleration.
8. If the centrifuge vibrates at any time during use, switch off and find the source of the problem.
9. Open centrifuge only after the rotor has stopped.
10. Clean spilled samples. Close lid.

### **Special precautions for our clinical centrifuge**

11. Metal shields and rubber pads must be the same shape and weight for opposite tubes (shields have their weight stamped).
12. Remove all unused shields, look for rubber pads.
13. Advance speed control knob one position every 5 seconds for the first three positions.

**Special precautions for our micro(centri)fuges**

- 14. To open safety interlock, the power cord must be plugged into an electrical outlet, the “on-off” rocker switch in the back must be switched to “on”, and you must hold down the release button on the side of the machine.
- 15. Turn tubes in the rotor so that Eppendorf tube hinges are facing outward.
- 16. An empty tube might be sufficient for balance.

**Special precautions for our high speed centrifuges**

- 17. This is the heaviest, fastest, and most dangerous of our centrifuges.
- 18. Never leave the lid open if centrifuge is unattended.
- 19. Separate cover must be in place before operation.
- 20. To start out, set switch to AMPS.
- 21. Next, set timer to desired time or to hold.
  - Turn speed knob to zero – pilot light should turn on.
  - Slowly advance speed knob while watching the AMPS reading – **do not exceed 10 AMPS** – switch **temporarily** to rpm, but go back to AMPS before advancing the speed knob if more speed is needed. When the correct speed is reached, set the timer.
- 22. After done – **wait** – the rotor might take 5-15 minutes to slow down.

**E. Review questions**

- 1. The rate at which particles suspended in a liquid will move depends on the following four factors:
  - 2. Which of these factors do you alter when using a centrifuge?
  - 3. By centrifuging a mixed suspension of particles at a specific speed for a particular time, the mixture will be separated into a \_\_\_\_\_ and a \_\_\_\_\_.
  - 4. Explain "differential sedimentation".
  - 5. Name the three centrifuges we will be using, their speeds and volumes accommodated


- 6. Why do you have to balance opposite tubes?
- 7. How do you balance opposite tubes?

## F: Background (Micro)pipettors

We will be using **graduated pipettes** (Fig. 1) to transfer 0.1 ml to 10 ml of liquids. Note the volume scale before using the pipettes. Both the largest and smallest volumes that a pipette can measure are marked on the upper end of the pipette. For example, “5 ml in 1/10” means that the pipette can measure a maximum of 5 ml. The smallest increment it can measure accurately is 1/10 ml. For 5 ml and 10 ml pipettes, hold the pipette at eye level – the volume must be read at the bottom of the meniscus.

