Laboratory #2
Pipetting Technique and Micropipette Calibration
Skills=40 pts

Objectives:

Upon completion of this unit, the student should be able to:

1. List and describe 3 categories of pipets.
2. List the steps for manual and mechanical pipetting.
3. Identify the components of a micropipette.
4. Properly handle and operate a micropipette.
5. Correctly select and read the appropriate volume in microliters on adjustable micropipettes.
6. Perform a micropipette calibration using the gravimetric method.

Materials:

1. Adjustable Micropipette
2. Pipette tips
3. Analytical Balance
4. Taring containers
5. Water
6. Serological Pipettes
7. Deionized water at room temperature
8. Controls

Background Information:

Types of Pipets

1. Volumetric
2. Graduated (measuring)
3. Micropipette

Volumetric pipettes:

- Volumetric pipettes are NOT graduated.
- They are calibrated to a specific volume.
- Volumetric pipettes are most frequently to deliver (TD) but NOT blown out. They may have a colored or etched ring at the top of the pipette. A color block
at the top of the pipette is for manufacturer identification and is NOT a ring. These pipettes are held vertically and drained by gravity with the tip of the pipet placed against the side of the container above the liquid.

- Volumetric pipets are designed to deliver a single volume precisely. They have a bulb-like enlargement which contains the majority of the liquid. The liquid must be drawn up to the fill line for accurate measurement.

**Graduated pipettes:**

1. **Serological pipettes:**
   - Measure various volumes with a single pipette.
   - Graduated to the tip. (Accurate measurement includes all fluid from the graduated fill line to the tip)
   - *To deliver/blow-out.* The vast majority of serological pipettes is TD/blow out and will have the letters TD and/or two etched (indicating blow out) or colored lines near the top of the pipette. After the pipette has drained by gravity, the last drop must be blown out with a bulb or dial device. **Note:** volumetric pipettes are TD/drain and serological pipettes are TD/blow out.
   - Graduated pipettes are not as accurate as volumetric pipettes, due to the fact that each graduation line is not individually calibrated and any imperfection in the internal diameter will have a greater effect on the volume delivered.

2. **Mohr pipettes:** (not frequently encountered in the clinical lab)
   - Only valid when filled to maximum graduation mark.
   - NOT graduated to the tip. Do NOT blow out.
   - To accurately transfer fluid with this type of pipette, the meniscus must be precisely on a graduation mark both at the beginning and at the end of a transfer.
   - Single painted or frosted line (don’t blow out) near the top off the pipette
Micropipettes

COMPONENTS OF THE MICROPIPETTE

- Also known as mechanical or automated pipettes
- Typically used to deliver volumes less than 1000µL
- MUST use a disposable tip
- Can be fixed- volume or adjustable for a range of volumes
- Can be single or multiple channel
Manual Pipetting Procedure

1. Hold the pipette several centimeters below the opening of the bulb with one hand.

2. With your other hand squeeze the bulb and touch the bulb opening to the top of the pipette. Do not insert more than 1/2 cm of the pipette into the bulb.

3. Holding the pipette vertically, place the tip into the liquid sample 2-5 mm beneath the meniscus away from the sides or bottom of the tube (container). **Note: it is critical to hold the pipette straight up and down. Insertion of the pipette tip that is too shallow, leads to sample vortexing and bubbles. Insertion too deep leads to droplets on the outside of the tip.

4. Slowly release the pressure on the bulb. By steadily releasing pressure on the bulb, liquid will to be drawn into the tube, slowly. **Note: Do NOT release your hand quickly or the liquid could flow into the bulb and be contaminated.

5. Pull the bottom of the curved liquid surface, the meniscus, a centimeter or two above the desired volume graduation mark.

6. Quickly, but carefully, remove the bulb and immediately slip your free index finger over the top of the pipette. **Note: Do NOT use your thumb. The index finger offers more control.

7. With your finger firmly on the top of the pipette, lift the pipette out of the sample.

8. Position yourself so that you are at eye-level with the liquid in the tube. **Note: Best practice is NOT to raise the pipette to eye-level, but rather to move your body to reach eye-level.

9. Slowly raise your finger to allow the liquid to drop in the tube until the bottom of the meniscus lines up with desired graduation mark. **Note: This MUST be performed with a vertical pipette at eye-level for accurate measurement.

