	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
Pipette Calibration **Module Hours: 2.5**	**Effective Date: 09/08/2023** **PRQs** **Lab Safety**	**Revision # 1.0** **M. Stowell** **Checked: M. de Vito & Z Hazlett**

BACKGROUND

Mastering the pipette is essential to successfully prepare and perform all other lab procedures. It is important to use proper technique when handling a pipette to make sure there is no contamination and that you measure as accurately and precisely as possible. Pipettes work by creating a vacuum in the pipette tip, allowing the liquid to move into it, this makes it easy to transport and dispense the liquid as necessary. There are a number of pipettes that hold different amounts of liquid ranging from P10 (holds 0.5 to 10µL) to P5000 (holds 1,000 to 5,000µL) so choosing and calibrating the right one ensures the best results. Sometimes a combination of different pipettes will more accurately deliver a specific amount of liquid, which will decrease the margin of error and improve your data quality. The goal of calibration is to ensure that a pipette is measuring the desired amount of liquid at varying measurements.

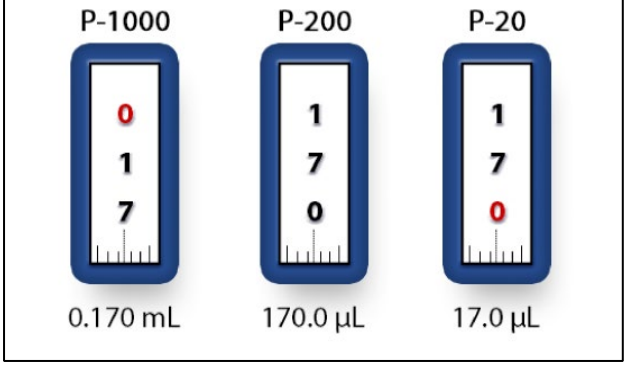

Figure 1: different pipette sizes measure different amounts of liquid

1. PURPOSE

To outline the procedures and best practices for pipette operation for properly dispensing liquids using a volumetric air displacement pipette. To provide procedures for calibrating pipettes.

2. SCOPE

This procedure applies to qualified skills center users.

3. RESPONSIBILITY

3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
3.3. It is the responsibility of the user to become trained on the procedure.

4. DEFINITIONS

4.1. Solvent - The substance which dissolves another to form a solution. For example, in a sugar and water solution, water is the solvent; sugar is the solute.
4.2. Solution - A mixture of two or more pure substances. In a solution one pure substance is dissolved in another pure substance homogeneously. For example, in a sugar and water solution, the solution has the same concentration throughout, ie. it is homogenous.
4.3. Mole - A fundamental unit of mass (like a "dozen" to a baker) used by chemists. This term refers to a large number of elementary particles (atoms, molecules, ions, electrons, etc) of any substance. 1 mole is 6.02 x 1023 molecules of that substance. (Avogadro's number).M
4.4. pH - The pH scale can tell if a liquid is more acidic or basic. The range of the pH scale is from 0 to 14 from very acidic to very basic. A pH of 7 is neutral. A pH less than 7 is acidic and greater than 7 is basic. pH is a log scale. Each whole pH value below 7 is ten times more acidic than the next higher value. For example, a pH of 4 is ten times more acidic than a pH of 5 and a hundred times (10 X 10) more acidic than a pH of 6. This holds true for pH values above 7, each of which is ten times more basic (also called alkaline) than the next lower whole value. An example would be a pH of 10 is ten times more alkaline than a pH of 9.

5. MATERIALS/EQUIPMENT

5.1. Deionized water
5.2. Buffers
5.3. Rainin pipettes (10, 200, 1000 uL)
5.4. Microcentrifuge tubes
5.5. Conical tubes (15mL, 50mL)
5.6. Analytical balance

6. PROCEDURES

6.1. Pipette Selection: Select the pipette that will dispense the ENTIRE desired volume for the task required (i.e. If 1000uL is required do not select 200uL pipette-select 1000uL pipette)

6.2. Dispensing Pipetting Solution: Affirm that the dispensing solution is NOT from the original stock solution. Pour the stock solution that requires pipetting into a clean vessel for dispensing.

