

Lab Two  
**Metabolism and Diversity**

*Wet Mounts - Phototrophs*  
*Chemoheterotroph Diversity - Glucose and Lactose*  
*Electron Acceptor Tests - Aerobes and Anaerobes*  
*Nitrogen Cycling Microbes*  
*Virus Homework Project*



**Part One - Phototrophic Microbes and Wet Mounts**

Cells that contain chlorophylls can be viewed live using wet mount procedures. Examples include algae (which house pigments in chloroplasts) and photosynthetic bacteria (which house pigments on their cell membranes), both described in your text and notes. Both of these important groups of microbes produce oxygen during photosynthesis, thus making aerobic life on earth possible.

**General Wet Mount Procedures Using Liquid Samples**

Using a transfer pipette, remove SMALL amount of sample - make sure you see green filaments  
Place coverslip on drop. If you used too much liquid, the coverslip will move/float – NOT GOOD

*Bad wet mounts will result in (1) the inability to focus because everything is moving; (2) more cleaning/liquid everywhere; (3) damage to microscopes as liquid corrode parts.*

**Live Organisms to View Using Wet Mount**

Cyanobacteria – Anabaena, view through 40X objective\*  
Green Algae - Spirogyra, view through 40X objective

*Anabaena make two different kinds of cells - a smaller green one that is photosynthetic and a larger colorless one that fixes nitrogen. Be sure to include BOTH cell types in your final drawing.*

**Part Two - Chemoheterotroph Diversity**

In contrast with most eukaryotes that primarily rely on their mitochondria to perform glucose-based respiration, bacteria are way more diverse in terms of their chemoheterotrophic abilities. MANY identification tests have been developed to analyze carbon use. Today, you will learn how to use glucose and lactose to distinguish between different bacteria. While some of these tests demonstrate respiration (i.e. using electron transport chains), others are examples of fermentation. It is important to remember that fermentation means the electron transport chain is not being used; although it is anaerobic, there are other ways to be anaerobic AND use the electron transport chain.

**Glucose Test Procedures**

Label two glucose ferment tubes – one for Escherichia and one for Providencia  
Inoculate with a loop by swishing anywhere in the liquid and growing until next week  
Organisms that ferment glucose to acid turn the red media yellow  
Organisms that respire glucose to carbon dioxide gas produce a bubble in the Durham tube

**Lactose Test Procedures**

Divide and label one MacConkey plate – one side for Escherichia and one for Providencia  
Inoculate each half with a loop of each culture by making a squiggle and growing until next week  
Organisms that use lactose appear purple; those that don't appear light pink to white.

### **Part Three - Aerobic vs. Anaerobic Respiration Testing**

Aerobic microorganisms use oxygen to accept final electrons at the end of their electron transport chains. These oxygen-accepting proteins can be tested for using a compound called oxidase. Many anaerobes use something other than oxygen to accept electrons. For example, sulfur can accept electrons in some bacteria, becoming sulfide. Although these tests sound esoteric, the SIM test detects Salmonella and the oxidase test detects Neisseria (e.g. gonorrhea and meningitis).

#### **SIM Test Procedures**

Label two SIM tubes – one for Salmonella and one for Escherichia

Inoculate with a loop by plunging throughout the semi-solid media and growing until next week

Sulfur acceptors producers produce black sulfide; non-sulfide producers do not change the media

*Yes - this is the same tube that we will use for motility testing next time. SIM stands for Sulfur, Indole (you will learn much later), and Motility - a three-in-one test.*

#### **Oxidase Test Procedures**

Obtain two oxidase disks; DO NOT HANDLE the disks with your hands - Use tweezers!!!

Smear/press a toothpick of Escherichia on one disk and watch carefully for color changes

Smear/press a toothpick of Pseudomonas on the other disk and watch carefully for color changes

Oxidase positive organisms darken within a minute (purple to black); negatives do not.

### **Part Four - Nitrogen Cycling**

Many different prokaryotes play important roles in the nitrogen cycle because they represent the only life capable of fixing nitrogen gas. Others uniquely process nitrogen compounds like ammonia, nitrite, and nitrate - frustrating because they convert fertilizers back into nitrogen gas (this is called denitrification) and/or acidic products (this is called nitrification). Having viewed nitrogen fixing cyanobacteria cells, you will now test a natural habitat (i.e. Natural Science building pond) for nitrogen cycling bacteria using the nitrate reduction test. Although these tests sound esoteric, denitrifying bacteria include medically important Pseudomonas (many skin lesions/boils and septic shock) and nitrifying bacteria include virtually all gut/enteric Gram negatives (e.g. Escherichia, Salmonella...).