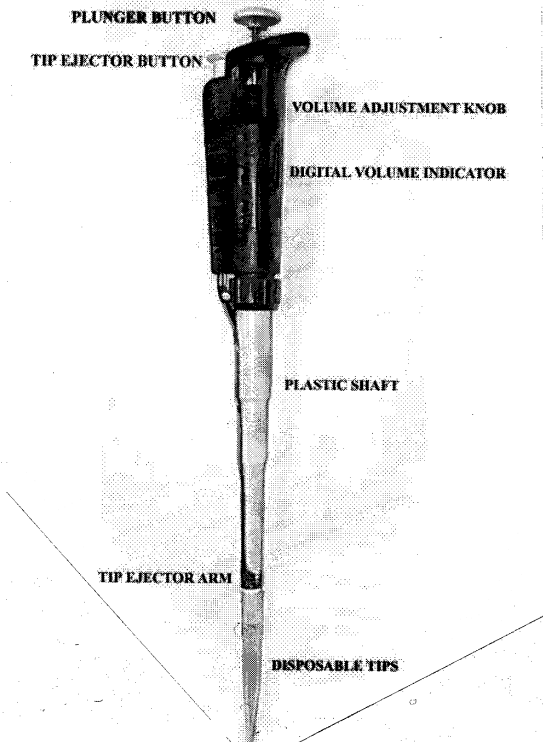


Figure 2: Diagram of a micropipettor

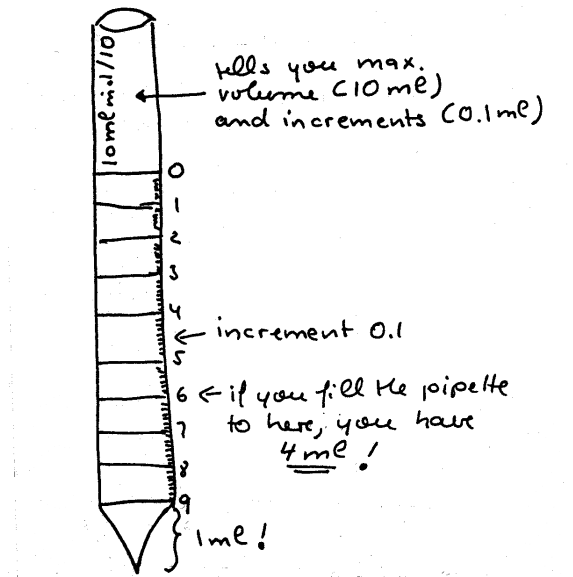
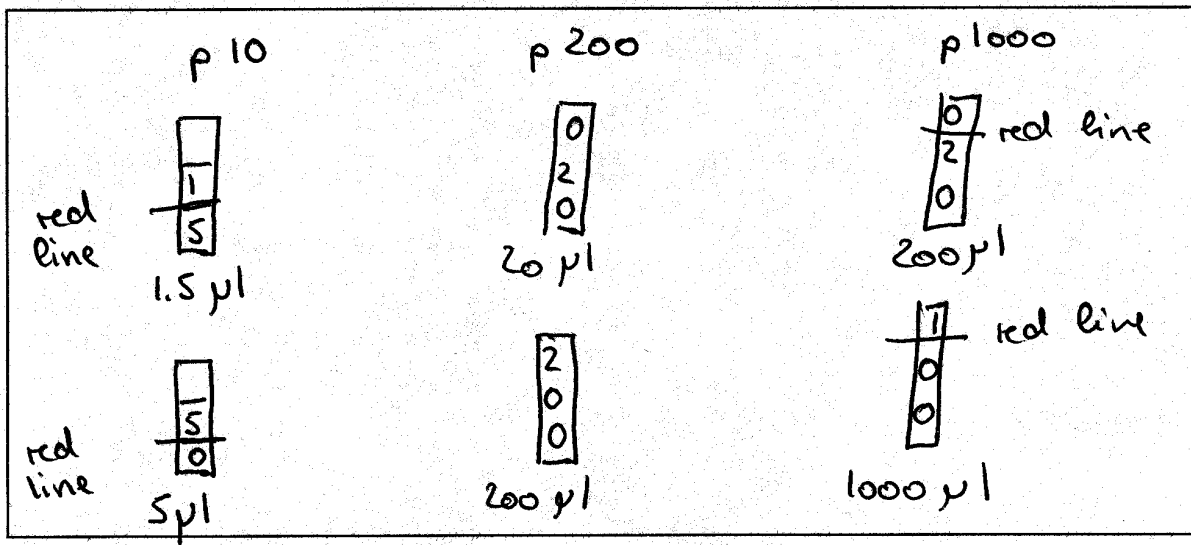


Fig. 1: Graduated pipette

The micropipettor is used to transfer small amounts ( $< 1$  ml) of liquids. The scales on micropipettors are in microliters ( $1000\mu\text{l} = 1$  ml). Our micropipettors come in three sizes which are capable of pipetting three ranges of volumes: **P10** = 0.5 - 10  $\mu\text{l}$ , **P200** = 20 - 200  $\mu\text{l}$ , and **P1000** = 200 - 1000  $\mu\text{l}$ . They are used in conjunction with disposable (often sterile) plastic tips; the smaller two micropipettors (P10 and P200) require the yellow tips and the P1000 pipettor uses the larger blue tips. The following is an illustration of a micropipettor:

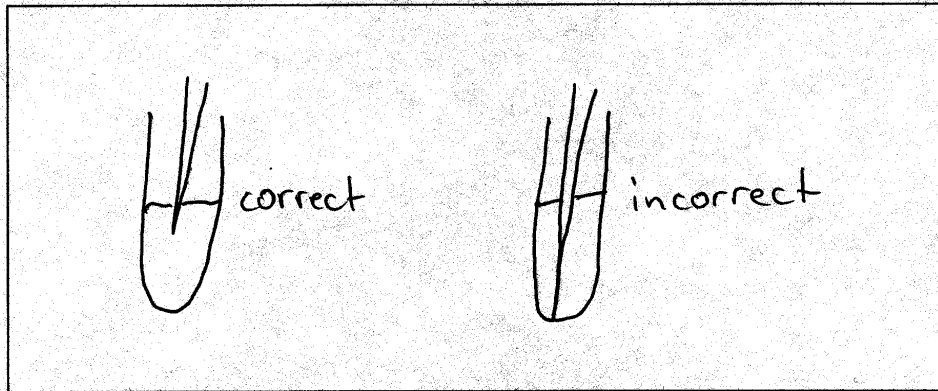
### G. Directions for use of the micropipettor:

1. **Never exceed the upper or lower limits** of these pipettors. They are very expensive and delicate instruments which we cannot afford to damage. The limits are:  
**P10:** 0.5 to 10.0  $\mu\text{l}$     **P200:** 20 to 200  $\mu\text{l}$     **P1000:** 200 to 1000  $\mu\text{l}$
2. Set the desired volume by turning the centrally located rings clockwise to increase volume or counterclockwise to decrease volume. Some examples are provided below:



