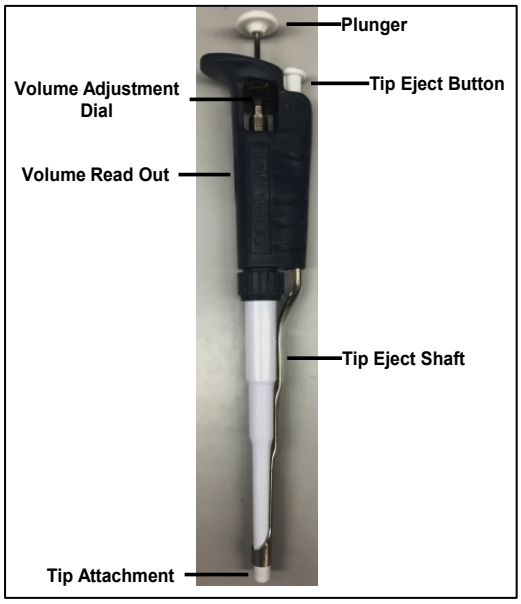
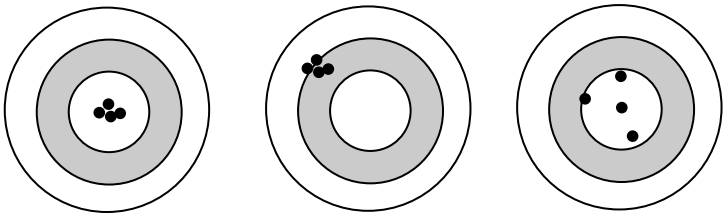
Pipetting Lab: Student Hand Out

Introduction:
The proper use of a micropipettor is one of the most important skills needed to work in a laboratory. Successfully dispensing the correct volume of sample or reagent with a micropipettor can improve the precision and accuracy of data (see targets below), as well as the repeatability of your experiments. Precision and accuracy can be estimated with an R-squared (R^2) value, which can, for example, tell you how well the data you generate matches the expected data, or a set of standards. The closer the R^2 value is to 1.0, the better the linear regression fits your data points, and the more precise and accurate your pipetting.



PRECISE & ACCURATE | **PRECISE NOT ACCURATE** | **NOT PRECISE ACCURATE**



For micropipetting, scientists use the volume of a 'microliter' (µL). To understand the size of a microliter – there are approximately 40 to 50 microliters in 1 drop of water.

Objectives:
- Practice basic skills important for most laboratory techniques.
- Understand precision and accuracy.

Materials Identify and check off that you have all of the following items before starting. Some of the items are shared between two people or the group:

- ☐ Gloves
- ☐ Micropipettor with ranges of: 0.5-10, 20-200, 100-1000 µL
- ☐ Pipette tips (3 different sizes)
- ☐ Water in 2 microtubes
- ☐ Yellow Dye #5 stock (in microtube)
- ☐ Empty 1.5 mL microtube
- ☐ Microtube rack
- ☐ 96-well plate
- ☐ Permanent marker for labeling
- ☐ Solid waste container
- ☐ Liquid waste container

Content developed by the Toxicology, Health and Environmental Disease Program at Rutgers University
http://eohsi.rutgers.edu/graduate-programs/toxicology-health-and-environmental-disease-thed-high-school-summer-program/