6.3. Aspirating Liquid:

6.3.1. Attach the pipette tip firmly; ensure good contact by gently pushing up on the tip.

6.3.2. Press the push-button of the pipette down to the intermediate stop position.

6.3.3. Immerse the pipette tip vertically into the water.

6.3.4. Use appropriate immersion depth for target Volume

101 to 100 µL immersion depth 2 to 3 mm
101 to 1000 µL immersion depth 2 to 4 mm
1.1 to 10 mL immersion depth 3 to 6 mm

6.3.5. Allow the push-button to move up to the top stop position slowly and smoothly.

6.4. Dispensing Liquid:

6.4.1. Place the pipette tip at an angle between 10-45°C against the inside wall of the receiving vessel.

6.4.2. Slowly press the push-button down to the first stop to empty the tip and wait 1 s.

6.4.3. To ensure the tip is emptied completely gently pull the tip along the side the vessel maintaining the angle from while pressing down the push-button to the final stop.

6.4.4. Allow the push-button to return to the top stop position.

6.5. Handling, Care and Maintenance:

6.5.1. When attached to a tip (filled or empty) do not lay down the pipette horizontally.

6.5.2. Avoid allowing a temperature difference between pipettes, tips and liquid as this may lead to incorrect dispensing volumes.
6.5.3. Do not allow any liquid to enter the pipette.
6.5.4. Do not clean the pipette with aggressive solutions (acetone, DCM, etc.)

6.6. Calibration

6.6.1. Pick the pipette you will be using to complete the procedures and the correct size tip for it
6.6.2. Fill a beaker with distilled water and record its temperature
6.6.3. Rinse the tip by aspirating and dispensing the set volume three times and push fully to remove any remaining liquid.
6.6.4. Set up a weight boat on a balance that weighs in micrograms
6.6.5. Aspirate the calibration volume (make sure there are no bubbles) and dispense the liquid slowly into the weight boat. Then, record the weight on the balance and repeat the process ten times, using **increasing** incremental volumes to test the accuracy of the pipette at different measurements. Increase each trial by the same increment.

Temperature (°C)	Z factor
20	1.0029
21	1.0031
22	1.0033
23	1.0035
24	1.0037
25	1.0039

6.6.6. Calculate the dispensed volume by converting values of the weight of water, in grams, to values of the volume of water, in milliliters, based on the density of water (1g water = 1ml water). Convert units of ml to µl where appropriate.
6.6.7. Temperature, atmospheric pressure, and humidity affect the volumes of water dispensed from a pipette. These variables can be corrected for using the Z-factor. Correct your values of the *volume* of water measured using the following equation V_raw x Z = V_corrected (V_raw is the volume of water calculated directly from the measured weight of water, Z is the Z factor, and V_corrected is the Z-factor corrected volume of dispensed water).

6.6.8. Next, determine the mean values of both expected volumes (VØavg) and measured volumes (Vavg) for each set of ten trials.

6.6.9. Finally, calculate accuracy by using the following equation:

$$A = 100\text{-}abs[100\text{-}100*(V_{avg}/V0_{avg})]$$

Where **A** is the accuracy of the pipette in %, $\mathbf{V_{avg}}$ is the average of the measured volumes and **V0** is the average of the theoretical volumes you attempted to dispense, **abs** is the absolute value. If the accuracy value lies in the 99-100% range, the pipette is considered in working order and calibrated.

7. TROUBLESHOOTING

7.1. My solution is viscous, and I always get bubbles. Make a more dilute solution by weight first and then pipette the desired quantity.

7.2. The pipette does not hold the solution. Make sure your pipette tips are mounting properly and securely, this is the most common cause of leaking. Next make sure you are not trying to pipette an organic solvent. Solutions for pipetting must have a certain surface tension to be accurately pipetted. If there is still a problem your pipette may require repair and calibration.

7.3. The pipette cannot aspirate any liquid. Make sure your pipette tips are mounting properly and securely, this is the most common cause of leaking

8. REFERENCES

ISO 8655-1:2002

ASTM E1154-14:2014

Saha, W. by S. (2018, May). *Performing pipette calibration yourself*. Bitesize Bio. https://bitesizebio.com/40766/performing-pipette-calibration-yourself/.

9. MODULE MASTERY TASK

This task will test your ability to make several standard solutions using a laboratory pipette.

1. Pick a P1000, P200 and P20 to calibrate
2. Calibrate the pipettes according to the calibration procedures
3. Create a curve on excel for each one of the pipettes, using each of the 10 trials as your data points
4. Finally, calculate the accuracy for each of the 3 pipettes
5. Described how the A values and R2 values can be used to evaluate the accuracy of the pipette versus the precision of the pipette and pipette operator
6. Make sure to provide conclusions and interpretations of the various calculations
7. Send your graphs, calculations, and conclusions to one of the proctors to check your results