# Microbiology Laboratory 10

## Observations from Last Week's Lab

### Serial Dilution Calculations Part 2

Count the number of colonies on a plate. Your goal is to count around 100 colonies.
Note the concentration from the plate you counted.
Count how many decimal places you need to move the decimal to get to 1.0 from the concentration on the plate you counted.
Take that number and add the same number of zeroes to your colony counts.
Report your result to your instructor.

For example: if you counted 145 colonies on a 0.0001 plate. You have to move the decimal point in 0.0001 four places to get 1.0. So add four zeroes to 145 to get 1,450,000. But you are not done yet.

Since you only transferred 0.1 ml (100 µl) of dilution to the nutrient agar plate you must add one more zero to your calculation. So 1,450,000 becomes 14,500,000. We would like to say that you had 14,500,000 bacteria per ml in your original sample but we can't be sure of that. It is possible that two bacteria were plated so close together that when they reproduced (binary fission) that they created only one colony. (In other words, we can not guarantee that each colony came from one bacteria). As a result we change the vocabulary term we use to **Colony Forming Units** (CFUs). So in the end, we had 14,500,000 CFUs per ml in the original sample.

### Filter Paper Disk diffusion or Kirby-Bauer Sensitivity testing

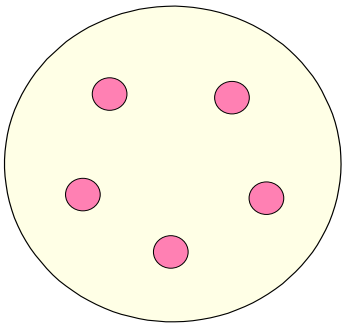
In this test paper disks containing specific concentrations of an antibiotic or an antimicrobial are placed on a lawn of bacteria on the agar surface. The compound in the disks diffuse out into the agar forming a concentration gradient. If the compound inhibits or kills the organism cultured in the lawn, 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 or sterile hockey stick 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

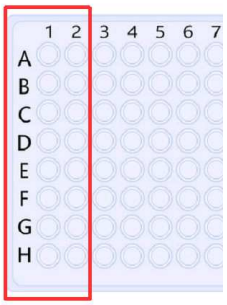
- Label the bottom of your plate with some identifying mark that you will be able to recognize next week.
- Transfer 100 µl of liquid culture to a labeled agar plate. Spread the culture around with an alcohol sterilized hockey stick.
- Let the agar surface dry before applying the disks.
- Using alcohol 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.



## **ELISA** - Enzyme Linked ImmunoSorbant Assay

Immunology is the study of the immune system and how the body protects itself against disease. Over 100 years ago, biologists learned that animals' immune systems respond to the presence of "foreign entities" now called antigens. When antigens are detected in the body, the immune system produces proteins called antibodies. These antibodies seek out and attach themselves to antigens, flagging them destruction by other cells of the immune system. The antigenic invaders may be any molecules foreign to the body, including components of infectious agents like bacteria, viruses, and fungi. Today, antibodies have become vital scientific tools, used in research and to diagnose and treat disease.
The number of different antibodies circulating in the blood has been estimated to be between $10^6$ and $10^{11}$, so there is usually an antibody ready to deal with any antigen. In fact, antibodies compose up to 15% of your total blood serum proteins. Antibodies are very specific, each antibody recognizes only a single antigen and binds it very tightly.
Scientists have applied the basic principles of antibodies to an assay for detecting antigens and other antibodies called an ELISA. Because ELISAs are antibody-based, they are often called immunoassays. ELISAs can detect minute amounts of antigens in body fluids or even swabs of body fluids. An ELISA is used for such diverse purposes as quantitating biological compounds, as well as, detecting the presence of disease or illegal drugs, testing indoor air quality, and determining if food is labeled accurately.
There are two types of ELISA tests, direct and indirect ELISA. The direct ELISA is used to quantitate or detect the presence of a compound. This is the type of ELISA used in home pregnancy tests to detect the presence of human chorionic gonadotropin (hCG) in urine. The indirect ELISA is used to detect infection by testing patients' blood for the presence or absence of patient antibodies against a particular pathogen. The presence of such antibodies indicates that the individual has been infected and that their body has launched an immune response against the disease-causing agent. This is the type of ELISA we will be conducting in lab.

## Lab Assignment



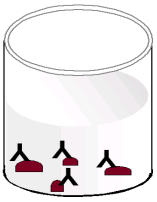

You will be working with the first two columns of a 96 well plate. Each test will be done twice, once in column 1 and the second same test conducted in column 2.

The plate has already come with the disease antigen adhered to the bottom of the wells.

Step 1
- Dump out the stabilizing buffer.
- Add 200µl of buffer to wash out the well.
- Dump out the wash buffer.

Step 2
- Add 200µl of patient serum A to row A, column 1 and 2.
- Add 200µl of patient serum B to row B, column 1 and 2.
- Continue through patient F.

- Add the positive control to row G, column 1 and 2.
- Add the negative control to row H, column 1 and 2.



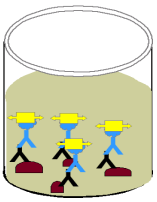
- Incubate for 10 minutes at room temperature (RT).
  If the patient is making antibodies against the disease their antibodies are binding to the disease proteins adhered to the bottom of the well.

Step 3
- Dump out the patient serum.
- Add 200µl of wash buffer to each well used.
- Dump out the wash buffer.

Step 4
- Add 200µl of the secondary (α-human IgG) antibody to each well used (all of column 1 and 2).

- Incubate for 10 minutes at RT.
  If human antibodies are present (captured in step 2), the secondary antibodies will bind the human antibodies. The secondary antibodies are made in goat to bind human antibodies. Once isolated from goat the antibodies are then covalently bound to the alkaline phosphatase enzyme.
If there are no human antibodies present there is nothing for the secondary antibodies to bind and will be washed away in step 5.

Step 5
- Dump out the secondary antibody.
- Add 200µl of wash buffer to each used well.
- Dump out the wash buffer.



Step 6
- Add 100µl of the substrate to each well used.

Incubate for a few minutes to see which wells turn color indicating a positive result.