Experiment #1
Pipette Calibration

Introduction

Automatic adjustable pipettes have become an indispensable tool in today's biochemistry laboratory. They are highly advantageous due to their accuracy, ease of use, and wide range of volume deliveries available.

Our lab is equipped with the Eppendorf line of pipettes. The identification numbers found on the side, as well as the color on the top button, give the pipette size. The pipettes provided allow for delivery of 0.5-1000 microliters in the following volume ranges:

PIPETTE	VOLUME RANGE (μL)
Eppendorf 10	0.5-10
Eppendorf 100	10-100
Eppendorf 1000	100-1000

Each pipette is to be used with a specific disposable tip provided in the plastic racks. **These pipettes must never be used without a tip!** The small clear tips are used with the Eppendorf 10, the medium size yellow tips with the Eppendorf 100, and the large blue tips with the Eppendorf 1000. Tips may be used more than once when delivering the same solution, but always replace the tip when changing solutions, when using sterile solutions, or if it becomes dirty, contaminated, or if liquid droplets seem to be retained along the pipette walls. Calibrating pipettes frequently is necessary to identify pipettes that are not delivering accurately.

Operation

Each pipette is equipped with a comfortable handgrip, a disposal button, a delivery button, and a grooved wheel for volume delivery adjustment. The delivery button, when pushed, is equipped with two "STOP" points, noticeable by the feel of gentle resistance. The first STOP is to obtain the

proper delivery amount, and the second to insure complete delivery. The volume to be delivered is adjusted with the grooved wheel, and read with reference to the numbers and vernier scale on the side of the grip. The designation of the numbers varies with each pipette as follows:

PIPETTE	PIPETTE READING					
Eppendorf 10	Max. reading	1	0	0	0	= 10.00 µL
		0	8	5	5	= 8.55 µL
Eppendorf 100	Max. reading	1	0	0	0	= 100.0 µL
		0	5	2	5	= 52.5 µL
Eppendorf 1000	Max. reading	1	0	0	0	= 1000 µL
		0	5	2	5	= 525 µL

A specific sequence of steps is followed each time a transfer is desired.

1. Set appropriate volume on appropriate model.
2. Carefully attach appropriate tip to model. Do not press down on tip too hard, but do insure that tip is firmly attached.
3. Press the large button until the first stop.
4. Place the tip in the solution to be transferred.
5. Slowly and smoothly release the button.
6. Inspect solution in tip to confirm that no air bubbles are present.
7. Place tip gently against the side of destination container.
8. Push large button to first stop.
9. Push large button to second stop.
10. If necessary, dispose of tip by pushing smaller button on the back of pipette grip.

These pipettes are very fragile and expensive. You are the primary users of these fine instruments and therefore, it is your responsibility to maintain the pipettes in fine working condition. You will be held responsible for any damage to your pipettes due to neglect. In addition to the guidelines already provided, the pipettes should never lay in a horizontal position with a full tip.

Experiment

All adjustable pipettes should have their calibration checked periodically. The easiest way to accomplish this task is to pipette an indicated volume of a solution of known density, weigh the amount transferred, and determine if the measured volume corresponds to the indicated volume. The density of distilled water at 25^{o}C is 0.997075 g/ml; an Eppendorf 100 pipette set to 0550 should deliver 55 μL, which will weigh 0.0548 g based on the formula:

Weight = Density x Volume

In order to establish the accuracy of your pipettes and to test the reproducibility of your pipetting technique, you and your lab partner will each pipette ten aliquots of distilled water from each of your pipettes, carefully recording the cumulative weight after each addition. One lab partner will utilize a volume in the lower third of the delivery range and the other partner will utilize a volume in the upper third.

Place a weighing boat on the Denver Instrument TR-104 analytical balance and accurately weigh the empty boat to the full resolution of the balance (0.1mg). Now, carefully pipette an aliquot of distilled water into the boat and record the weight. Repeat this process recording the cumulative weights of ten aliquots. Remove and dry the weighing boat and repeat the process for each pipette model. At the end of the experiment, each lab partner should have ten weights for each of the pipettes. Use your thermometer to measure the temperature of the distilled water and use the table of values given below to determine the density:

TEMPERATURE (^{o}C)	DENSITY (g/mL)
20	0.998234
21	0.998022
22	0.997801
23	0.997569
24	0.997327
25	0.997075
26	0.996814
27	0.996544
28	0.996264

Data Analysis

1. Using a computer program type in each of the datasets as (cumulative weight - weight of empty boat) versus (total volume added). Plot each dataset (linear option) and fit each plot with a linear regression. Record the slope of the plot, the correlation coefficient and obtain an error estimate for each dataset. Printouts of these plots should be included in your lab notebook.
2. For all three datasets, convert the cumulative weights into a table of weights per aliquot. Enter these data into the spreadsheet and obtain appropriate statistics for each set. Divide the mean weights by the density (corrected for temperature) to obtain the mean volumes delivered. Calculate the percent error for each of the three volume pipettes.

In your laboratory notebook you should include the plots prepared above and a table reporting slopes, and the mean, median and standard deviation for each dataset, as determined from your analysis. A second section of the table should report these same data as obtained by your lab partner. You should also comment on the accuracy and the reproducibility of your pipetting, a comparison of your error and your partner's and the correlation between the volumes indicated on the pipettes and the actual volumes delivered. Please note that your notebook will be scrutinized by your TA and you should utilize this opportunity to practice writing for future and more involved laboratory reports.