



The Life and Death of Bacteria



Background	<p>All living things contain cells. Eukaryotic organisms, things which contain more than one cell and contain membrane-bound organelles (plants and animals) have features unique to them, while prokaryotic organisms, things which are made of only one cell and do not have membrane-bound organelles (bacteria) have features unique to them. Bacteria come in many different shapes and sizes, which we can not see with our naked eye, but with the use of a microscope. In this class we will be learning about different types of bacteria, and where they grow, on our bodies and in our environment.</p>														
Objectives	<p>Students will learn about the structure and features of bacteria different than plant and animal cells. They will also learn basic aseptic technique used universally in microbiology laboratories, as well as safety precautions. Students will test different sources of bacterial growth, from their own body or their environment, to find out where bacteria considered “normal flora” are growing.</p>														
Materials	<table border="0"> <tr> <td>Nutrient broth</td> <td>Loop</td> </tr> <tr> <td>Nutrient plates</td> <td>Q-tips</td> </tr> <tr> <td>MacConkey plates</td> <td>Mannitol plates</td> </tr> <tr> <td><i>Serratia</i> cultures</td> <td><i>Micrococcus</i> cultures</td> </tr> <tr> <td>Dally rod</td> <td>Bacterial samples</td> </tr> <tr> <td>Antiseptics</td> <td>Disinfectants</td> </tr> <tr> <td>Sterile filter discs</td> <td>Forceps</td> </tr> </table>	Nutrient broth	Loop	Nutrient plates	Q-tips	MacConkey plates	Mannitol plates	<i>Serratia</i> cultures	<i>Micrococcus</i> cultures	Dally rod	Bacterial samples	Antiseptics	Disinfectants	Sterile filter discs	Forceps
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Preparation	<p>Wash hands and table tops before beginning any lab protocols today Wash hands after swabbing anything Wash hands and table tops after lab protocols are complete</p>														
<u>Procedure</u> Loop Transfer	<p>The purpose of this exercise is to learn “aseptic technique,” a method used in every microbiology lab setting. Aseptic technique is a method that: (1) prevents bacterial contamination of the environment (including you); and (2) prevents environmental contamination of the bacterial culture. It may feel tedious and awkward, especially if you are not dexterous, but you should strive for excellence in this method above all others. Here, we will use a LOOP to transfer media from one test tube to another test tube. Later, we will learn methods for handling other kinds of equipment.</p> <p><u>Materials:</u> Each student will receive two tubes of nutrient broth</p> <p><u>Procedures:</u></p> <ol style="list-style-type: none"> 1. Label your two tubes 2. Hold loop like a pencil and flame whole wire portion red hot. Cool 10 seconds. 3. Pick up a tube in other hand and remove cap with pinky of hand holding loop. 4. Flame mouth of tube 2-3 seconds and then place loop down tube to touch the liquid media. Carefully withdraw loop and re-flame mouth. Replace cap. 5. Set down first tube. Pick up second tube. Remove cap, flame lip, and deposit loop contents. 6. Flame loop and repeat procedure, passing back and forth 2-3 times. 7. Place all tubes in the incubator. 														
<u>Procedure</u> Streak Plating	<p>As you will discover from your ubiquity plates, there are LOTS of different kinds of bacteria everywhere. In order to study a particular species or strain, microbiologists need to isolate a “pure culture” of the organism of interest. This means physically spreading a population of organisms on an agar plate in such a way that a single organism can grow into an individual colony, significantly away from neighbors. Each colony is a population of “clones” that represent one bacterium which has separated from others and divided. For this exercise, we will make a streak plate from a liquid</p>														

