**Biology 422L Laboratory Experiments**

**Fall, 2020**

**Laboratory objective –**To learn some of the major techniques and procedures used in working with bacteria. Although the results you will obtain in these experiments are standard and easily verifiable in published papers and texts, the experiments are designed to provide you with experience in a variety of the most commonly used techniques of bacteriology. In a working microbiology laboratory if you wished to use a new technique you would check whether you were doing the technique correctly by using it in a standard well-documented experiment as you are doing here.

**Schedule for 422L**

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| **Date** | **Experiment** | **Hand In** |
| Aug. 11, 12 & Aug. 18 &19 | \*1) Sterile Technique  Viable cell count  Plate streaking  Separating individual colonies | Viable cell count (vcc) calculation |
| Aug. 25 & 26 | \*2) Growth Curve  Gram stain  Separating individual colonies | 2) Report (graph & 2 pages) |
| Sept. 1 & 2 | \*3) β-galactosidase Induction | 3) Report (graph, table & 2 pages) |
| Sept. 8 & 9 | \*4) Isolation of Bacteria from Nature | **---** |
| Sept. 15 & 16 | \*4) Isolation of bacteria from nature  \*5) Transposon mutagenesis Part 1 | 4) Report (2-4 pages) & Chart.  Due after experiment completed |
| Sept. 22 & 23 | \*5) Transposon mutagenesis Part 2 |  |
| Sept. 29 & 30 | \*5) Transposon mutagenesis Part 3 | 5) Report (2 pages) |
| Oct. 6 & 7 | 6) Biochemical Pathways | 6) Report (2 pages) |
| Oct. 13 &14 | Make-up labs | All lab reports must be in by Oct. 16 |
| Oct. 20 & 21 | Lab final |  |

Some labs require that you examine plates the next day. A TA or one of the lab prep people will photograph your plates and send you the photo. Since they will need to be able to identify whose plate it is, **PLEASE LABEL ALL YOUR PLATES NEATLY NEAR THE EDGE WITH YOUR LAST NAME.**

**General Information for Biology 422L**

**Before** each class meeting, read the directions and references for that laboratory. This will help you to use your time to the best advantage. All the laboratory experiments have preliminary worksheets included in the manual. The pre-lab worksheets are designed to require you to read the lab to complete them. **These are to be completed and turned in before you begin the lab.**

Laboratory is scheduled for 3 hours on Tuesday or Wednesday afternoons. Each lab will be divided into 2 sections. The first section will meet from 1:30 to 2:45. The second section will meet from 3:15 to 4:30. For some experiments, you will need to combine data or share cultures with the person in the other section working at your space.

The week following most of the laboratory experiments a **brief** report will be due. **All reports must be formatted in word (or excel for graphs) and emailed directly to the TA concerned.** A rubric for each of the lab reports will be distributed by your TA before the report is due. Some laboratory exercises will also include problems for you to work. The laboratory grade will be based on the laboratory reports and your plates, the lab final exam, and the teaching assistant’s evaluation of your microbiology laboratory skills.

Reports will be graded on:

1. Clarity and organization

2. Scientific correctness of results

3. Completeness - all questions answered. All results present.

All calculations made. All procedures described (if you used the procedures in the directions, it is adequate to state this).

4. All reports should contain your data from the experiment in a table or other easily readable form. If for some reason you were unable to obtain data, consult with your TA about what to do. If you use someone else's data or invented data, **this must be clearly stated.** In the case in which you use data from another student, they must sign the report to show that they consent to your use of their data. While it may be unavoidable and no fault of yours that you have to use someone else’s data, this should be a rare occurrence. If you are having trouble getting good data, consult with the teaching assistants or Dr. Matthysse regarding your lab technique. **Poor lab technique will affect your grade adversely.**

5. Late lab reports will lose 1 point for each weekday they are overdue. No lab report will be accepted after Oct. 16.

The final examination will consist of protocols and data similar to those from the experiments done in class. It will last 1 hour. Grades will be calculated with each laboratory from number 2 through 6 counting 10 points. The final will count 30 points and an evaluation of your work in the laboratory by the TA will count 40 points. **The TA evaluation will be based on their assessment of your lab skills and technique and understanding of the experiments.**

**Laboratory Safety**

**Before you come to the laboratory.** Good sense and OSHA safety regulations require that you wear sensible clothing which will protect you from chemical spills and **closed shoes**. You should not have long floppy sleeves or long hair which may hang into a burner flame and catch on fire. Fasten your hair back securely. Anyone with an impaired or suppressed immune system should not enter a microbiology laboratory.

**General Laboratory Safety.** You may not eat, drink or smoke in the laboratory. You should store your coat and backpack out of the way where no one will trip on them and they will not become contaminated with microorganisms. If you leave them out and live cultures are spilled on them, they will have to be disinfected.

