Medical Cellular and Molecular Biology I

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Practice: The use of micropipettes

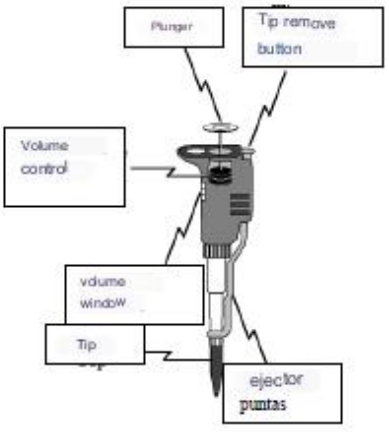
OBJECTIVE: Know the parts and operation of micropipettes; acquire skills in the use of pipettes to measure volumes between 1 and 1000 µL.

MATERIALS

3 sets of micropipettes P1000, P200 and P20
Deionized water
Glycerin or glycerol
Balances that measure 0.0001 g

2 mL plastic petri dish lids or 5 cm diameter watch glasses Tips for micropipettes (yellow and blue)

THEORETICAL AND PRACTICAL FOUNDATIONS



In biochemistry, the ability to accurately and reproducibly measure and transfer small volumes of fluids are critical to obtaining useful results. For volumes less than 1 mL, the most common method of measuring liquids requires the use of a device known as a micropipette.

A drawing of two micropipettes is presented. The pipets you use may not look like the ones shown. The micropipettes used in this laboratory come in three different presentations.

TYPES OF MICROPIETTES

The types: P1000, P200, and P20.

P1000 is useful for volumes from 200 to 1000 µL
P200 is useful for volumes from 20 to 200 µL.
P20 is useful for volumes from 0.5 to 20 µL.

Make sure you are using the correct micropipette for the volume you need. Also, make sure the micropipette is actually set for the volume you need by checking the "volume window", and, if necessary, changing the volume using the volume control "device" until the micropipette corrects the volume (the micropipette doesn't read your mind - several people will use the pipettes, you won't always find it the way you need it).

Do not try to set the micropipette for volumes greater than maximum, or for volumes less than zero, this will decalibrate and damage the micropipette.

All micropipettes use disposable tips (do not pipet without using the proper tip, doing so will contaminate the micropipette and may damage it).

Do not use the micropipette with liquids that attack polypropylene.

Do not use liquids that are emitting vapors.

The temperature of the liquids must be between 15 and 40 °C.

When inserting the tip, make sure the tip is the correct type and that it fits correctly.

Generally for P1000 the tips are blue, for P200 they are yellow and for P20 they can be yellow or white.

HANDLING THE MICROPIPETTE

Filling the pipette

Place your thumb on the pipetting knob or plunger.

Depress plunger. When the plunger is depressed you will feel a point of resistance. This is the first "high" or "top." If you continue to press you will find a point where the plunger no longer moves down, this corresponds to the second "top" or "top".

Depress the plunger to the first stop and place the tip into the liquid to a depth of 2-3mm. In a slow and controlled manner, decrease pressure on the plunger to allow it to travel up. Do not release the plunger abruptly, allowing it will cause liquid to splash inside the tip, producing inaccurate volumes and contamination of the pipet. Once the plunger has moved to the top, keep the micropipette in the liquid for one second, this prevents air from being aspirated at the end.

Sample ejection

Take the micropipette to the container in which you want to add the liquid. The tip rests on the wall of the container without preventing the sample from escaping. Press the plunger to the first stop and then to the second stop. Do this procedure at a moderate speed, doing it too fast will leave sample drops on the tip. If you watch carefully then you will notice that pressing to the second stop will expel all the liquid from the tip.

The above is true for most aqueous solutions, except for highly viscous solutions. For organic solvents or for solutions containing large amounts of protein (plasma and serum), it is difficult to remove all the liquid from the tip. In these cases, it is better to pipet the solution once, expelling it and then drawing up the liquid to be measured in a second time. For highly viscous solutions, filling and ejection must be slower.

If used improperly, the micropipette will transfer inaccurate volumes.

The micropipette may lose its calibration. Checking pipettor calibration is a simple procedure that can save considerable time, energy, and reagents. In this practice you will learn to use the micropipette of various sizes to measure its accuracy, precision and calibration.

PROCEDURE

For each micropipette, review the percentage accuracy (E%) and the coefficient of variation (CV%) over the entire range using at least two different volumes; by

Example: For the P1000 micropipette check the volumes of 300 µL and 1000 µL. For P200 review the volumes of 60 and 200 µL. For P20 check 3 and 10 µL.