<p>Testing</p>	<p><i>Staphylococcus epidermidis</i>, which lives on your skin. <i>E. coli</i> is Gram negative and lives in your digestive tract and <i>S. epidermidis</i> is Gram positive and lives on the skin. These experiments will allow you to determine if there are any obvious differences in how anti-microbials; like disinfectants and antiseptics we use in the house, affect a Gram positive vs. a Gram negative organism. We will be using <i>Serratia</i> (Gram negative) and <i>Micrococcus</i> (Gram positive) in this class for two reasons: they are less pathogenic and they produce distinctive colors which will increase our accuracy when reporting our results.</p> <p>The word “Anti-microbial” includes a variety of synthetic or naturally-produced chemicals: antiseptics, disinfectants, as well as antibiotics. Antiseptics are chemical substances that inhibit bacterial growth but are not normally harmful to human tissues. Disinfectants are substances that also inhibit bacteria growth but are also harsh to human tissues. Antibiotics are microorganisms, but we will not be working with them in this class.</p> <p><u>Materials:</u> 4 nutrient agar Petri plates per student, sterile filter discs and Q-tips, forceps and alcohol, 7 antibacterial compounds (antiseptics and disinfectants), sterile water, cultures of <i>Serratia</i> and <i>Micrococcus</i>.</p> <p><u>Procedures:</u></p> <ol style="list-style-type: none"> 1. Label each plate according to date, name bacterial organism place on plate (<i>Serratia</i> or <i>Micrococcus</i>) on the bottoms’ EDGE. 2. Divide each plate into four sections. (See diagram) Each area of the plate will be used for one antibacterial disc. 3. Label each section according to what is being tested (write a code and give a key to me); don’t forget a water control!! 4. Using a flame-sterilized dally rod, spread each culture over each plate. 5. Determine how you will apply your antibacterial to the filter discs. Discs SHOULD NOT BE DRIPPING WET 6. Using flame-sterilized forceps, remove filter disc from small jar, apply antibacterial, and lay in appropriate zone 7. Place the plates in the incubator
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Worksheet Assessment

Based on posted images that students reviewed on-line after the session was over.

1. Basic Practice Technique Results:

Look at the results you got from your basic practice techniques we learned about during this session!!

a. Loop Transfer:

The loop transfer exercise was a practice in our aseptic technique. Did your resulting liquid broth cultures show no growth (clear) or some growth (cloudy)?

b. Streak Plating:

The goal of doing the streak plating exercise was what?? Do you remember we wanted to take a colony of bacteria living in the liquid broth I gave you, and try to get a single colony growing on the plate at the end of the streak? Do you have some single colonies growing on your streak plates?

c. Dally Rod:

We practiced using the dally rod so we would know how when doing our antibacterial plates. We should see a well spread out colony of organisms with no visible clumps or bare spots, indicating we used the dally rod well and spread out our water sufficiently. What do you see?

2. Experiments and Their Results:

Look at the results you got from your experiments during this session!! We did several different types of things, all of which turned out great. If you have questions about anything on this page, please do not hesitate to contact me anytime shipleyk@wou.edu.

a. Survey Different Kinds Of Media:

In this formal experiment we swabbed a variety of places and then plated them on different types of media designed to grow different types of bacteria.

1. The nutrient agar plates were designed to grow what? Correct, they can grow just about everything.
2. The MacConkey plates were designed to grow on Gram negative organisms which grow mostly in the gut or inside your body. Look at your plates and where you swabbed to see if the plate only grew what it was supposed to. Are there any surprises?
3. The Mannitol Salt plates were designed to grow only Gram positive organisms which mostly grow on your skin surface. Look at your plates and where you swabbed to see if the plate only grew what it was supposed to. Are there any surprises? Think about where you swabbed, and what you thought might be growing there...

b. Antimicrobial Testing:

In this exercise we tested a variety of disinfectants and antiseptics to find out how effective they were against two different types of organisms, one which represents the type of bacteria normally living on your skin and the other representing a type of bacteria normally living in your gut.

1. Disinfectants are supposed to inhibit bacterial growth, but can also affect eukaryotic growth; can you see this effect when looking at the plates? (Look at the plates, as well as the corresponding measurements of the zone of inhibition)
2. Antiseptics are also supposed to inhibit bacterial growth, but not eukaryotic growth; can you see this effect when looking at the plates? (Look at the plates, as well as the corresponding measurements of the zone of inhibition)