

## Teacher's Guide for Pipetting Lab and Using the Microcentrifuge

**Time Allotment:** One period for explanation and prelab; one period for actual activity, additional periods for review activities as needed

**Preparation: (for basic activity)**

4 food coloring solutions aliquotted for each lab group in 1.5ml eppendorf tubes (in tube holders) labeled I, II, III, and IV.

1 glycerol + food coloring solution labeled tube V (consider using another kind of tube if available)

extra eppendorf tubes labeled A-D

p20 micropipets

yellow pipette tips (fit both p20 and p200 micropipets)

p200 micropipets

blue pipette tips (if using p1000)

p1000 micropipets

waste containers (paper cups or beakers)

paper towels to dry out tubes at end of period

Microcentrifuges

waxed paper for practice

**For Review Lab 1:** 4 tubes of colored solutions (3 labeled X, Y and Z), p20 and p200 pipets, tips, racks, waxed paper, PCR or other tubes, waste container, and paper towels

**For Review Lab 2:** 2 tubes of colored solution, racks, p20 and p200 pipets, tips, waxed paper

**Background for students:** Review metric units: particularly liter, milliliter, and microliter. Also review the abbreviations for these units: (L for liter, mL or ml for milliliter, and  $\mu\text{L}$  or  $\mu\text{l}$  for microliter)

Demonstrate the use of the micropipet, including all rules for use. When demonstrating use, exaggerate your motions so students don't forget.

Encourage students to REALLY observe the volume of liquid in the pipette at each step. This will allow them to "eyeball" whether they have the correct amount or not after some practice.

Students should also observe whether there is liquid on the outside of the tip. This should be removed CAREFULLY before transferring any liquid as it will change the total amount of liquid transferred.

Demonstrate the use of the microcentrifuge before student use. Be sure to emphasize balancing the tubes.

Watch student's technique. The two most common mistakes are:

- (1) When taking up liquid: Depressing the plunger to the second stop (instead of the first stop) before filling the tip (results in inaccurate measuring)
- (2) When dispensing the solution: Releasing the plunger before removing the tip from the liquid (results in sucking the liquid back up into pipette)

You may consider requiring that each student demonstrate his/her proficiency at each step before going on—No air in tip, no liquid left in tube, etc. Repeat until successful.

**Note on Microfuges:** If there are no white inserts available, cut the lid off of any eppendorf tube and insert into the holes. These will hold the 0.2ml tubes just as well as the inserts. It is very easy to tell if the students have balanced the load in the microfuges. You should not hear any noise, other than the whirl of the motor.

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Answers for Micropipetting and Microfuge PreLab:

1. 

a. $1\ \mu\text{l} = .001\ \text{mL}$	d. $1500\ \mu\text{l} = 1.5\ \text{ml}$
b. $100\ \mu\text{l} = .01\ \text{mL}$	e. $60\ \mu\text{l} = 0.06\ \text{ml}$
c. $250\ \mu\text{l} = .25\ \text{ml}$	f. $3\ \mu\text{l} = 0.003\ \text{ml}$
  
2. 

a. 2.5 ml, 250 $\mu\text{l}$ , 0.025 ml, 2.5 $\mu\text{l}$
b. 250 $\mu\text{l}$ , 100 $\mu\text{l}$ , 0.015 ml, 0.01 ml
  
3. 

a. setting the micropipette beyond its range will make it inaccurate, and may break the mechanism
b. without the plastic tip, the liquid you are measuring will enter the barrel of the micropipette and contaminate it (whatever that liquid is will continue to drip into whatever is measured after that time)
c. if the micropipette is held vertically, the liquid can run back into the barrel. (see above answer)
d. if the plunger is released too quickly, air bubbles may occur, causing bad measurements
  
4. 

The p20 is pipette b	its range is 0-20 $\mu\text{l}$
The p200 is pipette c	its range is 20-200 $\mu\text{l}$
The p1000 is pipette a	its range is 200-1000 $\mu\text{l}$
  
5. Reading left to right the measurements should be:  
2.5  $\mu\text{l}$ , 7  $\mu\text{l}$  (these could be reversed also), 150  $\mu\text{l}$ , 300  $\mu\text{l}$
  
6. If the centrifuge is unbalanced, it can damage the rotor mechanism.
  
7. Opposite or evenly distributed (see original handout)
  
8. Eppendorf tubes hold 1.5 ml of liquid, but the students should figure out a way to test this.