Manipulating Small Volumes of Glycerol

~ WEAR GLOVES ~

I. Practicing with a P-20

1. Label two empty reaction tubes A and B with a permanent ink marker, if not already labeled.
2. Add the amounts of solutions I, II, III and V to tubes A and B as shown in the table below.

Solution Volume in Each Tube						
Reaction Tube	**10% glycerol**	**25% glycerol**	**40% glycerol**	**60% glycerol**	**80% glycerol**	**Total in Tube**
A	4 µL	5 µL	2 µL			
B	6.5 µL	2.5 µL			2 µL	

3. Spin tubes A and B in the microcentrifuge for 1-2 sec to pool the solutions.

> REFER TO PREVIOUS CENTRIFUGE INSTRUCTIONS

4. Add up the total volume of liquid in Tube A. AS A CHECK OF YOUR TECHNIQUE, set the micropipet to that volume and withdraw all of the liquid in tube A. The contents should *just* fill the tip -- no air space at the bottom of the tip; no leftover liquid in the tube. Discard liquid and tip into waste beaker
5. How much should be in tube B? _____µl Check your technique by setting the pipettor to the correct volume and withdraw all (hopefully) the solution in tube B.

II. Practicing with a P-200

Reaction Tube	**10% glycerol**	**25% glycerol**	**40% glycerol**	**60% glycerol**	**80% glycerol**	**Total in Tube**
C	**20 µl**		**57 µl**	**110 µl**		

1. Label an empty reaction tube C, if not already labeled.
2. Add the amounts of solutions I, II and IV to tube C as shown in the table below.
3. Spin tube C for 1-2 sec. (Be sure to balance your tube.)
4. Check the accuracy of your micropipeting technique with the P-200: Set the pipet to _____ µL (the predicted "supposed to be" volume) & withdraw the contents of tube C.

III. Practicing with a P-1000.

1. Label an empty reaction tube D if not already labeled
2. Add the amounts of solutions III and V to tube D as shown in the table below.

Reaction Tube	**10% glycerol**	**25% glycerol**	**40% glycerol**	**60% glycerol**	**80% glycerol**	**Total in Tube**
D	**100 µl**		**320 µl**		**580 µl**	

3. Spin tube D for 1-2 sec. (Be sure to balance your tube.)
4. Check the accuracy of your micropipeting technique with the P-1000. Set the pipet to _____ µL and withdraw the contents of tube D.

IV. Practicing with all micropipets

1. Label an empty reaction tube E, if not already labeled.
2. Fill in the blanks in the following chart. Indicate on the line "P-_____" the appropriate micropipet to be used for each sample. Then write in the three boxes below each line, the numbers that should be dialed to give you the indicated volume.
3. Add the volumes of solutions I-V indicated above into tube E, using the *appropriate micropipet.*

Reaction Tube E	**10% glycerol**	**25% glycerol**	**40% glycerol**	**60% glycerol**	**80% glycerol**
Volume:	**15 µl**	**105 µl**	**2 µl**	**38 µl**	**350 µl**
Micropipet: (P-20,P-200,P-1000)	P-_____	P-_____	P-_____	P-_____	P-_____
Micropipet setting					

Check the accuracy of your micropipeting technique with the P-_____. Set the pipet to _____ µL and withdraw the contents of tube E.