University of Rochester
Summer Science Camp

1999

“MICROORGANISMS”

INFECTION OF PLANTS WITH TOBACCO MOSAIC VIRUS (TMV)

Materials Needed:

Young bean plants – pinto beans grown about 10 days
abrasive powder
water
diluted tobacco mosaic virus (TMV)
foam applicators
paper towels

Procedure:

(NOTE - you will be treating one leaf only)

1. Dip a clean, dry foam applicator into a vial of abrasive powder and dab (DO NOT RUB) the powder onto the upper surface of one leaf. Support the leaf from beneath with a pad of folded paper toweling while applying the abrasive. As quickly as possible thereafter, use a different clean foam applicator to paint (gently rub) a TMV suspension onto the abrasive-coated surface of the leaf. Support the leaf from beneath while you do this.

2. Allow the treated leaf to dry for several minutes, then rinse the leaf's surface for 3 seconds with a gentle stream of tap water from the dropper.

Evaluation of Results:

Lesions will begin to appear in the leaf 3-5 days after infection. These look like small black spots on the leaf surface. The lesions are best counted between days 4 through 7. Compare the results of the treated leaf and the untreated leaf.

Try this experiment at home!

♦ Soak 1 gram of dried tobacco leaves from a cigar or cigarette in 10 ml of water for 10-15 minutes. Grind the mixture with a mortar and pestle. Dilute 1 ml of this solution with 100 ml of water. (Alternatively, you can use a food processor or blender to grind up 1 gram of tobacco that has been soaked in about ½ liter of water).

♦ Grow bean plants from seeds until you have at least 2 leaves (this takes about 10 days for bean plants). Lightly scratch the surface of one leaf with 600 grit sand paper. Brush the leaf surface with the tobacco solution then rinse with water. Look for mottled leaves in 3-5 days. The tobacco mosaic virus in the tobacco solution will infect the leaves and cause small black spots to form.

♦ Do variations on this experiment to see how different factors effect the TMV virus. Try heating, boiling or freezing, or diluting the tobacco solution. Do you get less infection on the leaf? What happens if you don't scratch the leaf surface with the sand paper?

The Epidemic

An **epidemic** is caused by a contagious disease that spreads rapidly. In this activity, you will be demonstrating how easy it is to spread a contagious disease through the simple act of shaking another person's hands. We will be using a type of bacteria, called *Serratia marcesens*, that will be spread from one infected person to another. As part of this activity, you will try to figure out who was the initial person that started the epidemic.

In the lab today we will create an epidemic situation. One student in the lab will be "infected". The "disease" can be spread through handshaking. Each student will shake hands with a limited number of students and keep track of each contact and the order of the contact. The determination of whether you have the "disease' will be made by observing what grows on a petri dish.

Materials Needed

gloves
set of colored cards with the same number on each card
1 agar plate (2 if you are number 5, 10, 15, 20,)
a sterile swab (2 if you are numbers 5, 10, 15, 20)
a petri dish with a moist piece of gauze

Experimental Procedure

Day 1:

1. Write your initials on the bottom of the agar plate. Persons with 2 plates should label their plates #1 and #2.

2. Place your gloves on.

3. Remove the gauze with your **LEFT** hand and rub it onto your **RIGHT** hand, in particular the areas where you make contact when shaking hands.

4. Remove your **LEFT** hand glove and dispose of it. **DO NOT TOUCH ANYTHING WITH YOUR RIGHT HAND!**

5. Pick up your cards with your LEFT hand and walk around the lab to another classmate. ***DO NOT TOUCH THE OTHER CLASSMATE YET!!***

6. On the instructor's command, shake hands with the other classmate . Make sure you really come in contact with the inoculated area of the glove. After shaking hands, exchange a RED card with that person.
   DO NOT SHAKE ANYONE ELSE'S HAND YET!!

7. Walk over to another different classmate, and when the instructor says it is OK, shake that person's hand. Give that person your **ORANGE** card, and take their **ORANGE** card.
   DO NOT SHAKE ANYONE ELSE'S HAND YET!!

If you are number 5, 10, 15, or 20 you will need to take a sample of your hand at this point. (Other students will need to wait, and not touch anything) Sample your hand as follows:

Carefully remove a sterile swab from the wrapper. Rub your **RIGHT** hand, all over the palm and fingers, with the sterile swab and then rub the swab **gently** over the agar surface of your agar plate labeled #1.

8. Walk over to another different classmate, and when the instructor says it is OK, shake that person's hand. Give that person your **YELLOW** card, and take their **YELLOW** card
   DO NOT SHAKE ANYONE ELSE'S HAND YET!!

9. Walk over to another different classmate, and when the instructor says it is OK, shake that person's hand. Give that person your **GREEN** card, and take their **GREEN** card. You should now have 4 colored cards, each with a different person's number on it.
   DO NOT SHAKE ANYONE ELSE'S HAND!!

10. After 4 shakes, everyone will sample their **RIGHT** hand with a sterile swab, and then rub the swab gently over the surface of the agar plate. (Plate #2 for persons 5, 10, 15, and 20). Your instructor will store your plates in the incubator until tomorrow.

10. Remove your glove and dispose of it. Write down, in the table below, your "contact history" (who you shook hands with, and in which order).

