Objectives:

1. To learn basic microbiological techniques.
2. To determine if bacteria more likely to be resistant to older or newer antibiotics.
3. To allow each student to design and test a hypothesis of their choice.

I. Introduction

This week in lab you will be practicing bacterial streaking techniques, and designing and conducting two experiments. The first experiment will test a hypothesis about bacteria and antibiotic resistance (see Objective 2, above). The second experiment will test a hypothesis you develop. Since the bacteria you culture this week will need some time to grow, you will begin the experiments this week and use the first part of next lab to record your results.

Bacteria, properly the Eubacteria, are small, prokaryotic, single-celled organisms. Prokaryotes, from the Greek for “pre-nucleus”, are the oldest and most widely distributed group of organisms on Earth. Prokaryotes are found in almost every imaginable habitat: air, soil, water, and in extreme temperatures like the thermal vents of the sea floor and harsh chemical environments of radioactive waste. Some biologists estimate that five billion bacteria can be found in a single tablespoon of soil. Bacteria and Archea have a wide range of metabolic ‘lifestyles’, but most are heterotrophic, absorbing nutrients from the surrounding environment.

Although the individual prokaryotic cell is very small, we can observe them without high power microscopes by studying the colonies they form. By placing bacteria in a nutrient-rich environment (and keeping it at a growth-friendly temperature) we can encourage single bacteria cells to multiply (by binary fission) into thousands of genetically identical cells.
II. FORMING HYPOTHESES

1. Make observations about the natural world.
2. Ask questions about those observations.
3. Formulate a reasonable testable hypothesis to explain observations.
4. Create and execute experiments testing the hypothesis and generating data.
5. Analyze data and compare to hypotheses and draw inferences. This stimulates further inquiry and the cycle begins anew.

Part 1: Bacteria & Drug Resistance (Part 2 will be developed by you later.)

Observation:
Many antibiotics have become increasingly ineffective in treating bacterial disease compared to when they were first introduced.

Question:
Are bacteria more resistant to older antibiotics? Or is something else, apart from the length of time an antibiotic has been used, responsible for the bacterial resistance to an antibiotic.

Hypotheses:
Exposure over many years to an antibiotic has caused the evolution of resistance to that antibiotic.

Experiment:
We’ll grow various bacteria in the presence of antibiotics developed at different times over the last seventy years, and evaluate the effect the antibiotics have on growth.

Results:
At the beginning of lab next week, record your results for whether a bacterium exhibited resistance or not. It may be helpful to draw a diagram of your results. What inferences can you make about the antibiotics you are testing? Do your results agree or disagree with your classmates?
III. METHODS

Part 1: Bacteria and Drug Resistance

The purpose of this section of the lab is to allow you to practice your experimental design skills as you design an experiment to answer a question we pose to you:

Are bacteria more likely to be resistant to older (vs. newer) antibiotics?

The use of antibiotics combined with modern sanitation has greatly reduced the incidence of death due to bacterial infections. However, there is an increasing number of bacteria that are resistant to (are not inhibited or killed by) antibiotics. In this section of the lab you will test whether the length of time an antibiotic has been in medical use is correlated with its ability to inhibit bacterial growth.

In 1929, the British biologist Alexander Fleming found that a petri dish containing a bacterial culture had become contaminated by a mold called Penicillum. Noticing that the growth of the bacteria was inhibited around the mold, he hypothesized that the mold might be producing a diffusible chemical agent capable of inhibiting bacterial growth. Fleming later isolated this chemical and called it penicillin. Any chemical that kills bacteria or inhibits their growth can be referred to as an antibiotic. Since Fleming’s discovery, a wide range of antibiotics have been developed.

Because penicillin was such a panacea against bacterial disease, doctors began to prescribe this drug for everything. And over time some strains of bacteria acquired mutations that allowed them to survive in the presence of penicillin. As early as 1953 some bacteria strains showed resistance to penicillin; making this drug ineffective. Other antibiotics currently in use include streptomycin, chloromycetin, neomycin, novobiocin, erythromycin, kanamycin, and tetracycline. In each case, one or more pathogenic bacteria have evolved resistance, and new drugs are constantly being developed and tested. Some studies have concluded that we may only be as little as five years ahead of the bacteria.
Testing the hypothesis (from page 2):

1.1. You will be working in groups. Each group will work with 2 petri dishes, three types of antibiotics, and two types of bacteria. You will test all three antibiotics on each bacterial culture. To provide replicates and diverse testing, different groups will test different drug/bacteria combinations. Your TA will let you know which combination (of bacteria and antibiotics) you should work with. Please write your combination below.

Bacteria:  
Antibiotics:  
Year usage began:

Why do you think you will be asked to consider the entire class's data?

1.2 Before going further, please read Appendix 2 to see the protocol!

1.3 The antibiotics you will be using are available on small paper disks. What do you think happens when the paper disks containing antibiotics are placed on the damp surface of the petri dish? Will the antibiotics spread out beyond the paper disc or not? Please explain.