LABORATORY PROTOCOL

1. There are 3 different sizes of micropipettors that you may have in front of you, either 0.5-10 microliters (µL), 20-200 µL, or 100-1000 µL.
2. Practice loading and dispensing the tip. Hold the pipettor as pictured to the right.
   a. Press down on the tip firmly to load it onto your pipettor.
   b. Push the ejector button by your thumb to dispense the tip into a waste container.
3. Adjust the volume of water to be measured by twisting the plunger button on top of the device. Turn it to the smallest amount allowed for each device (i.e. 0.5 µL, 20 µL or 100 µL).
4. Now with your thumb, depress the plunger on top **half way,** which is the 'first stop'. This has made a space large enough for the amount of water you will be taking up inside the pipette tip chamber.
5. Insert the tip into the microtube of water and pull up the plunger by **slowly** lifting your thumb. Take your thumb off the plunger button and notice the very small amount of liquid in the tip.
6. Evacuate the tip into a separate liquid waste beaker (not the garbage can) by pushing the plunger back down to the first stop.
   a. This pushes out exactly the same amount you took up.
   b. If you push down to the 'second stop', extra air is forced out, which may be necessary to expel the little drop of water on the end of the tip.
7. Push the ejector button by your thumb to dispense the tip into a solid waste container.
8. Adjust the volume of water to be measured and extracted to the largest amount allowed for each device (i.e.10 µL, 200 µL or 1000 µL) and repeat steps 4-7.
   a. If you are dispensing liquid into a 96-well plate or loading sample into a gel, you will press the tip **lightly** against the inside wall of the well to ensure you are in the well.
   b. **ALWAYS** dispose of the tip after each sample so that you do not cross-contaminate your samples.
   c. **NEVER** use the pipette out of the range indicated on the pipettor.

MICROPIPETTING CHALLENGE

The Rutgers Environmental and Occupational Health Sciences Institute is hiring a laboratory researcher and you made it to the final round of interviews! The last test is to see how precise and accurate each applicant can be. Complete the following exercise to see if you will be the new hire!

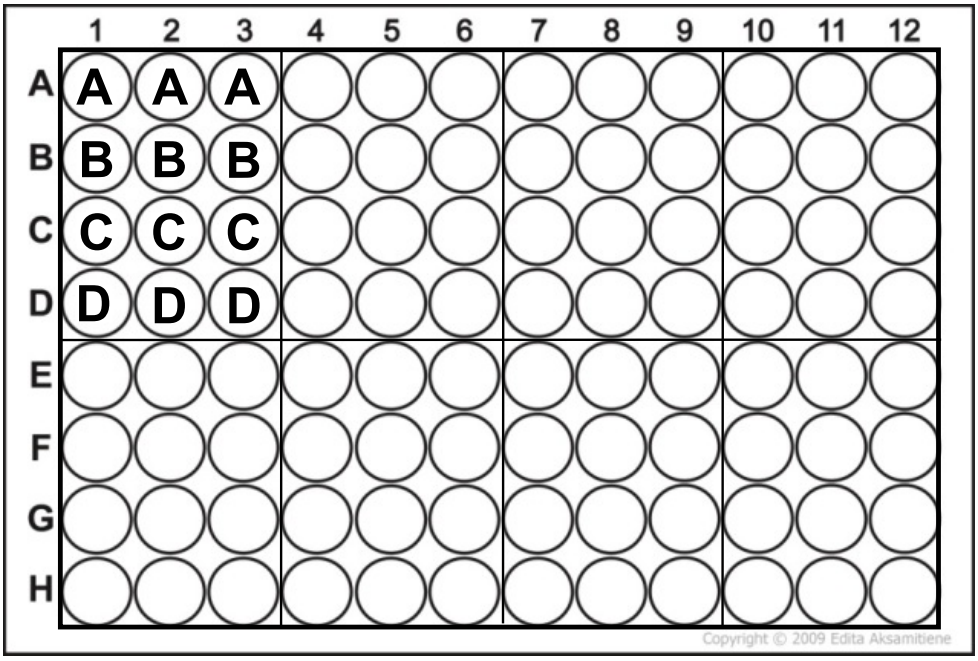
a. Each team must make the following dilutions of Yellow #5 from the stock solution provided, with each member performing a different pipetting task.
   i. Assign each member of the group a letter A, B, C, D and write the names of the group members in **Table 1** on the next page.
   ii. Each member should label a 1.5 mL microtube with the appropriate letter.
   iii. Each member should make a dye dilution by combining the water and dye volumes into the labeled 1.5 mL microtubes according to **Table 1**.

Table 1. Micropipetting Challenge Preparation

Group Member	Water (µL)	Dye (µL)
A:	900	100
B:	950	50
C:	990	10
D:	995	5

b. Pipette up and down to mix your diluted dye.
c. Each applicant will pipette 100 µL of each of the diluted dyes in triplicate into one 3 x 4 set of wells on a 96-well plate as indicated on the plate below. Circle your assigned section on the plate below and write your initials on the cover of the plate over your section with a marker.

