The Use of
General Lab Supplies & Equipment
Biol 2402 Lab

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I. General Equipment and Supplies

When doing the lab exercises in this class you will use the items below. You should gain some level of familiarity with them before you actually need to use them in the lab. This will save you considerable time and possible confusion later on.

Be able to locate, identify and know how to use the following items:

Electronic Balance	Graduated Cylinder	Volumetric Pipettes
Beaker	Pipumps/Bulbs	Pasteur Pipette and bulb
Erlenmeyer flask	Transfer Pipettes	Test Tubes and rack
Eppendorf tube	Petri dish (glass/plastic)	Spot Plate
Squeeze bottle	Parafilm	Weighing Boats
Microscope slides	Cover slips	Water Bath
Oven/Incubator	pH meter	iWorx Station

II. Making Volume measurements of Liquids

In many labs you will need to handle and measure solutions. There is more than one way to do this. For example, liquids can be manipulated using a graduated cylinder, a pipette or a beaker; the method you use depends on the amount and the accuracy required for the particular technique.

For small volumes such as 1 to 10 ml, using a calibrated pipette is the most accurate method. Graduated cylinders can accurately measure larger volumes; if exact amounts are not critical one can measure small amounts by counting drops from an uncalibrated pipette or estimating larger volumes using beakers of various sizes.

Before using solutions, it is always a good idea to gently shake the bottle of test solution, unknown, indicator reagent, etc. but before shaking make sure the cap is tightly sealed.

When mixing solutions a test tube or small bottle can be gently rolled between the hands or placed on a vortex mixer.

Learn to properly read the **meniscus** to accurately measure solutions

Never pipette by mouth, use a bulb or pipump

Never use a pipette for more than one kind of solution

Flasks and Beakers
: are crudely marked and are not normally used to measure volumes; the marks can be used when the amount needed is not critical but must be roughly proportional to other amounts used.

Centrifuge and test tubes
: some centrifuge tubes are calibrated and can be used to measure volumes of fluids such as urine or plasma

Graduated cylinder
: good for work that does not require a high degree of precision but more than when using a beaker or flask. Since water wets glass, the surface of the water will form a meniscus. Know how to properly read a meniscus.

Volumetric Pipettes
: are usually calibrated in milliliters and tenths of milliliters. They provide a much more accurate of smaller quantities(~.5 to 20 ml). Again read the bottom of the meniscus while holding it at eye level when measuring liquids

Transfer Pipettes and **Pasteur Pipettes**

generally not calibrated and used when only a few drops need to be transferred or roughly equivalent amounts of the same solution into different containers
The '1 ml' plastic transfer pipettes can hold a maximum of about 3 ml including the bulb
The droppers of the 30 & 60 ml brown glass dropper bottles hold about 2 ml when full & 1 ml when half full

Micropipettor

The most accurate way to measure small volumes in the microliter range
Different brands of micropipettors may show the amount to be delivered in different ways.

How it works:

There are two buttons that can be pressed down on the top end of the micropipettor. One is a "measuring plunger" that you will use to withdraw and then expel a small sample of liquid. The other is a "tip ejector" that allows you to quickly and easily pop off the disposable plastic tip. Operate both buttons several times until you are familiar with which button controls which function.

In order to measure an amount accurately, you must develop a good feel for the "first stop" and "second stop" of the micropipettor plunger. Press the plunger down gently until you feel some resistance. this is the first stop. Now continue to press harder until you cannot press any further. This is the second stop.

Practice using the plunger correctly with an Eppendorf tube containing water dyed with red food color: Press the plunger to the first stop, insert the tip into the solution to be transferred, and then slowly release the plunger

Procedure:

1. Place a plastic tip on the end of the micropipettor. Never use a micropipettor without a tip – this will ruin it.
2. Set the amount to be transferred. The number in the calibration window represents microliters of fluid. The white line represents the decimal. Have your instructor check that your micropipettor is set correctly before proceeding.

