Basic Lab Skills

AP Biology

Success in the science laboratory often relies on the little things: using common tools to measure solids and liquids with accuracy, calculating solutions correctly, and using specialized equipment safely and accurately. AP Biology students who are careless in preparing solutions and in collecting their data often force their lab partners to repeat experiments, fail to complete experiments on time, and earn the lowest grades. You were taught the correct ways to do all of these important tasks. This booklet is to remind you on the use of common lab equipment.

I. Measuring solids

The most basic of skills, measuring out solids is also the easiest to perform with our digital balances. Keep these basic principles in mind when using the balance:

- Never put the material being massed directly on the metal pan. Always tare a weighing boat or weighing paper first, and then place the chemical onto that.
- Avoid returning extra chemical back into the original container. This can lead to contamination.
- Pay attention to the precision of the balance. Include all digits in the measurement, as they play a role in calculating significant figures (*e.g.*, record 24.10 g, not 24.1 g)

II. Measuring liquids

A. Volumetric glassware: volumetric flasks and graduated cylinders

Chemists solely measure liquids with volumetric flasks. Biologists most often use graduated cylinders. Keep these basic principles in mind when using graduated glassware:

- Never, ever measure liquids using a beaker. The graduations on the side of a beaker are mere estimates, and can often have a 10% error or more.
- Use glassware that is close in size to the volume you are measuring: if you need 90 mL of a solution, use a 100- or 125-mL graduated cylinder, not a 500-mL one. This allows for greater precision.
- Since biology is an aqueous science, all of our solutions are in water. When you read the volume, always read from the bottom of the meniscus.
- Pay attention to the significant figures! Remember, when you read a measurement, you include all the places you are certain of, and one estimated digit. Figure 2 is a close-up of a graduated cylinder containing 73.0 mL of solution. You are certain of the 7 and the 3, and since the meniscus appears to touch the three graduation precisely, you can estimate zero-tenths.

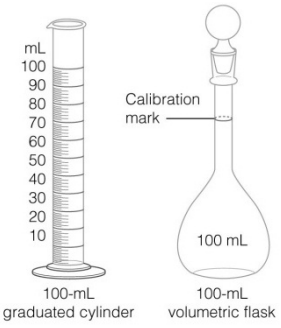


Figure 1. Volumetric glassware

Figure 2. Reading a graduated cylinder

B. Burettes

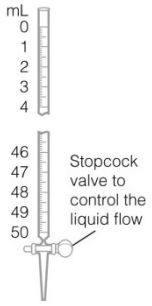


Figure 3. A burette

Burettes are read just like the graduated cylinder, though you must pay attention to the orientation of the graduations: notice that they are reversed from those normally encountered on graduated cylinders. Pay attention to this detail!

It is very important to get as precise a reading as possible from these devices, as they are always used to calculate an experimentally important result. Pay attention to the significant figures, and do not forget the final estimate value.

C. Micropipettes

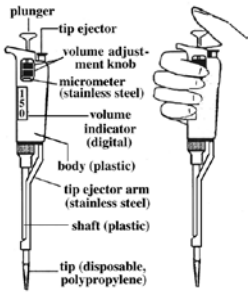


Figure 4. A micropipette

Digital micropipettes are used to measure precise volumes of liquid from between 1 mL to 0.1 µL. They are required pieces of equipment in the modern molecular biology laboratory. These devices are expensive (about $300 per pipettor) and easy to knock out of calibration, so care must be taken with their handling.

Setting the dispense volume

Different sized micropipettes deliver different ranges of liquid volume. You must choose the correct pipette for the job. The most commonly used micropipette in this course is the P-1000, which can deliver liquid in a range from its maximum of 1000 µL (thus the name, P-1000) to 10% of this volume, or 100 µL. Volumes exceeding this range are inaccurately delivered, and you can damage the device trying to set it.

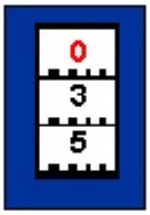


Figure 6. P-1000 set to 350 µL


Figure 5. Tip

You select the amount to dispense by setting a numerical display in the handle of the pipette. For the P-1000, you dial the first three digits of the measurement: for 1000 µL, you dial in 1 0 0. For 350 µL, you would dial in 0 3 5, as you see in Figure 6. If you had to set it to 352 µL (which is rare), you would use the fine ticks at the bottom to set the ones place.

Using the micropipette

Learning to use a micropipette is much simpler with one in your hand, so practice is important. Ask your teacher for an opportunity to practice.

