College of the Canyons: "Introduction to Biotechnology" Custom Lab Exercises

Data Gathering and Graphing Lab: Volumetrics and pH

Version: 7-12-12

- Accurate volume transfers are at the heart of any biotechnology protocol.
- Volumetric devices range from beakers to pipets. Any transfer must address the idea of accuracy verses time allotted. Researchers must choose the right piece of equipment that will give them accurate measurement, all the while be precise or more general when the process warrants this.
- Techniques for solution preparation range from direct measurement of dry reagents or through serial dilution from more concentrated liquid stocks. These common techniques can affect pH among other solution properties. The recently completed metric lab activity clarified this idea some.
- Using EXCEL and data replicates, researchers can investigate the effect of volume transfer on accuracy and pH graphically. Such data can be used to suggest how various techniques in a biotechnology lab may be modified to generate more reliable results.

- For more information on College of the Canyons' Introduction Biotechnology course, contact Jim Wolf, Professor of Biology/Biotechnology at (661) 362-3092 or email: jim.wolf@canyons.edu. Online versions available @ www.canyons.edu/users/wolfj

I. Objectives:

1. Become familiar with data gathering, concept of replicates, graphing and use of Excel spreadsheets and graphing programs.
2. Understand ideas inherent in data collection and graphing with respect to volumetric devices, transfer techniques, effects on accuracy, pH and media preparation techniques. This idea of volumetric devices and transfer also relates to time management and general lab efficacy.
3. Address effectiveness of solution preparation by direct preparation and serial dilution.
4. Address time management and learn to multitask and collaborate with your lab partners.

II. Background:

Rules for the exercise:

1. You will be working in groups of two.

2. At the end of the exercise, you will hand in the data set (Excel generated spreadsheet and histogram) and a brief summary statement (to be completed by each lab partner separately) on the volumetric portion of this lab. A separate histogram of pH verses concentration and manner of preparation (serial dilution verses direct preparation) will also be prepared. The metric system lab should be reviewed for pH data

3. A lecture about some of the pitfalls to avoid and general rules for graphing will be presented immediately prior to lab.

4. An additional short summary statement, written in the scientific style will also be turned in. This lab will NOT be graded. Subsequent writing labs (notebook, formal lab write –ups, presentation, etc.) will form the basis of some of your in-lab writing grade.

Volumetric Determination Protocol Notes:

1. For most solutions (under normal, atmospheric pressure), as volume decreases, the mass decreases. This allows one to use mass measurements as a way to verify volumetric measurements (provided the density of the solutions is known.) Fresh water is uniquely positioned with in the metric system (by design). One gram of water takes up 1 ml of volume and will occupy a space of one cubic centimeter (cc^3).

2. The standard error helps to avoid possible mistakes associated with a single erroneous sample. The larger the volume, the less the percent error will likely be. For example consider how a drop of solution would effect the measurement of 100 mls vs. 0.1 ml. In the first measurement the drop is almost inconsequential, where as in the second, it effectively may double the volume measured (a 200 % error!) This is true up to a point for a given set of measuring devices. For example, the smallest volume measured with a transfer pipette is also the least accurate (i.e. 0.1 ml transferred in a 10 ml pipette). Once a new device is used, the accuracy is restored up to point (as with a micropipette that has a maximum volume of 0.1 ml (100 μl) is very accurate at the top of its range (100 ul).

III. SOP:

1. Determine the weight of different volumes of water. The volumes are 10 µl, 50µl, 100µl, 500µl, 1 ml, 5ml, 10ml, and 50 ml Use the following devices: 2-20 µl micropipette (for 10 ul sample), 20-200 µl micropipette (for 100, 50 and 10 ul volumes) and 10 ml serological pipette for all of the remaining samples. Try to ensure as accurate a transfer of fluid as possible. Remember to record what device was used for each transfer (see space below the table). Use microfuge tubes to hold the smaller volumes and small beaker and centrifuge tubes (with caps) as needed for larger volumes. **NOTE: Be sure to check the scale range as some scales only go up to 50 grams, others to 300 grams.**