PLATE #:_____



d. While you are waiting for your turn to pipette into the plate, calculate the percent of each diluted stock that each group member prepared in the space provided.

e. The absorbance of dye in each well will be measured using a spectrophotometer at 422 nm, the peak absorbance wavelength of light for Yellow #5 Dye. The readings will be graphed and an R^2 value determined for each group.

Record your results in your notebook and answer the questions in the results and conclusions section on the next page(s).

RESULTS AND CONCLUSIONS

Answering these questions is important for any scientist carrying out an experiment.

Research Question (what is the question being asked?):

What aspects of the questions can we actually answer with this experiment?

Generate a hypothesis:

% Dye (calculated on previous page)	Absorbance (AU)				Mean	Standard Deviation
	Expected	Reading 1	Reading 2	Reading 3		
	2.0					
	1.0					
	0.2					
	0.1					

Group R^2 value (The closer this value is to 1.0, the more precise your collective pipetting): _____

	Mean	**Standard Deviation**		**Mean**	**Standard Deviation**
Group 1 R^2 Value:			**Group 4 R^2 Value:**		
A			A		
B			B		
C			C		
D			D		
Group 2 R^2 Value:			**Group 5 R^2 Value:**		
A			A		
B			B		
C			C		
D			D		
Group 3 R^2 Value:			**Group 6 R^2 Value:**		
A			A		
B			B		
C			C		
D			D		

- Which piece of data demonstrates your precision? Which demonstrates your accuracy?

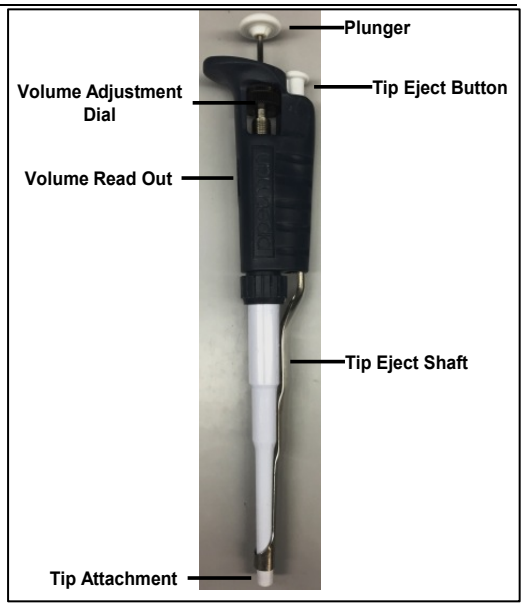
- What are some factors that could contribute to variation in readings?

Pipetting Lab: Instructor Script

Instructor Additions in Blue

Introduction:

The proper use of a micropipettor is one of the most important skills needed to work in a laboratory. Successfully dispensing the correct volume of sample or reagent with a micropipettor can improve the precision and accuracy of data (see targets below), as well as the repeatability of your experiments. Precision and accuracy can be estimated with an R-squared (R^2) value, which can, for example, tell you how well the data you generate matches the expected data, or a set of standards. The closer the R^2 value is to 1.0, the better the linear regression fits your data points, and the more precise and accurate your pipetting.



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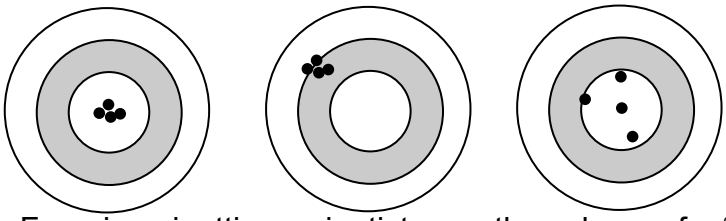

For micropipetting, scientists use the volume of a 'microliter' (µL). To understand the size of a microliter – there are approximately 40 to 50 microliters in 1 drop of water.

Objectives:

- Practice basic skills important for most laboratory techniques.
- Understand precision and accuracy.

