Appendix 2: Pipette Olympics

Adapted from Pipette Olympics: An Engaging Exercise for Undergraduate Laboratory Training. Richter, Wilkinson, Griffin, and Hunter. The Journal of Neuroscience Education, Fall 2022. 21(1) A81-A84

By this time, you have probably been taught several times how to properly use a pipette, but how can you know if your technique is correct? The following exercise is designed to give you feedback about the precision of your pipetting technique. In addition, you'll use a plate reader, graph some data, and calculate some simple statistics.

Learning goals:

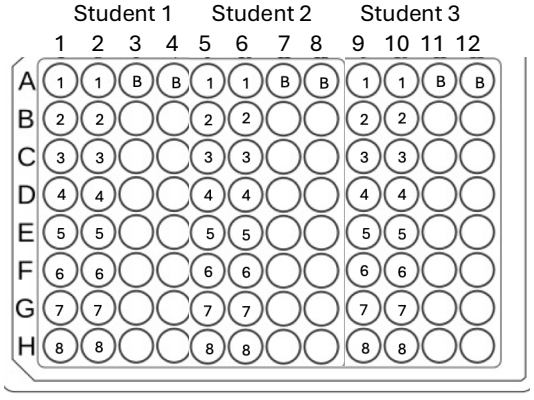
- Reinforce proper pipetting technique.
- Create serial dilutions of a colored liquid.
- Use a plate reader to measure the absorbance at 450nm of each well.
- Learn to do a standard background correction on the data from the plate reader, using wells containing water.
- Use data collected to create a scatterplot and fit a least squares regression line.

Materials needed:

- 96-well flat bottom plate
- P1000 and P200 with appropriate tips
- 1.5 mL microfuge tubes
- Solution with food coloring (50 µL of yellow food coloring in 10 mL of water.)

Plate map:

The plate map describes where each sample will be located. Note that it is customary to have two of each condition so that the measured values can be averaged.



Protocol

1. Label seven 1.5 mL microfuge tubes as numbers 2–8 and pipette 800 µL of water into tubes 2–8.

2. Provided tube 1 contains undiluted yellow sample.

3. Transfer 200 µL of the yellow sample from tube 1 into tube 2 and mix by vortexing.

4. Repeat step 3 (transferring 200 µL from tube 2 into tube 3, vortex; 200 µL from tube 3 into tube 4, vortex; etc.). **How much was the sample diluted in each transfer? What is the final dilution? Record these values in Table 1.**

5. Transfer 200 µL of the solution in tube 1 into both wells A1 and A2 in the 96-well microliter plate (replicates).

6. Repeat step 5 (transferring 200 µL from tube 2 into both wells B1 and B2, etc.) for the remaining vertical pairs of wells in columns 1 and 2. See Figure 1 for a visual guide.

7. Pipette 200 µL of water into wells A3 and A4 to serve as blanks.

8. Using a microplate reader, measure the absorbance at **450 nm** of each well. The wavelength varies depending on the color of the dye; the dye we are using, yellow 5, absorbs in the 400-460nm range. Set the microplate reader to measure at 450nm. Save your data (raw data → file → export as text) on provided thumb drive. Export the data from the thumb drive to your computer and analyze in **Microsoft Excel** (***please DO NOT use Google excel)***. Delete your data from the thumb drive and return to instructor.

9. In Microsoft Excel, average the absorbance of the blank wells. Then subtract this value from all other wells to eliminate background absorbance.

10. Take the average absorbance of each duplicate pair in columns 1 and 2 and generate a table (see Table 1 for an example).

11. Generate a **scatterplot** with these **absorbance averages on the y axis** and the **relative sample concentration in each solution on the x axis** (see Figure 2 for an example). If you have a value with a "!"in front, this value is out-of-range. You cannot use it in the graph because it will not have a linear relationship with the other values.

12. Add a **linear trendline to the plot along with an r^2 value and equation of the line**. Make sure that you have at least 4 decimal places in the r2 value. This can be adjusted by right-clicking on the trendline equation, choosing "Format Trendline Label", then choosing "Number" from the drop-down category.

Table 1. The background adjusted and averaged absorbance values from each replicate pair correlating to the relative sample concentration in the serial dilution.

Standard	Relative Concentration	Absorbance
1	1	
2		
3		
4		
5		
6		
7		
8		

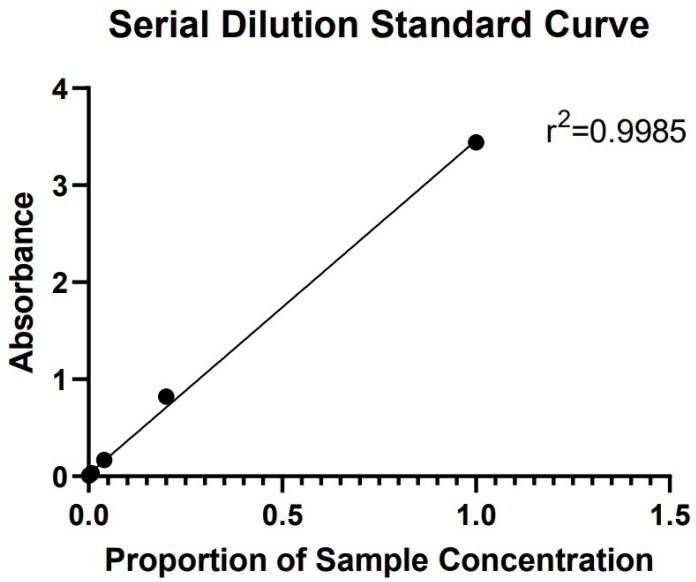


Figure 2. Example serial dilution standard curve with a linear regression line and r^2 value. You should also include the equation of the line. Note that your graph will have more points.