Microbiology Laboratory 12

Filter Paper Disk diffusion or Kirby-Bauer Sensitivity testing

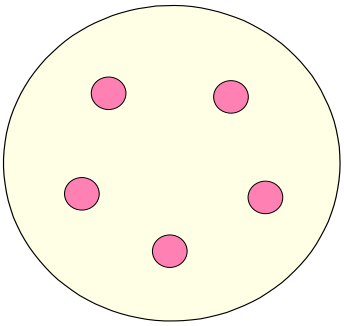
In this test paper disks containing specific concentrations of an antibiotic or an antimicrobial are placed on a lawn of bacteria on the agar surface. The compound in the disks diffuse out into the agar forming a concentration gradient. If the compound inhibits or kills the organism cultured in the lawn, there will be an area around the disk with no bacterial growth. This area of no growth is called the **zone of inhibition**. Next week after the plate has been incubated, students will measure across the entire zone, through the disk to assess the effectiveness of the compound on your organism.

Lab Assignment - Antibiotic testing

- Label the bottom of your plate so that you will be able to recognize next week.
- Transfer 100 µl of liquid culture to a labeled agar plate. Spread the culture around with an alcohol sterilized hockey stick.
- Let the agar surface dry before applying the disks.
- Bring your plate to the front to dispense the disks with an automatic dispenser.
- Remove the lid of the plate, and place the dispenser over the plate.
- Slide the arm on the side to dispense the disks.
- Remove the automatic dispenser and replace the lid of your plate.
- Back at your lab bench with a sterile forceps/hockey stick/tip tap each disk lightly to secure it to the medium.
- Invert your plate and place it in the 37°C incubator.

Lab Assignment - Antiseptic testing

- Label the bottom of your plate with some identifying mark that you will be able to recognize next week.
- Transfer 100 µl of liquid culture to a labeled agar plate. Spread the culture around with an alcohol sterilized hockey stick.
- Let the agar surface dry before applying the disks.
- Using alcohol sterilized forceps remove a sterile disk for the test tube aseptically.
- Dip the disk in your antiseptic of choice.
- Let any excess antiseptic drip off the disk then drag the disk along the inside side of the container to remove any additional excess.
- Place the disk on your agar surface with your lawn of bacteria.
- Place a total of four to five disks (each with a different antiseptic) in a circle on your plate.

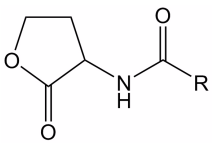


Quorum Sensing

Background

Many functions in bacteria are regulated by other bacteria in a process called autoinduction or quorum sensing (QS). In QS, the bacteria constantly produce small highly diffusible molecules which cross the plasma membrane to the external environment. At low levels these molecules produce no evident effect. As population density increases or as diffusion is constrained, as in a biofilm for example, the concentration of these molecules increases. At the correct concentration enough of the molecules will diffuse into other cells and be bound by special receptor proteins. Once the intracellular receptor proteins bind the diffusible molecule, they can then turn on or off certain genes. In this manner, prokaryotes can communicate with other prokaryotes.

Quorum sensing was first described as the mechanisms responsible for controlling bioluminescence in *Vibrio fisherii*, a Gram-negative marine bacteria. Initially, it was thought a quaint if interesting phenomenon. This view changed rapidly as other molecules were identified and were shown to be functioning in a great number of additional bacterial systems including virulence, motility, pigment production, and the development of normal biofilm architecture. One class of quorum sensing molecules that has been found to be expressed by several bacteria are the acyl-homoserine lactones (AHLs).



In this lab, we will use the Gram-negative bacterium, *Chromobacterium, violaceum*. This bacterium, can produce a water-insoluble purple pigment with antibacterial activity called violacein when properly induced by AHLs. We will use three strains of the bacterium.

The **wildtype** produces AHLs and responds to AHLs by producing the pigment, violacein.

The **reporter strain** can not produce any AHLs but does respond to AHLs produced by other bacteria (strains) by producing the pigment.

The **over producer strain** produces a bucket load of AHLs, but can not respond to the molecule and therefore does not produce the purple pigment.

Lab Assignment - Culture

Working in pairs . . .

1. Inoculate a NA plate, as shown in the diagram to the right. Be careful that your streaks get close to each other but without touching.
2. Be sure to flame you loop between each streak.
3. Label your plate, and incubate inverted at 37°C.