OPEN EDUCATIONAL RESOURCES @ DE MONTFORT UNIVERSITY, Leicester UK

TYPES OF LABORATORY PIPETTE

BY DR GRAHAM BASTEN

ASSOCIATED RESOURCES ON YOUTUBE

Part 1 of 3: Types of pipette
http://www.youtube.com/watch?v=_PoYvQTxaBk

Part 2 of 3: The pi-pump pipette
http://www.youtube.com/watch?v=m6zi_wJBVZc

Part 3 of 3: Micropipettes
http://www.youtube.com/watch?v=-WBRW2cwGiY

This document is a transcript of the above video with additional explanatory notes and diagrams.

PART 1 OF 3: TYPES OF PIPETTE

So in this session we are going to look at dispensing liquids and there are three different types of pipette that you'll see in the laboratory, and they each increase in sensitivity and accuracy. The first one is a plastic Pasteur or bulb pipette. The second is a graduated pipette and a pi-pump. And then the third type are the micropipettes which come with separate pipette tips.

The first one we are going to talk about is the plastic pipette; this one will dispense 1 ml. It is not very accurate but it is quick. So if we need to dispense roughly 1 ml or 0.5 ml then this is ideal. So in order to draw the liquid up, squeeze the air from the bulb at the top with your finger and your thumb, put it into the liquid and gently draw it up until it reaches this 1 ml point here. But this isn't very accurate – this is just for speed.

So to dispense, obviously we are wearing gloves because we don't know what this chemical is, you unscrew the cap of the vessel you are going to put it into, and then just gently squeeze the top (the bulb).

So to improve accuracy we can use the surface tension of the liquid. To do this we take the tip of the pipette and we place it on the edge of the vessel and gently squeeze the tube and you can see the droplets here are dragging down using the surface tension of the liquid. If I squeeze it like this (squeezes the bulb all of a sudden), not only does it shoot out of the top which is why I am wearing gloves, there are droplets around the edge of the vessel and there are also droplets still inside the pipette which is not very accurate.

PART 2 OF 3: THE PI-PUMP PIPETTE

Equipment: Air-dispensing pi-pump plus graduated pipettes. The pump is a thumb-operated dispensing mechanism and a long glass or plastic pipette is attached for the dispensing of fluid. The pumps and pipettes come in a range of sizes typically dispensing 1 ml, 10 mls or 50 mls of fluid. Automatic devices called pipettors are used in tissue culture and are operated via rechargeable batteries for the smooth dispensing of fluid and for easing repetitive work.

Top tip: It is important not to draw fluid past the zero point on the pipette scale, and especially with an automatic pipettor it is damaging to the equipment to draw fluid into the pump itself which can happen by mistake.

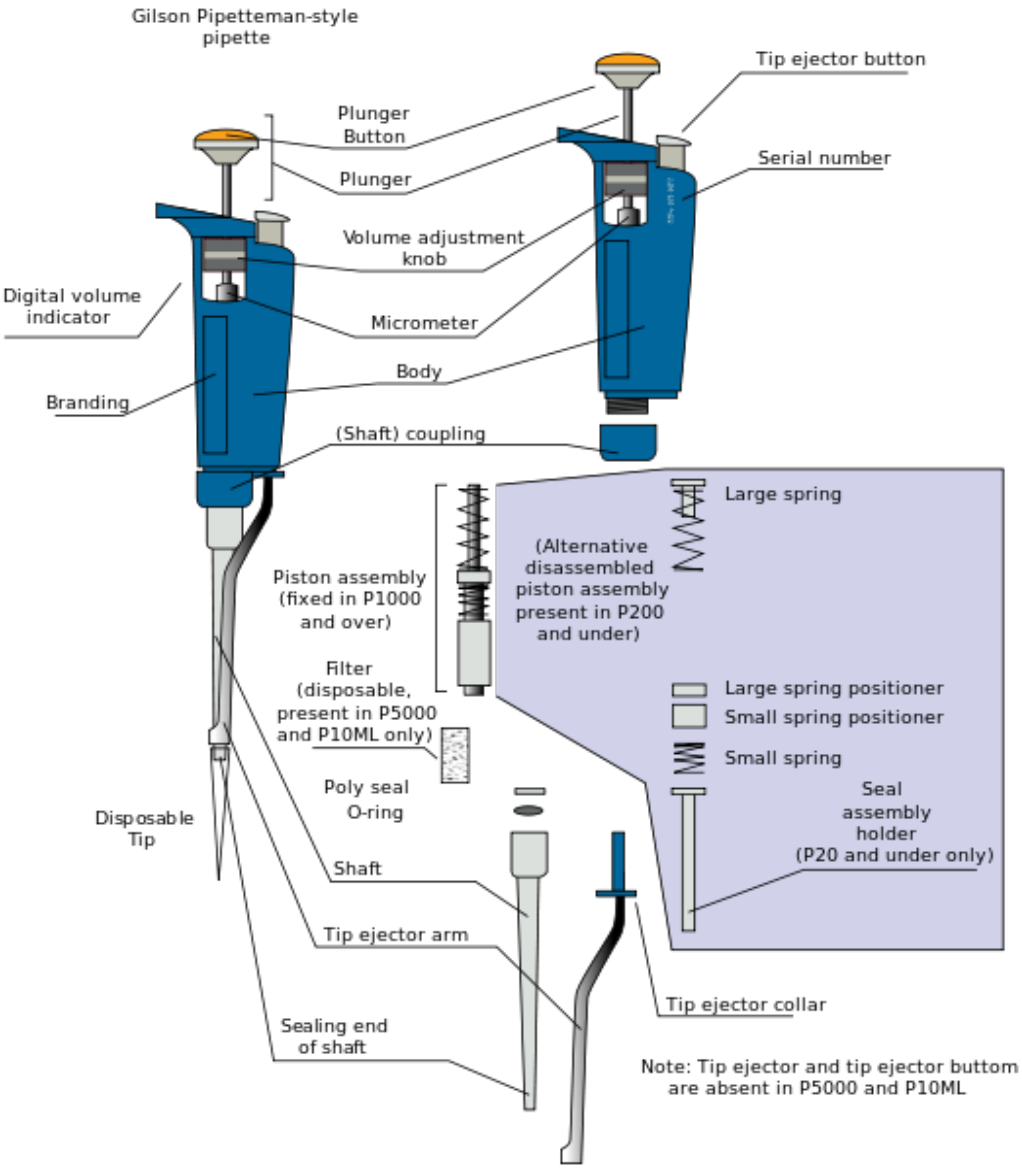
Safety: always dispose of the graduated pipettes in the correct manner as per your laboratory instructions.