1. Select the appropriate micropipette and tips.

2. Place the petri dish lid on the balance and tare to zero.

3. Take the volume of distilled water with the micropipette and dispense it on the lid of the petri dish. Record the weight of water added. The density of water is 1 g/mL at 25°C, therefore a volume of 1 mL corresponds approximately to a mass of 1 g. 300 µL at 0.3 g, 200 µL at 0.2 g, 60 µL at 0.06 g. 10 µL at 0.01 g and 3 µL at 0.003 g.

4. Repeat the procedure five times for each volume.

5. Repeat the entire procedure for glycerol. The density of glycerol is 1.2656 g/mL at 25°C. Calculate the expected mass for each volume.

CALCULATION OF THE ACCURACY (E%) AND THE COEFFICIENT OF VARIATION (CV%)

Average volume calculation

The values of the weights of the gravimetric control are only the mass of the dosed volume. To obtain the real volume, a corrective calculation must be made. The corrective calculation is made by multiplying the mean value of the weighing values (x) with the Z factor (mL/g, which is the same as ul/mg), which takes into account the density of the water, the control temperature and atmospheric pressure. Z is equal to 1.0032 µl/mg, referred to 21.5°C, 1013 mbar (hPa) and to the use of distilled water or z is equal to 1.0029 µl/mg, referred to 20 5°C and 1013 mbar (hPa). The Z factor is equal to 1/density.

Control values at 21.5°C (Z = 1.0032)

Controlled volume V. (ul): 200,0000

Face value (mg) = Controlled volume/Z

Face value (mg): 199.3620

x1 =200.2000 x2-199.6000 x3=199.4900 x4 199.7000 x5 199.7000

Middle value

$$X = \frac{So}{n}$$

Xi= result of the weighing
n = number of weighings

medium volume

$$\bar{V} = \bar{x} \cdot z$$

$$\bar{V} = \frac{x_1 + x_2 + x_3 + x_4 + x_5}{5} \cdot Z$$

$$\bar{V} = \frac{200.2 + 199.6 + 199.49 + 199.7 + 199.7}{5} \cdot 1.0032$$

V=199.738 1.0032

V=200.3772

Z factor of distilled water at different temperatures

Temperature °C	Factor z ml/g	Temperature °C	Factor z ml/g
18	1.00245	22.5	1.00338
18.5	1.00255	23	1.00350
19	1.00264	23.5	1.00362
19.5	1.00274	24	1.00374
20	1.00284	24.5	1.00386
20.5	1.00294	25	1.00399
21	1.00305	25.5	1.00412
21.5	1.00816	26	1.00426
22	1.00327		

Accuracy (E%)

$$I\,[\%] = \frac{V - V_{nominal}}{V_{nominal}} \cdot 100$$

$$E\,[\%] = \frac{200.3772-200}{200} \cdot 100$$

$$E\,[\%] = 0.189$$

Standard deviation

$$S = Z \cdot \sqrt{\frac{\Sigma(X_i - X)^2}{n-1}}$$

$$s = Z \cdot \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + (x_4 - \bar{x})^2 + (x_5 - \bar{x})^2}{4}}$$

$$81.0032\ U \sqrt{\frac{(200.2-199.74) + (199.6 - 199.74)^2 + (199.49 - 199.74)^2 + (199.7 - 199.74) + (199.7 - 199.74)^2}{4}}$$

$$s = 1,0032 \cdot \sqrt{\frac{0.29688}{4}}$$

$$s = 0,273$$

Coefficient of variation

$$HP\,[\%] = \frac{S-100}{\nabla}$$

$$CV\,[\%] = \frac{0,273 \cdot 100}{200,3772}$$

$$CV\,[\%] = 0,136$$

For the calculated example, the following result is obtained:

Controlled Volume (µL): 200.0000
Mean Volume (µL): 200.3772
E" [%] 0.189

CV [%]	0.136	
E [%] nominal*	0.500	
CV [%] nominal*	0.200	* tolerance limits of handling instructions

If the calculated values of accuracy (E [%]) and coefficient of variation (CV [%]) are less than or equal to the tolerance values, then the apparatus is OK.

*Limits of tolerance

Volume (µL)	E% rated	CV% nominal
5-10	1	0.8
20-50	0.7	0.4
100-1000	0.5	0.2