- Once the micropipette is set to the correct volume, place a clean pipette tip on the end (these look like large, plastic needles). These tips ensure that you have no contamination when you take your sample—as a result, you should use each tip only once.
- Depress the plunger with your thumb until you feel a stop.
- Submerge the tip into the sample of the liquid you need to withdraw. Smoothly release your thumb: you should see the liquid being drawn into the tip.
- Eject the liquid into the destination container by pressing the plunger firmly with your thumb. You will push past the first stop you used to measure the liquid: this extra burst will blowout any remaining liquid from the tip.
- Discard the used tip.

III. Performing Common calculations

Molarity

You will often be required to prepare your own solutions in this course. If you make a solution incorrectly, it will affect your entire lab group and possibly the class. In biology, solutions are molar (*M*). A 1.0 *M* solution is 1.0 mole solute/ 1.0 L of solvent. Remember, there is no device capable of measuring moles of a substance. You must always convert between moles and grams, which can be measured on a balance.

For example, let us assume you need to make 200.0 mL of a 0.15 *M* NaCl solution. To do this, you would perform the following calculation:

$$200.0\ \cancel{\text{mL}} \bullet \frac{0.15\ \cancel{\text{mol NaCl}}}{1000\ \cancel{\text{mL}}} \bullet \frac{58.4425\ \text{g}}{1\ \cancel{\text{mol NaCl}}} = 1.8\ \text{g of NaCl in 200 mL of water.}$$

IV. Operating with Significant Figures

Significant figures are extremely important in scientific research. They prevent scientists from relying on imprecise data; in other words, it prevents scientists from tricking you into accepting their horrible work. It is accepted as a given that you cannot exceed the precision of a measuring device. For example, assume someone is timing a race with a stopwatch that gives you seconds to one decimal place *(e.g.*, 25.2 s). If the result of the race was a time of 25.2 s but the timer gives the time as 25.22341 s, you cannot believe their value. The stopwatch is not accurate to this level of precision, and the timekeeper is trying to pass off their result as more precise than they actually are.

While this idea is obvious with single measurements, you must be very careful when you are using multiple measurements in a calculation. Your result can only be as reliable as your worst measurement. We control for this by limiting the maximum number of significant digits in a calculation to the term with the fewest significant figures.

Look at the calculation illustrated above to find the grams of NaCl needed to make a solution. There are five terms in this calculation: 200.0 mL (4 significant figures), 0.15 mol NaCl (2 sig figs), 1000 mL (infinite sig figs, because by definition 1 L is 1000 mL), 58.4425 g (6 sig figs), and 1 mol (infinite sig figs by definition: there are always the same number of grams in a mole as there are amus in a formula mass). The fewest number of significant figures is two, from the moles of NaCl.

Students sometimes get very nervous about significant figures. Remember that you only need worry about them when you are operating on measurements. Since you need only refer to the numbers involved in the calculation, the number of significant figures in the answer comes easily: it is the fewest number of significant figures you multiplied or divided.

V. Using the Microscope

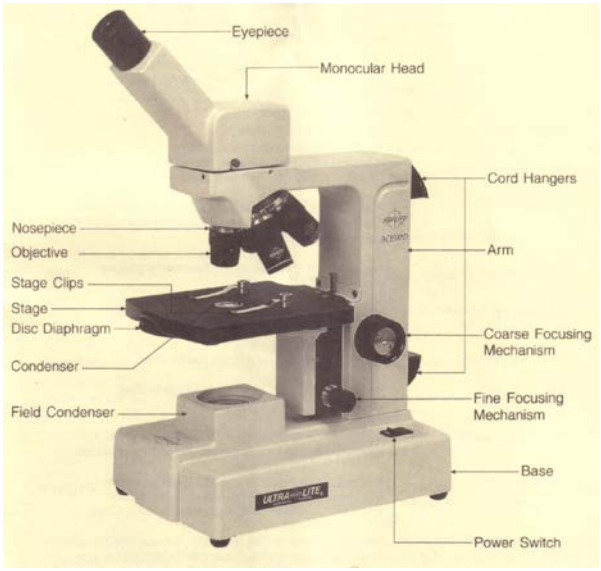
You are expected to use the microscope independently in the lab, and to measure samples under the eyepiece using the integrated ruler. You do not need to know how to use the oil immersion lens. Ask your teacher for a microscope and slide to practice.

Guide to Microscopy

The microscope is a very important tool in biological research. It allows you to study structures much too small to discern with the human eye. A high quality light microscope can magnify an image up to 2000× (though with slight distortion). You will be using a compound microscope in your biology studies this year. *Compound microscopes* contain more than one lens, typically an ocular lens in the eyepiece and an objective lens near the specimen. This guide is meant to serve as a reference throughout the year to aid your use of the microscope. Keep it with your biology notes so you can refer to it as needed.

Microscope structure

This diagram shows the microscope you will be using. You should become familiar with the various parts.



Your microscope also contains the following special features not on the diagram: a 100× oil immersion objective, an Abbe condenser, and a mechanical stage.