Volume	% Error			Average
	1	2	3	
10 µl				
50µL				
100µL				
500µL				
1mL				
5mL				
10 ml				
50mL				

Record the device used for each of the above volumes here:
10 ul _____, 50 ul _____, 100 ul _____ 500 ul _____
1 ml _____, 5 ml _____, 10 ml _____, 50 ml _____,

2. Repeat and record each measurement three times.

3. Calculate the % error from the theoretical weight (recall that water has a density of 0.9976 g/ml @ room T^o).

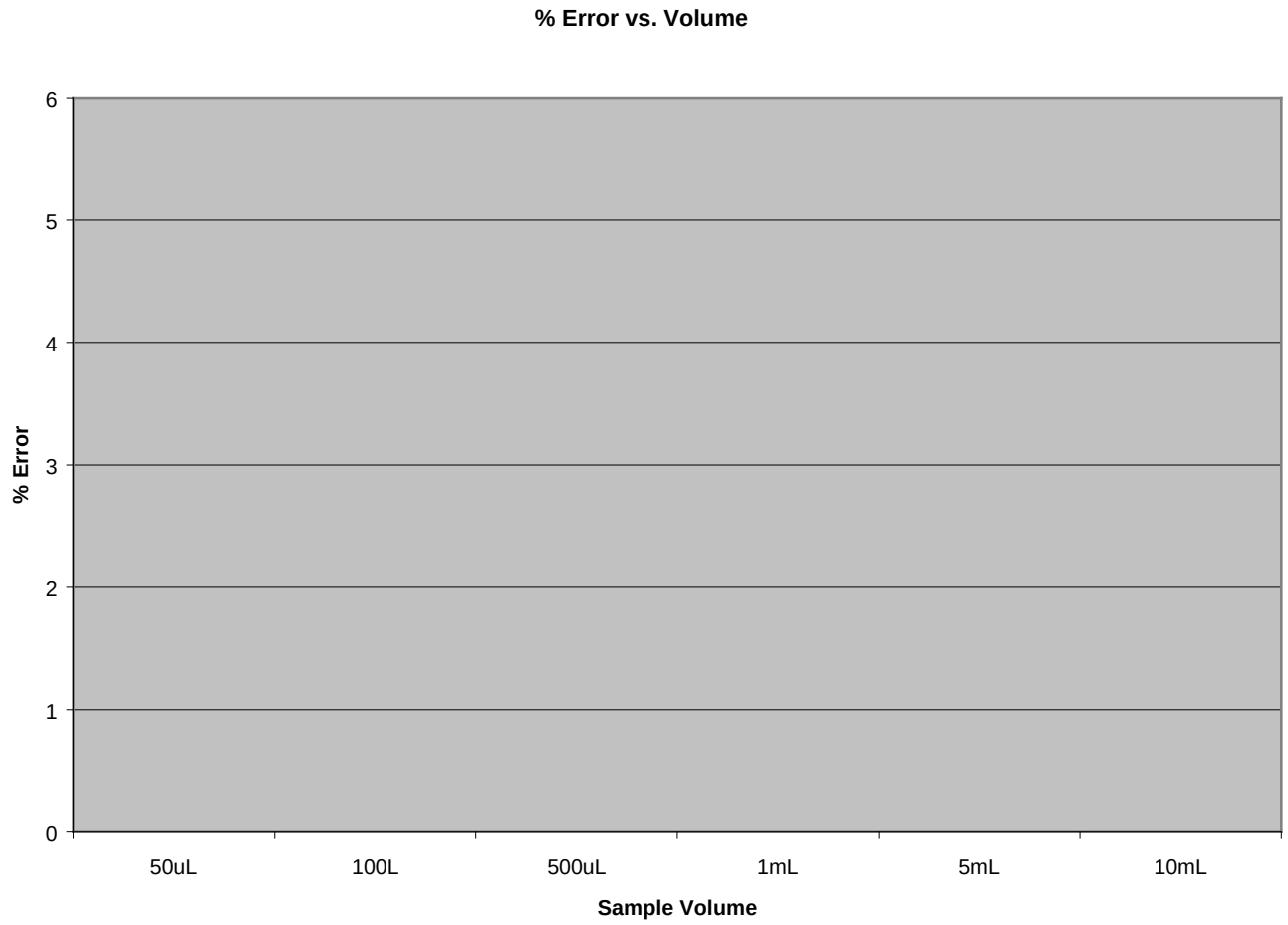
$$\%Error = \frac{ExpValue - TheoreticalValue}{TheoreticalValue} \times 100$$