Prep: Pre-label 3 96-well plates (plate 1, plate 2, plate 3) with clear covers - mark off sections students will be pipetting in with marker. Prepare and bring Yellow Dye #5 food coloring (can be purchased in baking section of any grocery store): **Add 250 µL of Yellow Dye #5 to 750 µL of water in microtube for each group (6)** for their 'stock solution'. Each pair or group should have a labeled solid waste container, liquid waste container, pipette tips, permanent marker, microtube rack, 1 empty microtube, 2 microtubes of water (~1 mL each), and 1 microtube **per group** with diluted yellow dye.

Materials Identify and check off that you have all of the following items before starting. Some of the items are shared between two people or the group:

- ☐ Gloves
- ☐ Micropipettor with ranges of: 0.5-10, 20-200, 100-1000 µL
- ☐ Pipette tips (3 different sizes)
- ☐ Water in 2 microtubes
- ☐ Yellow Dye #5 stock (in microtube)
- ☐ Empty 1.5 mL microtube
- ☐ Microtube rack
- ☐ 96-well plate
- ☐ Permanent marker for labeling
- ☐ Solid waste container
- ☐ Liquid waste container

-If available: Various instructors may stand with each group around the room with pipettor, solid waste container, liquid waste container, ***colored water****, and pipette tips. One instructor leads in the center of the room while the others demonstrate technique to surrounding students.*

Leading Instructor: Now we will begin the pipetting lab. The purpose of pipetting is to transfer a specific volume of a sample or reagent. Scientists do this using micropipettors, like the one your instructors and I are holding. For micropipetting, scientists use the volume of a 'microliter' (µL). To understand the size of a microliter – there are approximately 40 to 50 microliters in 1 drop of water. Every two lab benches has a set of three micropipettors that each measure liquid in three different ranges including 0.5-10 µL, 20-200 µL, and 100-1000 µL. Each pipettor has a plunger, a tip eject button, volume adjustment dial, the volume readout, the tip eject shaft, and the very bottom of the pipettor where you attach a tip to draw up the liquid (*all instructors point to part of the pipettor)*. Each micropipettor uses a different size tip. Each time you want to measure a specific volume you must choose the pipettor with the appropriate range of volumes. If you want to measure 400 µL of liquid you would choose the 100-1000 µL pipettor (*instructor with this pipettor, hold up*). And if you want to measure 5 µL, you will choose the 0.5-10 µL pipettor (*instructor with this pipettor, hold up*). Once you have the appropriate pipettor, set the desired volume by turning the volume adjustment dial so that the read out shows the volume you want to pipette. It is important that you only measure volumes in the designated range for each pipettor, even if the volume read out goes above or below the range, measuring these volumes will break the pipettor.

Once the volume is set you will hold the pipettor like this, placing your thumb on the plunger and your other fingers around the body of the pipettor. You then find the appropriate tip that will fit the pipettor and firmly press down on the tip to load it on the pipettor. ***Everyone pick up a pipettor and feel the difference between the 'stops.'*** Lift the tip up and press the plunger down to the first stop which makes enough room inside your tip for the volume that you set it to. You can then submerge the tip into your water and slowly draw up until the plunger is back to its original position. Move to the location you would like to dispense the liquid and slowly press down the plunger to the first stop to evacuate the tip of the exact same volume that you took up. You may need to push down to the second stop to get any remaining drops of liquid out of the tip. Once you are done ejecting the measured volume, keep the plunger down until you have removed the tip from the place that you evacuated it to avoid accidentally taking the volume back up into the tip. When you are done with the tip, place over the solid waste container and eject using the tip eject button with your thumb. Keep in mind that if you are pipetting the same liquid, then it is ok to use the same tip more than once, however it is important to change your tip in between different samples and/or reagents so that you do not cross contaminate.

In this lab you will be practicing pipetting and once everyone feels comfortable using the pipettors you will perform a micropipetting challenge to determine the accuracy and precision of your pipetting. The students with the most accurate and the most precise results as well as the group with the best r-square value will get prizes! You will be

sharing a set of pipettors between 4 people. If you have any questions, myself, *(name other instructors present)* will be around to answer them. You may now begin.

