Appendix C: CALIBRATION OF INSTRUMENTS

I. What are units of measurement?

A **unit of measurement** is an exactly defined amount of a property, such as grams, liters, meters, etc. In order to mean anything, we must all agree on our units of measurement, so that a gram in our country is the same as that in Europe or Asia. All of our units of measurement must agree. This agreement is manifested as a physical representation of a unit, called a **standard.** A standard is a physical embodiment of a unit. For example, a kilogram has been defined by international treaty to have as much mass as a special platinum-iridium bar located at the International Bureau of Weights and Measures near Paris. All other mass standards are defined by comparison to this special metal bar.

Standards are used to **calibrate** instruments or processes that have been designed to measure a specific unit of measurement. During the process of calibration the accuracy of the instrument or process of measurement is improved.

Many of the simplest measuring tools that we use in the biology lab have been calibrated by the manufacturer to within certain **tolerances**. Tolerance is the amount of error that can be tolerated in the calibration of that item. As a general rule, the lower the tolerances, the more expensive the instrument is. Many of the more complex and electronic instruments that are used must be calibrated frequently by the persons who use them. This is because there is a loss of accuracy over time with these instruments. While some of these instruments require special equipment and training for calibration, others are designed for the operator to routinely calibrate them on a daily basis.

In the BIOL1406 laboratory, we will need to calibrate the top-loading balance and the pH meter to agree with standards on a routine basis.

A. THE TOP-LOADING BALANCE

1. Make sure the balance is level and on a vibration-free surface.
2. Turn the balance on and adjust the balance to zero. This is done by first pressing the on button. The balance will go through an electronic self-check. When completed, the numbers displayed should be a series of zeroes. If not, press the TARE button and wait for the series of zeroes to appear on the display.
3. If the zeroes are appearing but the weight displayed is fluctuating, check for an air vent that is blowing on the balance. If you find one, you must either shield the balance from the air stream or move the balance away from the air stream.
4. Using forceps or gloved fingers, retrieve the standard weight from its container and place it on the center of the balance pan. If the weight displayed on the balance doesn't match the weight of the standard, press the "CAL" button. The instrument will automatically calibrate itself to that weight.

NOTE: Temperature can have a significant effect on weight measurements. If a sample and its surroundings are at different temperatures, air currents are generated that cause the sample to appear heavier or lighter than it really is. Also, balances can be affected by the heat from their own electrical components. For this reason, you should allow a "warming up" period to allow the balance to reach temperature equilibrium before use. Be aware that handling of things to be weighed can alter their weight from the dampness of your fingertips. For extremely sensitive balances, even the oils of your fingerprints can alter the measurement.

B. THE pH METER

When used properly, **a pH meter can measure the pH accurately to the nearest 0.1 unit** or better. A pH meter consists of a voltmeter that measures the voltage between two electrodes connected to one another through the voltmeter. One of the electrodes is internal to the other, so they appear as a single electrode. The inner electrode is a **reference electrode** for the outer electrode. When this dual electrode is immersed in a sample, an electrical potential (voltage) develops between the outer electrode in contact with the sample solution and the internal

electrode. This voltage is proportional to the pH and is measured by the meter's voltmeter. This voltage is translated into pH units during the process of calibration.

The pH electrode has a very thin, fragile glass bulb at its tip that must be handled with great care. It can shatter easily, so **care must be taken to avoid bumping it in any way**. A broken or cracked electrode cannot be repaired. Also, the bulb of a pH electrode is easily destroyed by a scratch, so it **should not be rubbed in any way**. It is important that this bulb doesn't dry out, and it **should always be immersed in solution**. Also, there are salt solutions that must be maintained inside the electrode. Towards the top of the pH electrode, there is a thin opening to allow the electrode to be filled with its salt solution. It is important that the **electrode be maintained in an upright position** so that this salt solution is not allowed to escape through the filling hole.

1. Allow the pH meter to warm up for at least 5 minutes. Until the instrument has warmed up, its readings will drift badly. If the meter has a "STANDBY" mode, press its button.
2. Make sure that the electrode filling hole is open and that the filling solution inside the electrode is nearly to the top of the electrode.
3. If the meter has a temperature setting, adjust it to the room's temperature.
4. Obtain two standard buffers; one that has a pH of 7.00 and the other that is either 4.00 or 10.00. Choose the buffer that will best bracket the pH that you will be using the pH meter to measure.
5. Rinse the electrodes with a squirt bottle of distilled water and blot the outside dry with a Kimwipe. Don't attempt to dry the bulb---you should avoid touching the bulb with your Kimwipe.
6. Immerse the electrode bulb in the pH 7.00 calibration buffer. Disengage the standby or follow the manufacturer's instructions. Allow the reading to stabilize.
7. Adjust the meter to read 7.00 using the correct knob, dial, or button, depending on the manufacturer's instructions. Reengage the standby mode, if present.
8. Remove the electrode, rinse with distilled water over a waste container, and blot dry with a Kimwipe (avoiding contact with the bulb).
9. Place the electrode in the second standardization buffer, set the meter to read pH, and allow the reading to stabilize. Adjust the meter to the pH of the second buffer with the proper method of adjustment, according to the manufacturer's instructions. Remove, rinse, and blot the electrode while on standby.
10. Recheck the pH 7.00 buffer as in Step 7 and readjust as necessary. Then, recheck the second buffer and readjust the meter as necessary. Continue to rinse the electrode after each solution. This process of calibration may require three rounds.
11. Return the electrode to its storage solution. This is usually a buffered KCl solution.

In general, a reading should stabilize within a minute. Many new pH meters have an "autoread" feature that automatically determines when the electrode has stabilized and locks on this reading. If your pH meter will not stabilize, check the electrode for breakage or missing solutions, and check to make sure that your sample solution has be adequately mixed.