# Working and best practices in a cell culture lab

Science, Biology

Learning the safety procedures is always the first and most important thing to do before entering a lab of any kind. Learning the safest methods and using the best practice methods is especially important in a cell culture laboratory. While handling and culturing cells, it is of the utmost importance to be as precise as possible and to limit any possible errors. Thus, throughout this lab experiment we practiced some of the basic techniques that will be important to use correctly in the future. These practices included using two kinds of pipettes to transfer solution, diluting a solution, and finding the absorbance of such dilutions.

The first type of pipette that was used was the pipette aid which is meant for larger amounts of liquids. The pipette aid can use serological pipettes of either 5ml or 10ml which are color coded accordingly. Then, in order to transfer smaller amounts of liquids we also learned how to properly use a micropipette. We were working with three different sizes of micropipettes: a P-20, a P-200 and a P-1000. The amount of liquid these devices can we use for increase as the number increases. For instance, the P-20 can transfer 2-20 microliters while the P-1000 can transfer 100-1000 microliters. The micropipettes also must have a tip attached to the end with different size tips for the different kinds of micropipettes. While using both types of pipettes, many different kinds of errors can occur. For instance, setting the pipette down, touching the pipette tips to the rim of the microfuge tubes, or allowing air bubbles to enter the tip by not pressing down to the first stop of the micropipette before entering the liquid. While taking in the solution into the device, be sure not to fill it higher than the highest capacity as it can damage the device or cause it to become unsterile. And make sure to

dispense liquid slowly while at a 45º angle to avoid splashing. In addition to the aforementioned procedures, try to read the level of the serological pipettes at eye level, and make sure to dispense all the liquid that was taken in, to avoid even more possible errors.

The procedures in this lab included transferring water and stock solution in order to dilute it and read the absorbance using a spectrometer, all while practicing good lab technique. First, to begin the experiment, the procedure called for a 10 mL serological pipette and a pipette aid to transfer 6. 5 mL of stock solution into one centrifuge tube. Here it was important to assure the pipette does not contact any tube surfaces to decrease the chance of contamination. The centrifuge tube was then placed into a holder and the serological pipette was disposed of. Note that caps of any tubes that are not being used should be capped at all times to avoid contamination as well.

Next, 7 mL of the stock solution was transferred using the serological pipette. 4 mL was placed in one test tube and another 3 mL was placed into another test tube. A new 5 mL serological pipette was used to dilute the 4ml solutions by 50% and also by 25% while both containing 2 mL of solution in both of the new 15ml centrifuge tubes. The accuracy of the dilution was then found by testing using a UV-vis spectrophotometry, and the results were recorded. In order to dilute 2 mL of solution by 25% it required to add roughly 0. 66 mL of water while still using the pipette aid. (2ml solution/2. 66 total *100 = 75% solution) Transferring 0. 66 ml of water with a pipette aid is not a precise transfer thus inducing error. A new 15 mL pipette and the pipette aide was then used to transfer 5 mL of the stock solution into the T-

25 flask. T-type flasks allow for gas exchange and provide a sterile barrier against contamination.

Using proper technique the T-25 flask was opened, flipped and the liquid was dispensed on the side opposite that the cells would be cultured on to avoid harming them. Similar to before, it was important to ensure the pipette did not touch the inside of the flask, to reduced contamination. A new 15 mL pipette was used to transfer 4. 0 mL of the previous solution in the T-25 flask to another T-25 flask. It is important to make sure that the pipette tip did not touch the side of the old T-25 flask when transferring the medium. The serological pipette was then discarded.

Then 3 mL was transferred from that T-25 flask into the first 3 wells of a 24 well plate, ensuring to never touch the sides of the wells with the pipette. A 24-well plate is a flat plate with multiple wells that are used as small test tubes. While transferring liquid into a 24 well plate we tried not to expose the entire 24 wells to the unsterile environment. Instead, we slid the lid over to uncover just the section of wells we were working with. The next section required the use of different size micropipettes.

First, a P1000 micropipette was used to transfer 2. 25 mL of solution into three microcentrifuge tubes at 750 uL each from the 4. 5 mL solution. It is important not to touch the micropipette tip to the edge of any contain to avoid contamination as well as pressing the micropipette to the first stop before entering the liquid to avoid air bubbling. Then, a new micropipette tip was then used to transfer 500 uL from each microcentrifuge tube into the first three wells of the 24 well plate. This was completed again by trying to

avoid uncovering all 24 wells to minimize possible contamination. Discard the tip. A new micropipette tip was used to transfer 150 uL from the previous three wells into the three wells of the next row. Lastly, using a new micropipette tip transfer 200 uL of water to each of the previous three wells. The tip was then discarded.