So the next pipette to look at is called the pi-pump and this must be used with a graduated pipette. And there are various types of these and you will be shown which one to use in the laboratory. So in order to use this ensure that the first cap is pushed down to the bottom, and with your hand quite near the top because we don't want to break the glass (or often they are plastic), just very gently squeeze it into position, so it is held. Transfer over to the liquid and place the tip of the pipette into the liquid, and using your thumb, very gently begin to draw the liquid up to the required level.

So for this example I am going to dispense 1 ml. So we draw it up to naught (0); remember to check the meniscus (the flat base of the meniscus should be level with the measure you wish to dispense), and then to dispense it is simply a matter of reversing the thumb action back down to 1 on here (using the scale on the pipette).

This pipette is also ideal for making sequential dilutions. So we have this filled to naught (0) and because this is a 10 ml pipette we can therefore get ten 1 ml units from this pipette, and we do this by simply using the thumb moving the rotor down to 1, and then we can take it down to 2, down to 3 and so on. It is not advisable taking it past the 9 point to dispense because as you'll see as I get to that point, there is often an air bubble forms at the bottom, so never use this portion to dispense your liquid accurately.

PART 3 OF 3: MICROPIPETTES



Gilson Schematic by Squidonius. Available: http://en.wikipedia.org/wiki/File:Gilson_schematic.svg

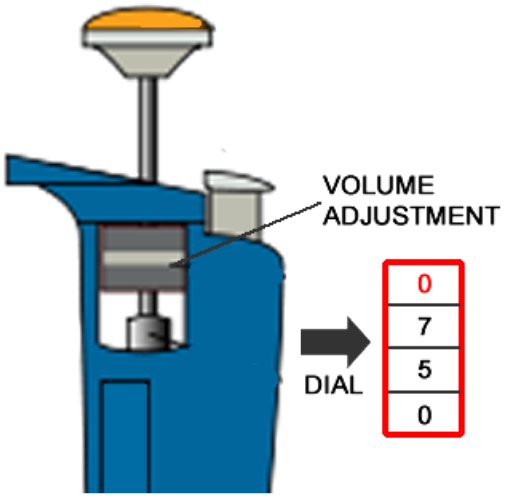
CC BY-SA 3.0

Equipment: Micropipettes come in a range of sizes and before dispensing you will choose the size appropriate to your needs. Each pipette will have its own appropriate set of tips. They range from dispensing 5 mls down to 1ul of fluid so are exceedingly accurate. For dispensing fluid into ELISA plates, a multiple micropipette may be used. The diagram by Squidonius provides a good understanding of the components and mechanics of a typical micropipette. The diagram below (Range of micropipettes by our team, HALSOER) shows some of the sizes of pipette available, and the volumes dispensed denoted by the figure on the top of the pipette usually in microlitres (µl).

P2, P10, P20, P100, P200, P1000, P5000 and P10000 (1 - 10 mls).

Range of micropipettes by HALSOER, De Montfort Univeristy
CC BY-SA 3.0

Once the size of pipette is selected, the volume required will be set on the volume indicator located on the barrel of the pipette. The diagram below adapted from Squidonius shows the location of the volume adjustment knob and the dial which is located on the barrel of the pipette. After use the tip is ejected using the ejector arm.



Micropipette volume setting. Adapted from "Gilson Schematic" by Squidonius.
CC BY-SA 3.0

Top tip: It is important not to draw fluid past the limit of the pipette tip into the device itself – this is often done by pipetting too quickly.

Safety: Pipette tips should be disposed of in the correct manner depending on the nature of the sample being pipetted.

So these are micropipettes often referred to as Gilson as a trade name, and these are extremely accurate but they are also very sensitive and very expensive, so care needs to be taken when using these.

The first thing we need to do is attach a pipette tip to the end and to do this we bring it down and very gently tap it in a vertical manner. Don't use excessive force and don't use any side to side lateral movement as this can damage the barrel (of the micropipette). You should see that the pipette can withdraw itself from the box without sticking to the side.

So in order to draw up the liquid we first need to expel the air from the barrel. If you think about all these pipettes it is all about using air and surface tension to dispense fluid. So the first thing to do is push air out with your thumb (by depressing the main stopper) until it reaches the first resistance point. Then insert the pipette into the liquid and simply return the plunger back to its original starting position. And this draws up the corresponding amount of liquid to the amount of air dispelled using the dial on the side of the pipette. In order to dispense the liquid push down to the first point slowly and then down to the second point so it won't go any further and then very slowly back to the top. When you do this return action it is important that the tip is not still in the liquid otherwise you will draw the liquid back out.

So again we can use surface tension to dispense the liquid so place the tip onto the edge and you can see the droplets are being drawn by surface tension, and again, at this point, my thumb is fully down to the second resistance point – the furthest position on the Gilson, and I can draw the tip away from the liquid and release. You should hear the click – and this is finished.