**Precautions in working with microorganisms.** You should treat all cultures of microorganisms as if they contained human pathogens since you do not know what contaminants you may have grown. Never mouth pipette cultures. Use a pi pump or rubber bulb. Wash the area where you will work with disinfectant before you begin work. If you spill a drop of culture, kill it with disinfectant or alcohol at once. Place all pipettes tip down in disinfectant immediately after use. Never vortex an open culture or carry out any other procedure which could produce an aerosol of a microorganism. Kill all liquid cultures, dilution tubes of microorganisms, etc. with disinfectant when you are done with them. Do not leave glassware carrying live organisms lying around. When you have used a loop or spreader to transfer or spread bacteria, kill the remaining organisms (in the flame or alcohol) before you place the loop or spreader on the bench top. At the end of the experiment wash the bench top with disinfectant. Wash your hands before you leave the lab. Bacteriophage are not killed by disinfectant; use NaOH or Clorox bleach instead to kill them.

**Precautions in working with chemicals.** Never mouth pipette chemicals. Use a pi pump. If working with organic chemicals wear safety glasses (in your drawer). Wear gloves to work with phenol or other caustic agents. Never engage in procedures which could cause caustic or organic solvents to splash such as vigorous mixing or shaking of open containers. Never leave unlabeled chemical solutions sitting around. If you wish to keep them, close them properly in a screw cap container and label them. If you wish to discard them, dispose of them in the sink or in marked containers and rinse the glassware before leaving it to be washed. Never put unrinsed glassware in the wash. The chemicals on it could react with those on other unwashed glassware to form hazardous compounds.

**Ultraviolet light.** UV light is harmful to your skin and eyes. Always wear a face shield, gloves, and long sleeves when working with UV light sources. Cover any part of the source you do not need. Limit your exposure time to that which is necessary. Long wavelength UV is less dangerous than short wavelength UV.

**Burners, alcohol fires, and paraffin fires.** Remember fire is dangerous. Before you turn on and light a Bunsen burner, check that there are not open containers of flammable liquids nearby. Also check that there are no containers such as squirt bottles which may respond to the heat from the burner by releasing liquid. Similar checks should be made before turning on a hot plate.

Ethanol catches on fire quite easily. Fortunately, such fires are not very hot. If you light alcohol in a Petri dish or beaker on fire, simply cover it with a glass lid to suffocate the flame due to lack of oxygen.

Paraffin if is heated too hot on a hot plate will burst into flames. Cover it to suffocate the flame. Turn OFF the hot plate.

**If you should somehow catch your hair or clothes on fire, put out the fire by smothering it. Do not run. Instead roll on the floor to put out the flames. Do not attempt to smother a fire with coats, etc. unless you know that the material is not highly flammable. Many synthetic fibers burn easily or even explode if exposed to fire.**

**General Directions for an Afternoon in the Laboratory**

Before you come to the lab read the lab manual carefully, do the pre-lab worksheet and email them to your TA, and make a flow chart of what you will do in the lab including tables to record your data.

When you come to the lab-

1. Hang your coat and store your back pack out of the way. Do not leave them out. If they become contaminated with bacterial cultures they will need to be disinfected.

2. Wash the area where you will work with disinfectant.

3. Lay out the materials and tools you will need for the experiment. Get out your lab manual and your flow chart.

4. Listen to the pre-lab directions and explanations from your TA.

5. Do the experiment. Always notice how your cultures and other materials appear. If anything does not seem right check with your TA.

6. When you have finished the experiment, place all cultures (carefully labeled) which need to grow in the proper location- the 37o incubator or in your cupboard for room temperature or in front of the light for photosynthetics.

7. When you are done, kill all cultures and other contaminated solutions, etc. with disinfectants. Kill organisms in side-arm flasks with ethanol. Rinse all glassware and either load it for the dishwasher or in the case of side-arm flasks wash it yourself and hang it up to dry.

8. Clean your work area. Throw away used disposable pipettes, etc. after they have been treated with disinfectant.

9. Check with your TA about when to check your cultures.

10. Wash your hands before you leave the lab.

When you check your cultures-

a. Once again, first store your coat and back pack away. Even if there are few people around materials could be spilled on your possessions.

b. Get out your lab notebook and turn to the data tables you prepared for this lab.

c. Get your cultures and record your data.

d. Kill all cultures with disinfectants or put them in autoclave bags. Wash any dishes. Wash your lab bench with disinfectant and leave it clean.

e. If your cultures look strange or unexpected, store them in your room temperature cupboard or the refrigerator and ask your TA or Dr. Matthysse for help. **Do not carry your cultures out of the lab to ask for help.** Instead, ask someone to come to the lab and look at them.

f. Wash your hands before you leave the lab.