If the opportunity arises introduce the concept of pipetting up and down to resuspend a pellet and/or mix samples. Pass out 1.5 mL microtube for each student:

Throughout the week you will be asked to pipette a small volume "up and down" to mix the contents. Choose a pipettor set to any volume and add that volume of water to an empty 1.5 mL microtube. After adding, push the plunger to the first stop and submerge tip into the water now in the 1.5 mL tube. Slowly pull the plunger up in order to bring the water back into the pipette tip. Once the plunger has reached the top, push the plunger back down to the first stop, evacuating the tip of the water. Repeat this a few times rapidly being careful not to make bubbles by keeping the end of the tip submerged in liquid. Once you are ready to remove the tip from the sample, evacuate the tip completely by pushing to the second stop and removing from the water before releasing the plunger.

LABORATORY PROTOCOL

9. There are 3 different sizes of micropipettors that you may have in front of you, either 0.5-10 microliters (µL), 20-200 µL, or 100-1000 µL.
10. Practice loading and dispensing the tip. Hold the pipettor as pictured to the right.
    a. Press down on the tip firmly to load it onto your pipettor.
    b. Push the ejector button by your thumb to dispense the tip into a waste container.
11. Adjust the volume of water to be measured by twisting the plunger button on top of the device. Turn it to the smallest amount allowed for each device (i.e. 0.5 µL, 20 µL or 100 µL).
12. Now with your thumb, depress the plunger on top **half way,** which is the 'first stop'. This has made a space large enough for the amount of water you will be taking up inside the pipette tip chamber.
13. Insert the tip into the microtube of water and pull up the plunger by **slowly** lifting your thumb. Take your thumb off the plunger button and notice the very small amount of liquid in the tip.
14. Evacuate the tip into a separate liquid waste beaker (not the garbage can) by pushing the plunger back down to the first stop.
    a. This pushes out exactly the same amount you took up.
    b. If you push down to the 'second stop', extra air is forced out, which may be necessary to expel the little drop of water on the end of the tip.
15. Push the ejector button by your thumb to dispense the tip into a solid waste container.
16. Adjust the volume of water to be measured and extracted to the largest amount allowed for each device (i.e.10 µL, 200 µL or 1000 µL) and repeat steps 4-7.

a. If you are dispensing liquid into a 96-well plate or loading sample into a gel, you will press the tip **lightly** against the inside wall of the well to ensure you are in the well.
b. **ALWAYS** dispose of the tip after each sample so that you do not cross-contaminate your samples.
c. **NEVER** use the pipette out of the range indicated on the pipettor.

MICROPIPETTING CHALLENGE

The Rutgers Environmental and Occupational Health Sceinces Institute is hiring a laboratory researcher and you made it to the final round of interviews! The last test is to see how precise and accurate each applicant can be. Complete the following exercise to see if you will be the new hire! Make sure to strategize with your group to achieve the best results. Help everyone make their dilutions by checking their technique because this will also affect your entire group's results. Think about what other factors might affect your accuracy and precision and try to control for them as best as possible.

a. Each team must make the following dilutions of Yellow #5 from the stock solution provided, with each member performing a different pipetting task.
   i. Assign each member of the group a letter A, B, C, D and write the names of the group members in **Table 1** below.
   ii. Each member should label a 1.5 mL microtube with the appropriate letter.
   iii. Each member should make a dye dilution by adding the water and dye volumes into the labeled 1.5 mL microtubes according to **Table 1**.

Table 1. Micropipetting Challenge Preparation

Group Member	**Water (µL)**	**Dye (µL)**
A:	900	100
B:	950	50
C:	990	10
D:	995	5

b. Pipette up and down to mix your diluted dye.
c. Each applicant will pipette 100 µL of each of the diluted dyes in triplicate into one 3 x 4 set of wells on a 96-well plate as indicated on the plate below. Circle your assigned section on the plate below and write your initials on the cover of the plate over your section with a marker.