Problems:

1. Avoid bowing bubbles: make sure that the plunger is pressed to the first stop before you insert the tip into the solution that you are withdrawing.
2. Avoid Drawing air into the sample: make sure to insert the tip deep enough into the liquid to be transferred so that the tip remains submerged until the plunger is completely released.
3. Avoid creating a vacuum: if the tip of the micropipettor is resting on the bottom of a container it may create a vacuum rather than draw in a liquid. To allow for this possibility, after the plunger has been released, raise the tip out of the solution very slowly. If a vacuum has been created, drawing the tip out slowly will result in liquid moving in to fill the vacuum, rather than air.
4. Dispose of the micropipette tip.

III. Microscope

Familiarize yourself with the basic "anatomy" of the microscope you will be using in this course. Use the illustrations provided to identify and review the functions of:

ocular, objective lenses including scanning, low and high power, mechanical stage, revolving nosepiece, condenser, iris diaphragm, illuminator dial, and coarse and fine focusing knobs

view the video and be able to define **magnification**, know how to make a **wet mount** and be able to find and focus objects at scanning, low and high power magnifications.
make sure you are familiar with how to use the microscope before using it. ASK FOR HELP if you need it, otherwise I will assume that you had plenty of experience from other biology courses.

You will not be using the oil immersion objective (100X) in this course.

1. Take out your microscope, note whether it was properly put away, if not notify the instructor
2. Write down the number of the scope you use for this exercise on your sketch sheet below

3. Select a slide of a typical human ORGAN, observe it under the 4x, 10x and 40x objectives
4. Make a sketch of the same organ under the three different objectives, label each and indicate the **magnification** of each drawing

IV. Electronic Balance

Make sure the balance is clean and free of spilled solids or liquids.
Never weigh a hot object; allow it to cool to room temperature
Never place any chemical or object to be weighed directly no the balance pan; select a suitable weighing vessel, such as a beaker, weighing paper, watch glass, or weighing dish as suggested in the lab exercise.

Procedure: Direct Weighing:

1. Turn balance on.
2. Place a weighing vessel on the pan and press ***tare*** to rezero the instrument and therefore automatically subtract the weight of the pan from the substance or object that you want to weigh.
3. Add material to be weighed and record its weight.
4. Turn off the balance when you are finished using it.

V. pH meter

You will need at least about 1" of solution in the container that you are using to measure pH in.
Be sure to remove the cap from the probe and rinse it with DI water before using it
Rinse the probe with DI water and recap it when you are finished.

1. lift cover/screen
2. turn meter on
   the meter should already be calibrated to a known standard and the measurements compensated for temperature.
3. remove cover from the bottom of the electrode
4. place electrode into solution to be measured and slowly stir electrode while watching the screen for an accurate reading, the solution should cover the lower 15 to 20 mm of the electrode
5. When the pH value stabilizes record the value
6. Turn off the machine
7. Rinse the electrode with DI water again.
8. Replace the electrode cover
9. Turn off the pH meter when you are done using it

VI. Deionized Water Tap

Both sinks in the PIN labroom are equipped with a deionized water tap to refill DI water bottles as needed. Turn the tap on slowly to prevent a powerful stream of water that could knock a beaker or flask out of your hand.

VII. iWorx/204 Equipment and LabScribe Software

Many of the laboratory exercise will involve the use of this equipment and software for data gathering and analysis. Data can be accumulated, displayed and analyzed on a computer screen and selected sections can be printer to turn in with your lab report.

In most cases the computer, iWorx station and needed accessories will already be set up, connected, and preset for you and the software opened to the proper window to begin your experiment. You may occasionally however have to restart the software. The parameters being used are included with your instructions for each lab – see your instructor if you need help

The software has several windows. Your exercises will use mainly the following windows:

main – record incoming signals and performs data analysis

marks – reviews typed annotations entered during data gathering

stimulation – change stimulator settings

These and other windows can be accessed at anytime using the labscribe toolbar (fig 2-5). You can also drag away any windows that you are not using during a particular exercise.