1.4. In addition to providing you with the antibiotic paper disks, we are also providing you with paper disks that have no (known) antibiotic on them. You should definitely incorporate these "blanks" into your experimental design. Why?

1.5. Before continuing, it is important that you think about how you will actually recognize resistance and non-resistance. Your TA will discuss how to score resistance and non-resistance.

![Diagram of Petri dishes and antibiotic disks]
Part 2: Posing an original hypothesis and designing an experiment.

2.1. In this section you will be developing your own hypothesis about bacteria. You can ask questions about the relative abundance of bacteria in different locations, or you can ask questions about how different variables affect the growth of bacteria. You are encouraged to be original; however, you will need to work within the following limits.

The limits:

- You will have only two petri dishes.
- All of the petri dishes must be incubated at the same temperature.
- All of the petri dishes will have been prepared in a standard manner.
- If you are collecting bacteria from outside the lab, please do so in and around Marsh Life Sciences.
- You will be using DAMP q-tip swabs to collect bacteria and inoculate your plates.
- In the interest of safety (you never know what you might find out there!) all plates used in part 2 should be permanently sealed with parafilm after inoculation.

2.2. Please take a moment to develop a question you would like to explore and write it below. Be sure it is a question that you are able to address given the limits outlined above. You are encouraged to think of your own experience in the world and use your past observations to lead you to questions. It may be helpful to brainstorm several questions and then choose one to pursue further.

Question:
2.3. Reframe your question so that it is a realistic mechanism or hypothesis that you can test with the supplies available. The hypothesis can be either experimentally driven (as in Part I) or it may be observational.

2.4. Design protocol: What variable will you manipulate and what variables should you keep constant to test your hypothesis?

2.5. Now, consider your hypothesis, and your experiment, and make a prediction of what you think you will observe:

2.6. Having decided what variables you will hold constant, please use the space below to briefly describe how you will do so.

2.7. In order to be able to illustrate results graphically and make comparisons, it is often helpful to have them in a quantitative (numerical) rather than a qualitative (verbally descriptive) format. Please explain how you will quantify your results (i.e. measure them!).
2.8. **WRITING OUT YOUR PROCEDURE**: (The last step before you conduct your experiment.)

- Articulate (in numbered steps) how you will set up an experiment to answer your question.
- Provide a sketch that shows how you will label your plates, AND
- Include an orderly data chart in which you will record your data next week.

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<thead>
<tr>
<th>Variables to be held constant:</th>
<th>How will you keep it constant?</th>
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<th>Variable(s) manipulated:</th>
<th>How will you manipulate the variable?</th>
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<th>Variable(s) measured:</th>
<th>How will you measure the variable?</th>
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Predictions?

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Appendix 1: Terms

**Prokaryotes:** Meaning “pre-kernel” or “pre-nucleus”- a reference to cells that do not have a bound nucleus. Prokaryotic chromosomes are found in a localized area of the cell but are not separated from the rest of the cytoplasm by a membrane. Bacteria and Archaea comprise the whole of this domain.

**Bacteria:** A member of the prokaryotic domain found in almost every conceivable habitat. These diverse and tiny cells are responsible for the most important and rudimentary of life’s functions, including; the first oxygenation of earth’s atmosphere, the constant fixation of nitrogen in our soils and disease in humans.

**Binary Fission:** The asexual division of a cell into two genetically identical daughter cells.

**Agar:** A nutrient-rich medium derived from seaweed; used to encourage the growth of bacterial cultures.

**Antibiotics:** Translating to “against life”- molecules that inhibit the growth of, or kill bacteria.

**Colony:** A group or population of bacterial cells originating from one original cell.
Appendix Two

Methods for the inoculation of a Petri dish with Bacteria and treatment with Antibiotics

1. Obtain a Petri dish containing nutrient agar.

2. Your TA will provide you with one of the following 4 bacterial cultures suspended in fluid (bacterial suspension):
   - *Escherichia coli* (E. coli)
   - *Micrococcus luteus* (M. luteus)
   - *Serratia marcescens* (S. marcescens)
   - *Bacillus subtilis* (B. subtilis)

3. Shake the bottle/tube to re-suspend the bacteria.

4. Using a pipette, place 1 ml of the bacterial suspension onto the surface of the agar. Be careful not to pierce the surface of the agar.

5. Use the clean, flamed (but cooled) "hockey sticks" (best glass rods) to spread the droplets of bacteria across the surface of the agar.

6. Let the bacteria settle into their new home on the surface of the agar for no less than 10 minutes. During this time please move on to the next question.

7. Partially lift the cover and, using flamed (but cooled) forceps, place the antibiotic disks into their predetermined locations.

8. Incubate the plates at room temperature. Carefully examine the plate to obtain your results next week in lab.