# Antibiotic Susceptibility Testing

The purpose of this lab is to learn how to test the susceptibility of different bacteria to different antibiotics. Different classes of bacteria are susceptible to different antibiotics. For example, you should remember from earlier in the class that gram positive bacteria have a very thick layer of peptidoglycan. You will see in this lab, that it is the gram positive bacteria that are the most susceptible to penicillin which acts by inhibiting the crosslinking of the peptidoglycan chains.

You will need to learn some terms associated with antibiotics.

**Bacteriostatic** means to inhibit growth and inhibit protein synthesis. However, if you remove the agent, growth can continue.

**Bacteriocidal** means to kill bacteria.

**Bacteriolytic** means to lyse bacteria.

If you are sick and prescribed and antibiotic, you should take the full course of antibiotic or the illness may return.

Some antibiotics are known as **broad spectrum** antibiotics while other antibiotics are **narrow spectrum** antibiotics.

**Broad spectrum** antibiotics will affect both gram positive and gram negative bacteria while a **narrow spectrum** antibiotic will only affect specific organisms.

Organisms can also develop a **resistance** to different antibiotics. This can occur for a variety of reasons:

-Resistance can develop if antibiotics are used too often.

-Microorganisms carry plasmids (type of DNA) for antibiotic resistance

-These plasmids can be transferred to other microorganisms

-Certain microorganisms don't have the structure that the antibiotic targets

-Certain microorganisms can pump out the antibiotic so that it cannot affect the microorganism.

-Certain microorganisms can modify the target of the antibiotic.

For this section of the laboratory, you will work in groups. Each group will be assigned a culture by instructor. The cultures are SA, EC and PA.

SA is a gram positive organism and both EC and PA are gram negative organisms. The different composition of the cell wall will influence how susceptible the organisms are to different antibiotics.

In order to perform this experiment, we will use MH plates (Mueller-Hinton). These plates allow the antibiotics to diffuse throughout the plate. If the plates are not labeled, you will want to label them so that you do not confuse them with other plates used in this laboratory.

**Specifically, what you will do is the following:**

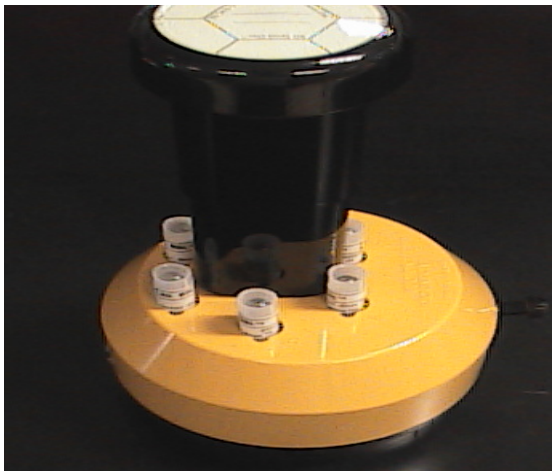
**-Use a cotton swab to swab your organism across the entire plate (you do not want to streak for isolation).**

**-Use the disc dispenser to dispense the antibiotic discs**

**-Invert the plates and incubate them at 37 degrees C until the next class**

The picture below shows the device that will be used to dispense the antibiotic discs.

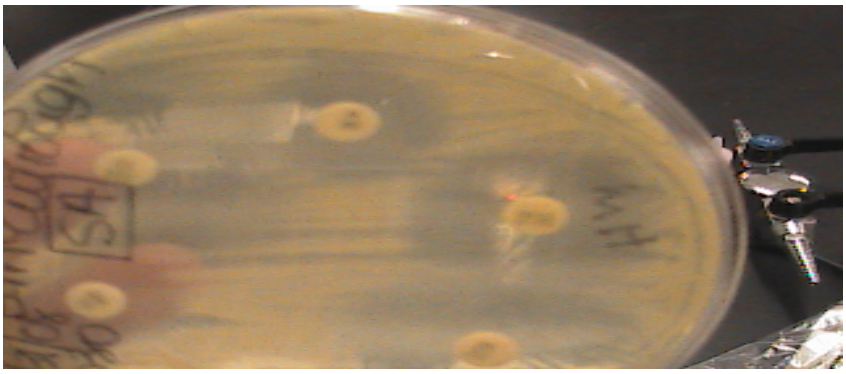
After you have swabbed your organism on the plate, you will put this device in the center of the plate and press it down to dispense the discs.

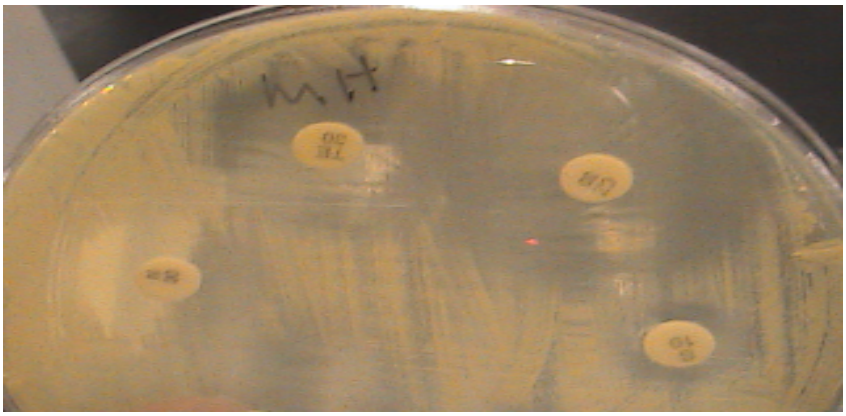


In the next lab period, you will measure the **zone of inhibition** produced by the antibiotics. This is a region surrounding the antibiotic disc where there is no growth of bacteria. You will be measuring the **diameter** of this ring and express your results in **mm.**

It is important to understand that when you measure the zone of inhibition, there must be complete inhibition of growth surrounding the antibiotic disc. If there is not, then you will record the results as have a 0 mm diameter of inhibition.

I have shown below some pictures of possible results that you will obtain.





Notice that the initials on the discs correspond to specific antibiotics. After you measure the mm of the zone of inhibition, you will classify the bacteria as sensitive (S), resistant (R) or intermediate resistance (I) to the particular antibiotic.

Place the data received into your lab notebook.

**Expected results:**

**PA**

Most resistant to antibiotics

Gram negative – 2 layers; outer membrane

Efflux pump: pumps out antibiotics and foreign particles

Selective porins

**EC**

Intermediate sensitivity to antibiotics

Gram negative

Porins not as selective

**SA**

Most sensitive to antibiotics

Gram positive