#### **Nitrate Reduction Test Procedures**

Label two Nitrate tubes and inoculate each with 1 ml Natural Science pond water

Grow for 1 week at room temperature on side counter - an area will be designated

Next week, examine the tube for evidence of denitrification and/or nitrification

Organisms that denitrify (e.g. nitrate to nitrogen gas) produce a bubble in the Durham tube

Next, add 3 drops Nitrate A and 3 drops Nitrate B - the combination of which detects nitrite

Organisms that nitrify (e.g. nitrate to nitrite) turn red when mixed with Nitrate A/B

*Both denitrification and nitrification also represent anaerobic metabolisms (i.e. nitrate is accepting electrons at the end of electron transport chains - not oxygen).*

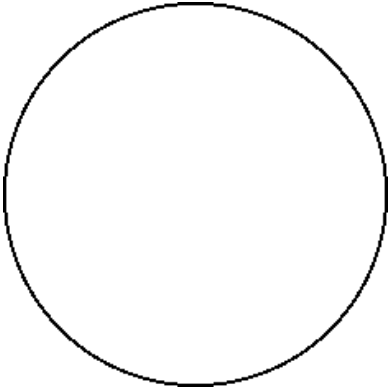
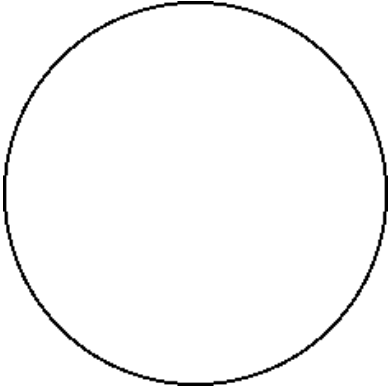
**Biology 318 Worksheet Due Next Lab - TURN IN ONE WORKSHEET ONLY PER PAIR**

**DON'T FORGET ABOUT INDIVIDUAL VIRUS ASSIGNMENT - NO LATE ASSIGNMENTS WILL BE ACCEPTED!!!!!!**

Names:

(1) 6 pts. Phototroph Table - Remember: view through 40X only, NO OIL!!!!!!

*Special Instructions: One of these has organelles and the other makes specialized nitrogen fixing cells. Determine which has which and specifically label respective structure in final drawings.*

	40X Objective
<p style="text-align: center;"><u>Spyrogyra</u></p> <p>Kind of microbe:</p> <p>Kind of cell:</p> <p>Cell wall components:</p> <p>Example disease caused by this organism or relative:</p>	
<p style="text-align: center;"><u>Anabaena</u></p> <p>Kind of microbe:</p> <p>Kind of cell:</p> <p>Cell wall components:</p> <p>Example disease caused by this organism or relative:</p>	

(2) 8 pts. Metabolic Tests

<b><u>Glucose</u></b>	Describe What You See	Significance (+ or -)
<u>Escherichia</u>		
<u>Providencia</u>		
<b><u>Lactose</u></b>		
<u>Escherichia</u>		
<u>Providencia</u>		
<b><u>Sulfur</u></b>		
<u>Escherichia</u>		
<u>Salmonella</u>		
<b><u>Oxidase</u></b>		
<u>Escherichia</u>		
<u>Pseudomonas</u>		

(3) 3 pts. Nitrogen Cycle - Dentrification and Nitrification

<b>Denitrification Test</b>	Describe What You See	Significance (+ or -)
Tube One		
Tube Two		
<b>Nitrification Test</b>		
Tube One		
Tube Two		

(4) 8 pts. Virus Homework - Individual Assignment.

*No viruses highlighted during the class lecture - i.e. no Influenza, Papillomavirus, HIV, or Herpes!*

Viruses are not considered living because they lack metabolic genes and rely on a living host cell for most functions associated with what it means to be alive. Each member of the team will, using any number of virus tables in the book, pick a different virus that infects animals or humans and complete the following tasks about this virus. Beyond the book, you will only be allowed to use the CDC website ([www.cdc.gov](http://www.cdc.gov)) and its excellent Public Health Image Library (PHIL) sub-site.

- a) Locate, print, and attach an image of this virus. Make sure the image shows the actual virus - not just pathological effects of infection. You probably should start here because if you can't find a virus image, the rest of the project is moot.
- b) Describe the structure of the virus - addressing shape, size, genetic material, envelope status - all categories of virus classification described in the book/lecture. Limit this description to 1 double spaced page.
- c) Summarize the major disease caused by this virus, including describing typical host(s), tissues affected, timecourse, and at least one piece of statistical data. Limit this description to 1 double spaced page.
- d) Each individual will turn in his/her own report (with picture) separately next week at lab.