Magnification and Resolution

Your microscope has a 10× ocular (10× means magnifies 10 times). It has four objective lenses (red 4×, yellow 10×, blue 40× and white 100×). Because you have a compound microscope, you must take into account both lenses to determine the magnification of the image.

Total magnification = magnification of ocular • magnification of objective

The quality of a microscope is determined not by its magnification, but by its resolution. A microscope's *resolution* is its ability to distinguish clearly two closely spaced points as separate points.

Use and care of the microscope.

- Always carry the microscope with two hands, one under the base and one grasping the arm.
- Before and after use, always clean the ocular lens and all the objective lenses. *Only use lens paper!* Even Kim-wipes or Kleenex can scratch the lens.
- Never touch the lenses with your fingers. The normal oil on your skin can attract dirt and dust, which can scratch the lens.
- Clean up any spilled chemicals on the stage or substage condenser immediately, especially if they contain stains.

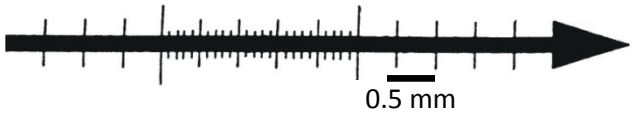
Focusing the microscope

This is a *parfocal microscope*, meaning that you need only slightly adjust the fine focus to bring the image into view when you change the objectives. If you are having a hard time focusing your scope, follow these steps:

1. Center your slide over the condenser using the mechanical stage.
2. Even if you require higher magnification, use the large coarse focusing knob to focus first using the low power 10× objective. It is much easier to focus the scope at lower magnifications.
3. Switch to higher magnification. Make sure that the nosepiece "clicks" into place.
4. Look through the eyepiece and slowly focus the image using the small fine focusing knob.

   Hint: *remember that as you go from low to high power, you see only a small part of the field you were viewing before. You may have to move the slide with the mechanical stage to recenter the object you were studying.*

Measuring with the microscope



One powerful tool your scope possesses is a ruler integrated into the moveable pointer in your eyepiece. Each regular mark is 0.5 mm long. Simply move the object you want to measure until it is alongside the arrow, and then measure it as you would with a ruler.

You **must** make adjustments due to the magnification by the objective lens:

- *To get the true measurement with the 10× objective, divide the scale reading by 10.*
- *To get the true measurement with the 40× objective, divide the scale reading by 40.*
- *To get the true measurement with the 100× objective, divide the scale reading by 100.*

Oil Immersion

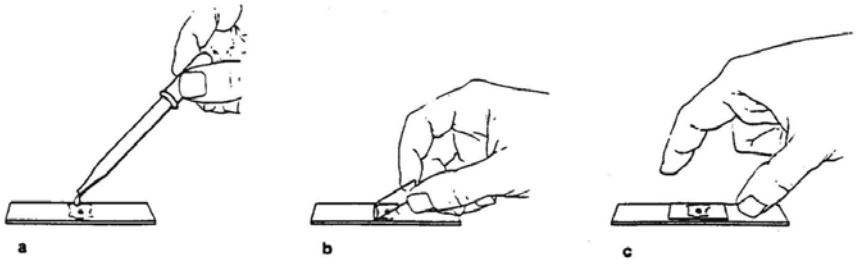
When working at very high magnification, the rays of light from the sample are so tightly focused that they can be bent out of focus simply by passing through the air. To prevent this from happening, the space between the slide and the bottom of the objective is replaced by a drop of clear oil. This allows the microscope to resolve the image at 1000× magnification. To use the oil immersion lens:

1. Center the object you want to look at under the 40× objective.
2. Turn the nosepiece halfway, moving the 40× objective out of the way so you have room to work.
3. Place a drop of immersion oil on the coverslip in the area that would be under the objective.
4. Carefully turn the 100× objective into place. It should end up very close to the slide, but should not hit either the slide or the coverslip.
5. Very carefully focus the image using the fine focusing knob.

Hint: *You may have to adjust the light with the iris diaphragm under the stage to see the image with the greatest detail.*

Wet Mounts and Staining

The wet mount is the most common technique you will use to prepare samples for viewing under the microscope.



1. Get a *clean* slide and cover glass.
2. If the sample you are studying is liquid (like pond water), place a single drop on the center of the slide.
3. If the sample is dry, place a drop of distilled water on the slide. Using a toothpick or other tool, mix the sample you wish to study into the water drop.

   Hint: *Your samples must be transparent so that light can pass through. This requires very small or thinly-sliced samples.*
4. Grasp the cover glass by the edge. Holding the glass at a 45° angle, slowly lower it over the water drop. Do not drop it—you want to avoid trapping air bubbles.

Often you must stain the sample in order to see the cells clearly under the scope. If you are going to stain the sample, add the stain before you lower the cover glass. Be careful not to add too much stain.