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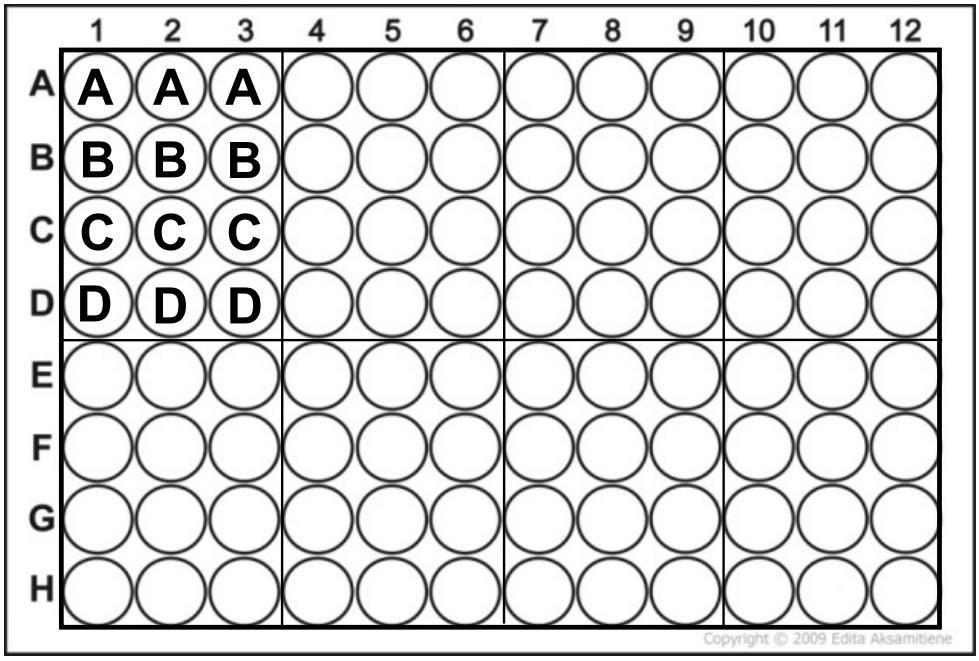

d. While you are waiting for your turn to pipette into the plate, calculate the percent of each diluted stock that each group member prepared in the space provided.
   Group member A: 10% dye
   Group member B: 0.05% dye
   Group member C: 0.01% dye
   Group member D: 0.005% dye

e. The absorbance of dye in each well will be measured by a spectrophotometer at 422 nm, the peak absorbance of Yellow #5 Dye. The readings will be graphed and an R^2 value determined for each group. One instructor takes plates to be read on spectrophotometer and plots points to determine R^2 value for each group using Microsoft Excel Template. Give handout of results to students - one per group.

f.

MAKE SURE STUDENTS WRITE THEIR INITIALS ON THE COVER OF THE PLATE OVER THEIR SECTION

Record your results in your notebook and answer the questions in the results and conclusions section on the next page(s).

RESULTS AND CONCLUSIONS

Answering these questions is important for any scientist carrying out an experiment.

Research Question (what is the question being asked?):

How precise and accurate is my pipetting?

What aspects of the questions can we actually answer with this experiment?

The mean and standard deviation of your readings, and overall R^2 value.

Generate a hypothesis:

My pipetting will be consistent

My pipetting will be inconsistent

% Dye (calculated on previous page)	Absorbance (AU)				Mean	Standard Deviation
	Expected	Reading 1	Reading 2	Reading 3		
	2.0					
	1.0					
	0.2					
	0.1					

Group R^2 value (The closer this value is to 1.0, the more precise your collective pipetting): _____

	Mean	Standard Deviation		Mean	Standard Deviation
Group 1 R^2 Value:			**Group 4 R^2 Value:**		
A			A		
B			B		
C			C		
D			D		
Group 2 R^2 Value:			**Group 5 R^2 Value:**		
A			A		
B			B		
C			C		
D			D		
Group 3 R^2 Value:			**Group 6 R^2 Value:**		
A			A		
B			B		
C			C		
D			D		

- Which piece of data demonstrates your precision? Which demonstrates your accuracy?
  - Precision - standard deviation of 3 readings
  - Accuracy - mean as compared to expected absorbance reading
- What are some factors that could contribute to variation in readings?
  - There are multiple factors that affect accuracy, see if they can think of a few:
    - Human error
    - Pipette calibration
    - Improper preparation of dilutions