A UV-vis spectrophotometry was used to measure the absorbance of the stock solution and the two diluted solutions. However, the stock solution should have the highest absorbance according to Lambert-Beer’s law. This law states that when concentration increases, absorbance increases. This drastic error could be a result of many things. If the dilutions were done incorrectly due to human error and improper lab techniques, these numbers could have been affected. Instead of using solely the pipette aid to do the dilutions, using the micropipette could have resulted in a more accurate result. Instrumental error in this procedure could include errors with the spectrometer machine itself while reading the absorbances or even with the tubes that were put into the machine. If the tubes were smudged or dirty than the resulting absorbances could be lowered.

## Conclusion

The purpose of this experiment was to gain practice in using micropipettes and a pipette-aid while becoming familiar with safety precautions and proper lab techniques while working in the cell culture laboratory. Each student was required to transfer stock solution and water with pipettes from different containers including: a T-25 flask, 24 well plate, centrifuge tubes and microcentrifuge tubes. Dilution was also performed in order to measure the

absorbance of a stock solution, a 50% diluted solution and a 25% diluted solution. When the solutions are put through a UV-vis spectrophotometer, you should see that as you dilute the solution the absorbance also decreases, due to Lambert-Beer’s law, which also indicates the dilutions were done properly. This was not the case for our particular results, indicating attention to detail and utilizing proper lab techniques has to be a point of emphasis going forward.

The serological pipettes come in 5 ml and 10 ml sizes and are paired with the pipette aid. The micropipettes come in three different sizes, P-20, P-200 and P-1000. P- 20 has a range of 2 microliters to 20 microliters, P-200 has a range of 20 microliters to 200 microliters and the P-1000 has a range of 200 microliters to 1000 microliters. Centrifuge tubes can hold approximately 15 ml. Attach the serological pipette to the pipette aid by opening in slightly at the top of the pipette (the side with the cotton). Then grab the pipette, with the paper still on, and attach it to the pipette aid by slowly twisting it. Save the paper to allow yourself to discard the serological pipette later on. If you tilt the pipette back and the liquid goes past the red mark, you should contact your TA or lab instructor immediately because the device can be damaged or become unsterile.

In order to transfer 23 ml of liquid, use the 10 ml serological pipette. To be more precise, you can use the 10 ml pipette to transfer the first 20 ml and then use the 5 ml pipette to get a more accurate 3 ml transfer. The best practice of withdrawing liquid and dispensing liquid while using a serological pipette is to withdrawal at 90º and dispense at 45º while pressing the

buttons very slowly to avoid splashing. Also read the level of the liquid at eye level. While dispensing liquid into a T-flask slowly turn it upside-down and at a 45º angle to avoid damaging the cells. Be sure not to set the flask lid down to avoid contamination. If you spill liquid while transferring, tell the lab instructor or TA immediately and prepare to clean it up. When withdrawing liquid using a micropipette, press the plunger until the first stop, then place the tip in the liquid and release the plunger. When dispensing, slowly press the plunger until the second stop and hold it for a few seconds to ensure all liquid is out of the tip. Withdrawal liquid while holding the micropipette at a 90º angle and dispense around a 45º angle to avoid splashing. Also avoid touching the micropipette tip to the lid of the container or the outside of the container. The best way to dispense liquid using a micropipette is slowly, at an angle and against the side of the container so that the liquid doesn't splash or harm any of the cells.

Errors while using the micropipette include contamination by dirtying the tip, air bubbling by using improper techniques or measuring the wrong amount of liquid. If your sterile pipette tip touches the outside of the bottle be sure to dispose of the tip and grab a new sterile tip. If you plunged the tip into the media bottle, the media would become contaminated and should be replaced or restarted. It could cause the experiment to fail. To avoid bubbling, be sure to slowly press the plunger to the first stop before entering the liquid, and then slowly press the plunger to the second stop to dispense liquid. Pressing the plunger while already in the liquid or pressing the plunger too quickly can cause air bubbles which may cause errors in calculations. Using a pipette may mix liquids while the second liquid is

dispensed but it should not be used to stir liquids together due to possible contamination.