10. Touch the tip of the pipette to the inside of the container to remove a hanging drop.

11. Touch the tip of the pipette to the second container and release your finger.

12. If the pipette is a serological pipette, verify that it is a TD pipette and gently place the bulb on the end of the pipette and squeeze slightly to "blow out" the remaining liquid. Then remove the pipette from the second container. **Note: If the pipette is volumetric TD, then DO NOT BLOW OUT or shake the pipette, allow to drain, withdraw.
Mechanical or Micropipetting Procedures

Selecting the desired volume in adjustable micropipettes:

1. Dial (turn) the Selecting Ring (Figure 1, pg 3) or volume adjustment knob until you are 1/3 revolution above the desired volume setting. Then return clockwise to the desired volume. This will ensure accurate volume selection.

2. Verify the pipette is set to the desired volume according to the display descriptions below.

SMALLEST MICROPIPETTES

- Some adjustable pipettes with a volume range of 1-20 µL will often have a display arranged as follows:

<table>
<thead>
<tr>
<th></th>
<th>Tens of microliters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ones of microliters</td>
</tr>
<tr>
<td>4</td>
<td>Tenths of microliters</td>
</tr>
<tr>
<td>5</td>
<td>Hundredths of microliters</td>
</tr>
</tbody>
</table>

  The setting of the pipette reading illustrated above would be 13.45 µL.

  These types of pipettes use a line to indicate a decimal point.

- Other adjustable pipettes have a 3 number dial system seen in the image to the right. The number is read with the most significant digit at the top and the least significant number at the bottom. If the lowest number is red, this often indicates tenths (one place to the right of the decimal point).

  The large pipette to the right has a range of 1-20 µL and is set at 5.0 µL (the top number is 0, the middle number is 5, and the 0 at the bottom is red)

MEDIUM MICROPIPETTES

- Some adjustable pipettes with a volume range anywhere between 10-200 µL will often have a display arranged as follows:

<table>
<thead>
<tr>
<th></th>
<th>Hundreds of microliters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Tens of microliters</td>
</tr>
<tr>
<td>0</td>
<td>Ones of microliters</td>
</tr>
<tr>
<td>3</td>
<td>Tenths of microliters</td>
</tr>
</tbody>
</table>

  The setting of this pipette would be 150.3 µL.
LARGEST MICROPIPETTES

Adjustable pipettes with a volume range anywhere between 100-1000 µL will often have a display arranged as follows:

<table>
<thead>
<tr>
<th>0</th>
<th>Thousands of microliters</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Hundreds of microliters</td>
</tr>
<tr>
<td>2</td>
<td>Tens of microliters</td>
</tr>
<tr>
<td>6</td>
<td>Ones of microliters</td>
</tr>
</tbody>
</table>

The setting of this pipette would be 926 µL. **Note: The largest micropipettes do not have decimal points; therefore, usually do not have a line through the setting display.

Forward Pipetting Technique:

This is the most frequently used technique in the clinical lab. In forward pipetting, a desired set volume of liquid is aspirated into the disposable tip and then delivered into the new container.

Procedure:

1. If using an adjustable micropipettor, select desired volume.
2. Push the appropriate disposable pipette tip on the end of the pipettor snugly, but not forcefully.
   - If the fluid is NOT viscous (Ex. water, plasma, control material), see Pre-Rinse steps below before proceeding to Step 3. If the fluid is viscous (Ex. whole blood or body fluids) proceed to Step 3 now.
3. Press the Control Button (plunger) to the FIRST stop.
4. Dip the tip 2-5mm under the sample meniscus.
5. Release the button slowly to aspirate liquid into the disposable tip.
   - **Note: Do NOT “pop” the plunger. This will lead to fluid entering the shaft which can damage the piston and reduce accuracy. Fast aspiration can lead to bubbles reducing accuracy.
6. Remove thumb from the Control Button.
7. Lift the pipette tip out of the sample slowly, but deliberately.
8. If there is residual fluid on the outside of the pipette tip, wipe it off gently. **Note: Do NOT touch the pipette tip opening because this will cause capillary action of the fluid into the wipe and inaccurate transfer volume. NEVER tilt or lay a pipette down that has fluid in the tip. This leads to liquid in the shaft, piston damage, and inaccuracy.

9. Move the pipette tip to the second container (tube) and touch the tip gently to the wall of the tube or vial. **Note: Do NOT let the tip touch the contents of the transfer container.