Most of the time you will be using the **main window** to collect and process your data. The main window is displayed when the application is first opened. Notice that ***each channel*** has its own **recording area** with a **title area** above containing "**autoscale**" and "**full scale**" buttons. Above the channel one title area on the left side is a "**time value**". Toward the right of the time value is the "**mark**" button and a "**start**" button.

- Use the **red triangles** to the right of the screen, between each window to enlarge or reduce the size of a particular window

- The default value for the time for a signal to cross the screen is 10 seconds (see display time). This can be changed to half or double time using the two display controls in the toolbar at the top of the screen (fig. 2-5).

- After making a recording and stopping, click autoscale to enhance the tracing for easier analysis

- If the tracing in "upside-down" compared to the illustrations, right click the mouse and select <invert> to flip the image.

- At the end of each recording activity, save your recording by selecting <save as> from the file window, give it a name, and **save it to the desktop**. That way you can go back later using the <marks> pulldown to find your data for further analysis

- The default value for the number of samples taken per second is 200 (see speed at top of screen). It can be changed under edit - preferences - sampling rate

A. <u>Attaching and Connecting the Equipment</u>

Most of the time the equipment will be set up and plugged in for you. To practice using the iWorx station you will record your pulse using a plethysmograph:

1. Locate the plethysmograph.
2. Place the unit on the volar surface (where the fingerprints are located) of the distal segment of a middle finger and wrap the velcro to attach the unit firmly to the end of the finger.

B. <u>Recording data</u>

1. Click <Start>
2. note the rhythmic signal in the channel recording area. If the deflection is very small, click <autoscale> to enlarge it
3. click <stop> to halt the recording
4. click and drag the red arrows (in the right margin of the window) up and down to make the channel recording window as large or small as desired.

C. <u>Making marks on your data</u>

1. type "first attempt" on the keyboard and notice that the words appear in the "marks-area" to the left of <Start> button
2. click <start>
3. Press the <enter> key on the keyboard and notice that the words disappear and a vertical line appears in the LabScribe window
4. type "second try" and press <enter>
5. click <Stop>

D. <u>Saving a file</u>

1. click on the file menu and select <save as>
2. type a name for the file, e.g. " Fred's finger pulse" and select <save>. Make sure you save it to the **desktop**. Otherwise it will be buried on the hard drive and you may never find it!

E. Finding Your Saved File

You can find your desired data in two ways:

1. use the cursors and arrows at the bottom of the screen to scroll back to the appropriate marks. This could take a long time depending on how long you recorded. Its good to use if you are doing your analysis after each major lab activity.

or

If you did all your recording for all the day's activities, making marks or labels for each "treatment" and saving after each set then the method below is the easiest way to get to your data so that you can analyze it

1. click the <marks> icon at top of screen
2. you will see a pull down menu listing the times and any labels or "marks" that you made at that time
2. click on the **time** for the mark you want and press <GoTo>

Note:

marks can be moved vertically and place anywhere in the recording by clicking and dragging the mouse. Marks in a given view can be reset by selecting: 'View-- Reset Marks'

F. Analysis: Making Measurements on your Data

measurements are taken using the **cursors**, there are two cursor buttons; for one or two cursors (fig 2-5):

Essentially **all** the measurements that you make will involve measuring the **amplitude** of a wave or a **time interval** of an event.

The **amplitude** is the **vertical deflection** of your tracing and is usually recorded as volts in the window to the upper right of the screen you are recording your data on.

The **time interval** of an event is the horizontal distance between two repeating marks. The time interval is read as T2-T1 in a window to the upper left of the screen as hours: minutes: seconds: tenths. To calculate the number of events/minute take this interval and divide it into 60.