**Figure 3:** Micropipettor volume settings

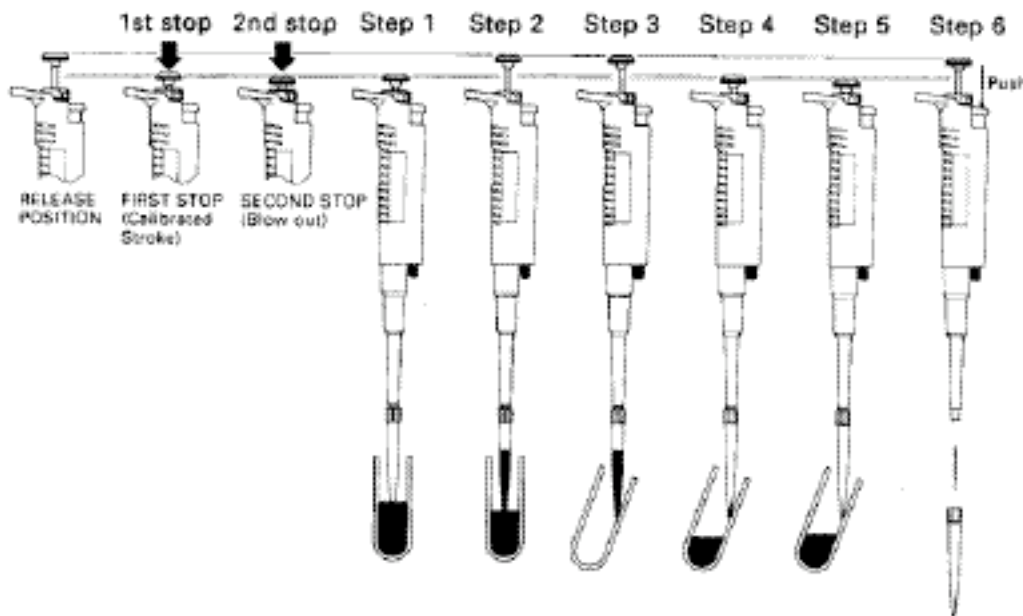
3. Place a tip on the discharge end of the pipettor. NOTE: If sterile conditions are necessary do not allow the pipette tip to touch any object (including your hands).
4. The plunger will stop at two different positions when it is depressed. The first of these stopping points is the point of initial resistance and is the level of depression that will result in the desired volume of solution being transferred. Because this first stopping point is dependent on the volume that is being transferred, the distance you have to push the plunger to reach the point of initial resistance will change depending on the volume being pipetted. The second stopping point can be found when the plunger is depressed beyond the initial resistance until it is in contact with the body of the pipettor. At this point the plunger cannot be further depressed. This second stopping point is used for the complete discharging of solutions from the plastic tip. You should not reach this second stop when drawing liquid into the pipettor, only when expelling the last drop. Before continuing, practice depressing the plunger to each of these stopping points until you can easily distinguish between these points.
5. Depress the plunger until you feel the initial resistance and insert tip into the solution, just barely below the surface of the liquid and not as deep as possible.



**Figure 4:** Withdrawing a sample with a micropipettor

6. Carefully and slowly release plunger. NOTE: If the solution you are pipetting is viscous, allow the pipette tip to fill to final volume before removing it from solution to avoid the presence of bubbles in the plastic tip which will result in an inaccurate volume. If you release the plunger too quickly, it will suck liquid up into the pipettor and damage it.
7. Discharge the solution into the appropriate container by depressing the plunger. This time, depress the plunger to the point of initial resistance, wait one second, and then continue pressing the plunger as far as it will go in order to discharge the entire volume of solution. Do not insert tip into any liquid already present in the container. Drag the tip of the micropipettor along the wall of the container to remove any drops hanging off the tip. Withdraw the micropipettor
8. Remove tip by pressing down on the tip discarder.

REMEMBER TO CHANGE TIPS BETWEEN SOLUTIONS TO AVOID MIXING OR CONTAMINATING THE SOLUTIONS USED!!



**Figure 5:** Steps in micropipetting

## H. Micropipettor exercise

1. Obtain three microfuge tubes.
2. Label the tubes 1 through 3.
3. Choosing the appropriate micropipettors and tips, dispense the following amounts of colored water into the tubes:

	Tube 1	Tube 2	Tube 3
red	5.5 $\mu\text{L}$	82 $\mu\text{L}$	116 $\mu\text{L}$
green	125 $\mu\text{L}$	8 $\mu\text{L}$	550 $\mu\text{L}$
yellow	800 $\mu\text{L}$	650 $\mu\text{L}$	6 $\mu\text{L}$
Total volume			

4. To check the accuracy of your measurements, set the 200 - 1000  $\mu\text{l}$  pipettor to the appropriate total volume for each tube and withdraw the contents of the microfuge tube into the pipettor. If you have measured correctly, the entire contents of the tube should be withdrawn into the pipette and there should be no bubbles in the pipette tip.
5. If you measured incorrectly, repeat the practice exercise.
6. Report your **numerical** results in your notebook.