10. Press the button to the first stop and pause.

11. Firmly and quickly push the button to the second stop. **Note: No droplets or liquid should remain in the pipette tip.

12. Withdraw the pipette tip from the tube.

13. Point the tip into a biohazard/sharps container.

14. Press the Ejection Button (see Figure. 1) to remove the disposable pipette tip from the shaft.

★Pre-Rinse, if applicable

When pipetting non-viscous fluids, such as diluents, calibrator, and control material, the pipette tip should be pre-rinsed after Step 2. This coats the inside of the tip with a fine layer of sample; thereby, reducing the effects of evaporation and lost residual sample. Do NOT pre-rinse when pipetting more viscous liquids such as whole blood or body fluids.

Pre-Rinse steps

1. Press the button to the first stop

2. Place the tip 2-5mm beneath the meniscus of the liquid to be measured

3. Slowly release the control button (DO NOT POP IT)

4. Press the button firmly to the second stop.

5. Proceed with Step 3 in the Forward Pipetting procedure

Reverse Pipetting Technique
In the reverse technique, a larger volume than the desired volume of solution is aspirated into disposable tip. An exact (set) amount is delivered, but liquid remains in the tip. This is infrequently used, but may be preferred when pipetting very viscous or foamy liquids. Pre-rinsing is NOT necessary.

**Procedure:**

1. Select desired volume, if applicable
2. Attach appropriate pipette tip.
3. Push the Control Button to the second stop.
4. Dip the tip 2-5 mm below the meniscus of the liquid.
5. Remove the tip from the liquid.
6. Wipe off excess liquid from the outside of the tip.
7. Place the tip of the pipette against the wall of the transfer container (tube, boat, etc).
8. Depress the Control Button just to the first stop.
9. Keep holding the Control Button in this first stop position and remove the tip from the tube.
10. Return the remaining liquid to the original vessel by pressing the Control Button to the second position or dispose of the remaining liquid in a Biohazard/sharps container by pressing the Ejection button.

**Micropipette Calibration**

Calibration of Pipettes, officially means determining the difference between the dispensed volume and the selected volume. Adjustment means altering the pipette so that the dispensed volume is within certain specifications.


**Summary of Method:** The correct functioning of a micropipette is determined by checking the accuracy and precision of the volumes it delivers. The weight of water samples delivered by the instrument is converted to a volume based on the density of water. Calibrations should be
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checked at two volumes: one at the **maximum** setting and another at **half** the maximum setting—so this means two sets of measurements for each micropipette.

**Micropipette Calibration Activity:**

1. Work in pairs and select an adjustable micropipette. Each member of the group will perform the pipetting for one of the two settings described above (max and ½ max volume.)

2. The analytical balance used to check the micropipette must be well-maintained and properly calibrated. It must also be in a draft-free, vibration-free environment.

3. Document all relevant information including date/time, micropipette serial number, pipette volume range, temperature, and name of the person performing the evaluation. **Note: If the water and micropipette are not stored at the same temperature, allow at least 2 hours for temperature equilibration.**

4. Set the micropipette volume (maximum volume for first setting, then 50%).

5. Tare(zero) the balance to a weighing boat or beaker.

6. Using your best **forward pipetting** technique (see procedure above) measure and deliver a volume of deionized water into the weighing boat.

7. Immediately record the weight measurement.

8. Tare(zero) the balance between each measurement.

9. Working quickly, repeat steps 5-7 for a total of 10 weight measurements. **Note: Avoid prolonging this process, as you want to avoid evaporative losses.**

10. Repeat the 10 measurements at the second volume setting for your micropipette.

11. For each volume setting, calculate the mean delivered weight ($\bar{W}$) of the water for the 10 measurements.

12. Calculate the Mean Volume Delivered ($V_1$) at the mean water temperature of 22.0 °C using the following formula. Please note, “$Z$” is the conversion factor (in µL/mg) for the mean water temperature. “$Z$” for this lab is 1.0033.

$$V_1 = (\bar{W}) \times Z$$

13. Determine the % inaccuracy or the “inaccuracy” by calculating the percent error of the micropipette at each volume setting as follows:
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- Percent error = \( \frac{\text{mean volume delivered} - \text{expected volume}}{\text{Expected volume}} \times 100 \)

Log your result on the report form.