1. After you have the portion of your recorded data on the screen, Click the <two cursor> icon
2. two vertical lines appear over the recording window, one with a star and the other with a square where the bar intersects the trace
3. click and drag either or both lines to the left or right to display the **difference in time** (horizontal distance between the points on each cursor) or **difference in voltage** (vertical distance between the points on each cursor)
4. The **time** between two points is show in the **top left margin above channel one**, giving the difference in time (T2-T1) between the two cursors.
5. **Voltage** is displayed in the value area on the right, above the channel recording window, giving the difference in voltage (V2-V1) between the two cursors. *Be sure that you are reading the voltage from the proper display window, i.e. the one you are recording in.*
6. Set the cursors so that each is on two consecutive "peaks" of your pulse recording. Read the time between peaks and record it on the data sheet. Calculate your pulse/minute.
7. Now, set the cursors so that the first is at the peak of a beat and the other is at the trough just after the peak. Read the "voltage" difference in the appropriate window and record it on your data sheet. This value is a relative indication of the size of the peak of your pulse. Measure the size of three or four peaks; are they all the same or do they vary? Record your results on the data sheet

G. Printing Your Data

If your computer is connected to a printer you can print samples of your data as requested.

1. Find and go to the portion of your 'saved file' that you would like to print as described above
2. Select <print> under the file menu to print a copy of whatever you have on the screen.
3. Attach a copy of your pulse printout to your data sheet.

Name:_____

Date Due:_____

The Use of General Lab Supplies and Equipment & Laboratory Safety

Bio 2402 Lab Data Sheet

You will have two weeks to show that you have familiarized yourself with the various supplies and equipment discussed in this exercise. Keep this sheet with you until you complete all the activities listed in this exercise.

	Item completed	**initials** of lab partner or **actual value** – as needed
1	A flask with 250 ml of water in it	
2	A graduated cylinder with 55 ml of water	
3	A 10 ml volumetric pipette with 7 ml of water in it	
4	A plastic transfer pipette with 1 ml of water in it	
5	The dropper of a 60 ml amber dropper bottle with about 1 ml of water in it	
6	A micropipette with 30µl of water in it	
7	Record the weight of 2 nickels in milligrams	wt =
8	Record the pH of Frog Ringers solution	pH =

iWorx and Finger Pulse

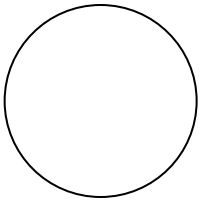
1. What was the amplitude of your pulse?

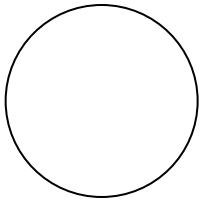
2. What was the duration of a single pulse?

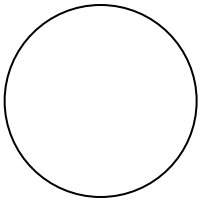
3. How many pulses/minute did your data indicate?

4. Attach a printout of *your* finger pulse to this data sheet

Microscope sketches: Organ:_____







Magnification:_____ **_____** **_____**

Location of Lab Safety Equipment

Assume the blackboard is at the "front" of the room and the windows are on the "left" side

	Describe The Specific Location of Each
nitrile gloves	
safety glasses/goggles	
eyewash station	
sinks	
disinfectant spray bottles	
paper towels	
biohazard bag	
glass disposal boxes	
deionized water spigots	
fire extinguisher	
first aid kit	
hazardous materials spill kit	
microscope slides & coverslips	
exhaust fan vent switch	
emergency gas shutoff switch	

Answer the following questions on a separate sheet of paper:

1. Describe ***each*** of the specific precautions that should be taken when handling blood in the lab.

2. What is the most precise and least precise method for measuring liquids in the lab?

3. Look in your lab manual, select a chemical used for any one of the labs and then look up information about that chemical in the material safety data sheets. Describe the hazard level and the precautions to be taken when handling that chemical.

4. Make a "flow chart" for this specific lab activity: **The Use of General Lab Supplies & Equipment and Laboratory Safety**. Your flow chart should be no longer than one side of one page.