APPENDIX A
CORRECT USE OF DIGITAL PIPETTES

The digital pipettes currently being used in this laboratory are Eppendorf Reference pipettes and are significantly more expensive than many other types. Those with blue tops can be used to transfer volumes of 100-1000μL, those with yellow tops can be used to transfer volumes of 10-100μL, and those with white tops can be used to transfer volumes of 0.5-10μL. The sterile tips used in association with these pipettes are colored to match the pipette tops, i.e., blue (100-1000μL), yellow (10-100μL) and white 0.5-10μL.

1. Before making any transfer using a digital pipette, be sure to check the digital volume indicator to be sure it is correctly set. In order to set the pipette to measure the correct volume, hold the pipette body in one hand, compress the "lock release" button and carefully turn the volume adjustment knob until the desired volume is shown on the digital volume indicator. **PLEASE NOTE** – Our digital pipettes require that you compress the "lock release" button (blue or black and located beside the volume indicator) before you adjust the volume. If you do not do this, the **pipette will be damaged**. **CAUTION** - make certain you know the volume parameters of your pipette before you turn the adjustment knob. Do not try to exceed the volume limitations printed on the instrument! Inappropriate volume adjustment may result in damage to the pipette.

2. Attach a clean, sterile, disposable tip to the shaft of the pipette by **tapping** the pipette into the tip while it is sitting in the tip box (**DO NOT LEAN ON THE BOX**). You may wish to press the tip firmly in place with a slight twist to insure a positive, airtight seal.

3. Carefully depress the plunger to the first positive stop. You will feel an increased resistance within the instrument indicating the position of this stop. This part of the stroke is the calibrated volume displayed on the digital volume indicator.

4. While holding the pipette in a vertical position, carefully immerse the disposable tip in the solution to be transferred. The depth of immersion should be less (1-2 mm) with a small volume pipette (.5-10μL) and greater (2-4 mm) with a larger volume pipette (100-1000μL). At no time should the pipette shaft make contact with the liquid being transferred.

5. Slowly release the pressure on the plunger so that it is allowed to return to the "up" position. Do not allow the button to "snap" up, as this will allow air bubbles to enter the tip and will cause your volume measurement to be inaccurate. If you are transferring a large volume (1-5ml) allow 1-2 seconds for the tip to fill before removing the tip from the liquid.

6. Remove the tip from the sample liquid and wipe any liquid adhering to the outside of the tip on the inside of the sample container. If necessary, you can wipe away excess liquid with a lint-free cloth or paper, but you must be careful not to touch the tip opening as this will draw liquid from the pipette via capillary action, and will contaminate the tip.

7. To dispense the sample liquid, place the open end of the pipette tip against the side-wall of the receiving container and carefully depress the plunger to the first stop. Allow 1-2 seconds for the liquid to exit the tip and then depress the plunger to the second stop to expel any residual liquid from the tip.

8. With the plunger still depressed to the second stop, carefully withdraw the pipette from the receiving vessel. Allowing the tip to slide along the vessel wall will insure that any residual liquid is pulled from the tip.

9. Allow the plunger to return slowly to the "up" position, or if the tip is to be ejected, depress it to the third stop. In most cases this will cause the disposable tip to slip off the pipette shaft. Be sure your disposable tip is placed in the appropriate container.

NOTE - In order to acquire proficiency with the digital pipettes, students must pay close attention to technique. Factors such as speed and smoothness during depression and release of the plunger, immersion depth of the tip and angle at which the pipette is held during filling will influence the accuracy of your volume measurements.

If, during a liquid transfer, you note the presence of an air bubble within the tip, return your sample to its original vessel. Then while checking tip immersion depth and pipette angle, slowly take up the sample again. If a bubble appears the second time, discard the tip and obtain a new one.

APPENDIX B
CALCULATIONS ON SURFACE AREA TO VOLUME RATIOS

Cells are limited in size by the ratio of their surface area to their volume. In the example below, a spherical cell or coccus is allowed to increase in size, and as it does so, cell volume increases more rapidly than does surface area. Eventually the surface area is too limited to allow nutrients, gasses and other materials to pass quickly enough to keep pace with metabolism. The cell will not be able to grow beyond that point.

(Assume constants -- units and shape, as labeled)

INPUT RADIUS OF SPHERE	EQUALS	SURFACE AREA OF SPHERE	VOLUME OF SPHERE	RATIO OF AREA TO VOLUME S.A./VOL
(Shape = Sphere/coccus)				
.0001	===	.000	.000	30,000.075
.001	===	.000	.000	3,000.008
.01	===	.001	.000	300.001
.1	===	.126	.004	30.000
.5	===	3.142	.524	6.000
1.	===	12.567	4.189	3.000
3.	===	113.101	113.101	1.000
5.	===	314.170	523.615	.600
10.	===	1,256.680	4,188.923	.300
20.	===	5,026.720	33,511.383	.150
50.	===	31,417.000	523,615.358	.060
100.	===	125,668.000	4,188,922.861	.030
1000.	===	12,566,800.000	4,188,922,861.000	.003

REFERENCE LITERATURE ON MICROBIOLOGY

The following sources of information may be of additional help as needed. Many of these works served as sources of information for this syllabus.

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