14. Determine the precision (repeatability) of the micropipette at each volume setting by first calculating the standard deviation (SD) as follows:

\[
SD = \sqrt{\frac{1}{n-1} \sum (x_i - \bar{x})^2}
\]

- \( x_i \) = each weight measurement
- \( \bar{x} \) = mean delivered weight measurement
- \( n \) is the number of measurements (10)

Log your result on the report form.

15. Determine the imprecision by calculating the coefficient of variation (CV%)

\[
CV = \frac{SD \times 100}{\bar{W}}
\]

Log your result on the report form.

**Note: You may use scientific calculator functions to determine SD and CV. You may also use SD calculation websites:
http://www.mathsisfun.com/data/standard-deviation-calculator.html

16. Check the manufacturer’s specifications, below, for the micropipette to see if your measurements are within the expected range. Document if “out” of manufacturer’s limits.

<table>
<thead>
<tr>
<th>Manufacturer’s Inaccuracy Limits</th>
<th>Manufacturer’s Imprecision Limits</th>
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<tbody>
<tr>
<td>Pipette Range in µL</td>
<td>Limit at 50% Setting</td>
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<tr>
<td>10-100</td>
<td>±1.0%</td>
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<tr>
<td>20-200</td>
<td>±1.0%</td>
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<tr>
<td>100-1000</td>
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**Additional Manual Pipetting Practice Activity**

Reconstitute (rehydrate) one (1) lyophilized (freeze-dried) specimen.
1. Each student will take **one (1)** vial of lyophilized control material.
2. Determine the amount of deionized water required by looking at the control vial or package insert.
3. Choose the **appropriate manual pipette(s)** to reconstitute each vial.
4. Obtain a pipette bulb from the supply shelf.
5. Each pair or grouping of students will need a **beaker** filled with **deionized water** from the container/faucet at the sink.
6. Work with one vial at a time. If vials have silver aluminum tops or collars, carefully open the vials by peeling back the silver tops and collars (Take care not to cut fingers!). Carefully remove the stopper and place it upside down on a kim wipe on the counter.
7. Using good technique and the appropriate pipette, pipette the correct amount of deionized water into each vial.
8. Restopper the vials and gently invert each 10 – 15 times.
9. Label tubes with **initials, date and time**.
10. Have the instructor verify that the vials have been properly reconstituted, restoppered, and labeled.
Micropipette Calibration Report
40 pts.

Pipette Serial number:_________________  Pipette Volume Range:_________________

**Setting 1:**

Name of person calibrating:_________________
Calibration Date/Time:_____________________
Room Temperature:_______________________

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<th>RUN</th>
<th>Weight Measurement</th>
<th>Mean Weight Delivered ($W$)</th>
<th>Mean Volume Delivered ($V_l$)</th>
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<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (CV)</th>
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</table>
**Setting 2:**

Name of person calibrating: ___________________
Calibration Date/Time: _______________________
Room Temperature: _________________________

<table>
<thead>
<tr>
<th>RUN</th>
<th>Weight Measurement</th>
<th>Mean Weight Delivered ($\overline{W}$)</th>
<th>Mean Volume Delivered ($V_i$)</th>
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<tbody>
<tr>
<td>1</td>
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<table>
<thead>
<tr>
<th>% Error (imprecision)</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (CV)</th>
</tr>
</thead>
</table>
Pipetting and Micropipette Calibration

Study Questions

13 points (1 pt each answer unless otherwise stated)

1. Below are readings from a 1-20µL pipette. What volume is represented in each display?

   | 1 | 0 |
   | 5 | 8 | 0 |

2. Below are readings from a 10-100 µL pipette. What volume is represented in each display?

   | 1 | 0 |
   | 0 | 4 | 2 | 0 |

3. Below are readings from a 100-1000 µL pipette. What volume is represented in each display?

   | 0 | 8 |
   | 7 | 1 | 0 | 0 |

4. What is the name of the type of pipette that is calibrated for a specific volume and does not have graduations?

5. What type of specimen requires micropipette pre-rinsing for most accurate results?
6. What might happen to a pipette if the Control Button (plunger) is released suddenly (rather than slowly) and “pops”?

7. Which finger is used to plug the top of the serological pipette?

8. Volumetric pipettes without an etched line are TD/blow out. True/False (circle one)

9. Standard deviation=Imprecision. True/False (circle one)

10. Explain why slow sample aspiration is better than fast sample aspiration in micropipetting.