Volume	% Error			Standard Error
	1	2	3	
10 µl				
50µL				
100µL				
500µL				
1mL				
5mL				
10 ml				
50mL				

4. Calculate the standard error of each weight. For the purpose of this exercise, the standard error is simply the average of the three % error calculations.

$$\text{Standard Error} = \frac{(\%\ \text{Error 1} + \%\ \text{Error 2} + \%\text{Error 3})}{3}$$

5. Determine a correlation between mass and volume by graphing the data and standard error. Your graph should be a histogram with axis like the one below (note; some of the values have been omitted for brevity.)



SOP Phase Two:
Revisit the pH data from the "metric system" lab. Ideally, you should have collected the pH data during this lab. If you did not complete the activity, there may be time in the lab to do the exercise. Please see the instructor for the pH meter, SOP, etc.

Using the pH data, create a histogram of the pH results. Along the X-axis place the concentration of the various pH solutions (2.5 M, 1.0 M, 0.1 M, etc.). Make two columns since there are two pHs for each concentration (diluted and directly prepared). On the Y-axis, place the pH value.

On the last page of this lab is a summary statement similar to the format of the summary statement you are being asked to create for the purpose of volumetric analysis. Please do not read the summary statement until you have had a chance to create your own graph of the pH lab and to consider its

implications. Also, the summary statement is only a approximation of the data and may not agree exactly with your data. This said, it is still very useful to help you to understand the general format and structure of the requested summary statement.

IV. <u>Questions</u>: Answer all of the following (points 1-4) in your summary statement. NOTE: Do not literally answer them 1,2,3, etc. Use them as ideas to help you formulate your summary statement.

1. What is the relationship between the mass and volume of water?
2. Why do you calculate standard error (as opposed to just % error?) Is there any relationship between it and the volume measured?
3. What can you deduce about need for critical measuring as a function of volume?
4. If micropipettes are often more accurate than serological pipettes, why not use a micropipette for all lab measurements? List three reasons as to why this may not be such a good idea.
5. Using computer lab resources, recreate above graph in Excel and hand in. Looking at the data, comment on the trends. Consider the major shifts in the data as related to the volumes measured and the devices used. Now write a 1-2 paragraph summary statement and include all of the aforementioned ideas. Each partner should write and <u>submit their own</u> summary statement. This is good practice for your pending lab notebook writing. These papers will be edited and returned to you by the next lab.
6. Lastly, remember to use size 12-courier font, double space with one-inch margins all the way around the paper. Keep it under one page and consider going through at least one edit before you turn it in?

pH of solutions prepared via direct measurement and serial dilution.

Nine solutions ranging from 10 M to 0.1 um (in order of magnitude concentrations) were prepared by measuring out needed amounts of sodium bicarbonate ($NaHCO_3$) and bringing to a final volume of 10 mls. A second set of identical concentration solutions were prepared by using serial dilution of the most concentrated, fully soluble sample. Both techniques presented limitations to the researcher including the inability of very concentrated solutions to solubilize completely and for very small concentration solutions to be directly measured on a scale. Serial dilution samples are more quickly prepared and as evidenced by the graph, has a more consistent pH when compared to samples prepared by direct measurement. Assuming the first solution solubilizes completely and is accurately prepared, serial dilution is a superior's technique to creating a range of solutions quickly and accurately. However, serial dilution can be wasteful if only a few solution concentrations are needed and/or the first solution (in dilution series) is inaccurately prepared or incompletely solubilized.