BIOCHEMISTRY II (Chemistry 332)

To: Product Testing & Quality Control

From: The Management

RE: **Micropipette Calibration and Protein Assays**

As you may know, your team has been hired to do several projects in DNA technology requiring the use of micropipettes. Since many of these experiments require accurate and precise solution transfers of μL volumes, it will be important to calibrate your pipettes.

We will leave the details of how to design these tests to your team. (See the references[1-3] below for help.)

Accordingly, your team will have access to adjustable micropipettes of 2-20 μL, 20-200 μL or 5-50 μL, simple 100 μL pipettes and 5, 10 and 20 μL Wiretrol® pipettes. You may request additional supplies and reagents as needed, but must plan around their absence, as Management can guarantee little in this budgetary environment. Any instrument access must be scheduled and cleared with the analytical chemist on staff.

Your manager has suggested the following to aid the investigation, but again solicits your input on how to proceed.

1. Is there a technique for setting the volume on the pipette that yields more consistent volumes delivered?
2. Does accuracy vary with repetitive measurements and with varied volumes set on the pipette (for example, 2, 5, 10, 15 and 20 μL)?
3. Does the accuracy vary within the temperature range likely to be needed? What temperatures are likely - 0°C, 20°C, 32°C, 100°C?
4. How accurate are the different pipettes you have?
5. Does the volume delivered vary significantly with viscosity? Can you compensate for this technically or by adjusting the volume settings?
6. Will you measure volumes by mass, dye absorbance or another technique?
7. Will evaporation compromise your results? If so, how might you compensate?
8. Is pre-rinsing or reverse mode pipetting more accurate or precise?[2]

When your team is satisfied with accuracy and precision of your pipettes, you will test them by determining the concentration of proteins in standard and unknown samples.

Determination of the concentration of protein in samples is one of the most common tasks in any biochemical lab. There are several methods for measuring protein concentrations. For many years a method devised by Lowry *et al.*[4] was the most common, and this paper may still be the most cited scientific paper ever. More recently a method devised by Bradford[5] has become more popular, due primarily to its ease and simplicity. Bradford's paper describing this method has been cited more than 100,000 times.

The Bradford method is based on binding of a Coomassie blue dye to proteins. In the presence of protein, this dye turns from a reddish color to a brilliant blue, with an absorbance maximum at 595 nm. Several companies have developed assay kits based on this method. One of the most popular reagents is marketed by a company called Bio-Rad, and this is the method you will be using.

Method

You will be provided with a pure, solid protein that can be used to construct a standard curve. Although the most common protein standard is bovine serum albumin, the Bradford method gives anomalous results with this particular protein, and so γ -globulin is more commonly used.

The method is very simple. Take 1.6 ml of sample and add 0.4 ml of the Bio-Rad reagent. Mix, wait 5 minutes to 1 hour, and then measure the absorbance (OD) at 595 nm. The OD should be proportional to the protein concentration.

You will be provided with three "unknown" samples. Your task is to determine as accurately as possible the concentration of protein in these three samples.

I suggest you construct a standard curve consisting of 5, 10, 20, 30 and 40 μg of γ-globulin in 1.6 ml. Your first task will be to determine how you will prepare these standards. How much protein will you weigh out? What will you dissolve it in? How will you dilute it? How will you get the right amount of protein in each standard?

Next you will need to determine what sample size to take from the three unknowns. Will you do more than one for each? How about replicates?

References:

1. http://www.pipette.com/Manuals/AccuPetPro.pdf. Accessed 1/19/09
2. http://www.pipette.com/Manuals/rainin_classic_manual.pdf; accessed 1/19/09
3. Helser, T.L. **2009 (**http://employees.oneonta.edu/helsertl/pipette.html) TBA
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with the Folin phenol reagent. *J.Biol.Chem.* **I93**:265-275, 1951
5. Bradford, M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal.Biochem.* **72**:248-254,1976

Since we are an equal opportunity employer, management will assign the leader, technical and data specialist positions to begin, but the position will rotate when new projects begin. As this is a high priority project, you must plan the use of your time carefully. Good luck!