Measuring Micropipette Accuracy

Being able to accurately measure and dispense small volumes of liquid is essential for the success of experiments in a biotechnology lab. In this lab, you will learn a method to **calibrate**, or measure the accuracy, of your micropipettes. By comparing the volume set on the micropipette with what was actually dispensed according to the weight of the water, you will find out whether you can really trust your pipette!

Materials

- P200 or P1000 micropipette and tips
- Weigh boat
- Thermometer
- 50ml or 100 ml beaker
- Spray bottle with 70% ethanol solution and paper towels
- Electronic balance

Protocol

- 1. Clean your micropipette with ethanol, wiping gently with paper towels.
- 2. Add approximately 20 ml of water to the beaker and measure the temperature of the water. Record the temperature in your lab notebook.
- 3. Make a data table in your lab notebook to record your measurements. You will measure the weight of water pipetted into the weigh boat ten times.
- 4. Place a weigh boat on the balance and tare the balance.
- 5. Set the micropipette to the middle of its volume range and attach a tip.
- 6. Pre-wet the tip by inserting the tip approximately 2 mm into the beaker of water, aspirating water into the tip and dispensing it back into the beaker. **Repeat this step three times.**
- 7. Using the same form as in Step 6, aspirate the full volume you have set, waiting at least one second after releasing the plunger to remove the pipette tip from the water. Inspect to ensure there are no air bubbles in the tip. (If you observe air bubbles, dispense the water back into the beaker and try again.)

- 8. Dispense the liquid into the weigh boat as follows:
 - a. Press the plunger to the first stop to deposit the majority of the liquid;
 - b. Move the tip to a different part of the weigh boat;
 - c. Depress the plunger to the second stop to expel any liquid remaining in the tip. (**Do not remove the tip** when you have finished this step.)
- 9. Record the mass of the liquid in the table in your lab notebook.
- 10. Wipe the weigh boat until it is dry. Replace it on the balance and re-tare the balance.
- 11. **Repeat steps 6–10 at least nine more times.** You will need at least ten measurements to calculate the accuracy of your micropipette.
- 12. Dispose of the weigh boat and pipette tips in the trash bin.

Calculations

To determine the accuracy of your micropipette, we first need to calculate the average volume of water that was actually dispensed using the following formula:

 $V = w^*Z$ To solve for V = Average volume of water dispensed, multiply

w = Average weight of water*

Z = factor for distilled water corresponding to its temperature**

* Use the average over your ten replicates. To calculate the average, add up all of your measurements for weight and divide by the number of replicates.

* * The temperature of the water, as well as the humidity and air pressure in the room, impact the water and thus your results. These variables are grouped together in a Z-factor. Consult the table provided below to determine the Z-factor for distilled water that corresponds to the temperature you measured.

w (average weight in grams for 10 replicates)

Z-factor

Solve for V (average volume dispensed)

1.0029
1.0031
1.0033
1.0035
1.0037
1.0039

Image from <u>Colorado.edu</u>

Now that we know the average volume dispensed, we can compare this to the volume you set on the micropipette and see how close the two values are (i.e. the accuracy of your pipette). The accuracy for a properly calibrated pipette should be between 99 - 101%.

Use the following formula to calculate the accuracy of your pipette:

$\%A = \frac{V_{avg}}{V_O} \times 100\%$	A = Accuracy (%)
	V _{avg} = Average volume dispensed
	V ₀ = Volume set on micropipette (in ml)
V _{avg}	
V _{0,} express	sed in ml
% A	

Protocol is based on this *micropipette calibration tutorial* from WikiHow.

BioTechBuilder.org

BioTechBuilder prepares students with the skills and knowledge they need to be successful in state-of-the-art biotechnology labs.

With this curriculum, instructors can

- introduce students to core concepts in biotechnology.
- build student proficiency in a range of lab skills from foundational to advanced.
- prepare students to earn industry-recognized microcredentials.
- set students on a path toward post-graduation employment in the biotech industry.

COURSE MODULES

LAB SKILLS

This module introduces students to fundamental concepts, math, and techniques needed to operate in any laboratory environment. Beginning with lab safety and chemical handling, students progress through weighing solid chemicals, preparing common solutions, and culturing bacteria using aseptic techniques.

- TOPIC: **BUILDING A SOLID FOUNDATION** (4 LESSONS + 1 LAB PRACTICAL)
- TOPIC: MAKING SOLUTIONS (8 LESSONS + 2 LAB PRACTICALS)
- TOPIC: GROWING CELLS (4 LESSONS + 2 LAB PRACTICALS)

FOUNDATIONS

This module uses a molecular framework to introduce techniques in DNA and protein analysis. Students culture bioengineered bacteria, and then use DNA gel electrophoresis, restriction digest and sequencing to verify its construction. To study the strain's performance, students can induce gene expression, purify the expressed protein using column chromatography, and assess its purity using protein gel electrophoresis.

- TOPIC: ANALYZING DNA (12 LESSONS + 3 QUIZZES + 2 LAB PRACTICALS)
- TOPIC: ANALYZING PROTEINS (14 LESSONS + 2 QUIZZES + 2 LAB PRACTICALS)

APPLICATIONS

This module introduces students to production and manufacturing of bioproducts, covering upstream and downstream processing, quality control, and documentation using batch records. Students are also introduced to the Design-Build-Test-Learn cycle in biodesign through BioBuilder kits and work as a group on a novel product design.

- TOPIC: **BIOMANUFACTURING** (14 LESSONS + 3 QUIZZES + 3 LAB PRACTICALS)
- TOPIC: SYNTHETIC BIOLOGY (20 LESSONS + 3 QUIZZES + 3 LAB PRACTICALS + 1 FINAL PROJECT)