CONTACT HISTORY

shake #	color card	number on card
1	red	
2	orange	
3	yellow	
4	green	

Day 2:

1. Record your history on the blackboard.

2. Observe your agar plates for sign of bacterial growth. Determine if you were infected, and then add this information to the blackboard chart.

3. After all of the data has been reported, attempt to determine who was the initially infected student. If you can't figure out who that was, explain why.

person #	red	orange	yellow	green	+ or -
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					

Microscopic Things in Pond Water

Materials Needed
pond water
depression slide
microscope

1. Place a drop of pond water on a plastic or glass depression slide (a slide with a small well in it).

2. View the slide under both the dissecting microscope and the compound microscope

3. How many different types of microorganisms can you find?

4. Are they still or are they moving?

5. Can you identify any?

6. Draw what you see.

The Protists: Protozoa and Algae

The kingdom of single-celled microorganisms, called protists, are divided into two groups. Protozoa, are the animal-like protists. Algae are the plant-like protists.

Protozoa are a diverse group of one-celled animal-like microorganisms that have a tremendous variation of cell shapes, means of movement, and ways of life. Protozoa are found in every kind of environment - marine, fresh water, and in the soil. Some are parasites, others are predators, and some are decomposers. Some protozoa cause human diseases, while others play important roles as decomposers in the ecosystem.

All protozoa are single-celled, but they have different means of locomotion (movement). Some move by using a "false foot" (called a pseudopodia). An example of this type is ***Amoeba***. Other protozoa have a long whip-like tail (called a flagella) that beats around to move the protozoan forward through its watery environment. Some protozoa, such as ***Paramecium***, are covered with tiny hairs (called cilia) that they beat to propel them.

Algae is a general term for a group of plant-like microorganisms. These include single-celled forms or giant seaweed that grow as big as a tree. Algae are mostly found in water, but some do grow on land in moist places. Many kinds of algae are important to marine and freshwater ecosystems because they are the producers that begin the food chain. Many food and industrial products come from algae. Some algae, such as ***Euglena***, exhibit characteristics of both plants and animals. Euglena has a tail (flagella) that enables it to swim.

You will be observing several different types of protists under the microscope. These may include: *Amoeba, Paramecium, Euglena, Stentor, and Blepharisma.*

Materials Needed
samples of protists
depression slide
microscope

1. Place a drop of protist sample on a plastic or glass depression slide (a slide with a small well in it).

2. View the slide under both the dissecting microscope and the compound microscope

4. Are they still or are they moving?

6. Draw what you see. (Use space below, or the back of this page)

7. Repeat steps 1-6 for each sample. Rinse off your microscope slides between samples (do not throw out the slides!)

Onion Skin Cells

Cells are crammed with a great variety of specialized structures that have various functions. These cellular structures are referred to as organelles (or "little organs") and their functions maintain the cell in a way similar to the organs that keep your body functioning. Most organelles are colorless, with the exception of green chloroplasts found in plants. Many kinds of special stains are used to color the organelles so that we can see them under the microscope.

We will look at onion skin cells, stained to see the organelles.

Materials Needed
Piece of onion
Tweezers
Microscope slide & coverslip
Stain (methylene blue or iodine)
Microscope

1. Obtain a slide, coverslip, and a small piece of onion.

2. Use tweezers to carefully peel off the thin epidermis ("skin") from the inner curve side of the onion layer.

3. Place the onion epidermis onto the slide carefully, to avoid any wrinkles.

4. Add a drop of stain on top of the onion epidermis then place a coverslip on top.

5. View the slide under the microscope. The epidermis resembles a brick wall, with each "brick" being a cell. The stain is absorbed by the cell nucleus, making it appear as a small colored sphere within the cell. The nucleus contains the cell's genetic materiel (DNA) that controls all of the cell's functions.

6. Draw what you see under the microscope

Human Cheek Cells

The lining of the human mouth (cheeks) is composed of **squamous epithelium cells**. The term "squamous" means flat, which describes these thin, transparent cells. Within the mouth, these cells fit closely together as tiles on a floor, forming a protective sheet of cells. In many areas of the body, such as the epidermis (outermost skin layer), and the mouth, squamous epithelium cells are continuously being rubbed off by friction. These cells are replaced by new cells, which rise to the surface as a result of cell growth activity in the lower cell layers.

We will look at squamous epithelium cells from the inside of your cheeks.

Materials Needed
Microscope slide & coverslip
Wooden tongue depressor
Saline solution
Stain (methylene blue or iodine)
Microscope

1. Obtain a slide, coverslip, and a wooden tongue depressor.

2. Place a drop of saline solution on the center of the slide.

3. Gently scrape the tongue depressor along the inside of your cheek. This will remove some surface cells.

4. Rub the tongue depressor onto the drop of saline on the slide. This will disperse the cheek cells onto the slide.

5. Add a drop of methylene blue stain to the slide. This will allow you to see the cell's nucleus and other organelles.

6. Place a cover slip on top.

7. Observe the slide under the microscope. Notice the prominent nucleus within each cell. You may see that some cells are clumped together. Draw a picture of one of your cheek cells.

Bacteria

People commonly think of bacteria as harmful disease-causing microorganisms. Actually, there are many more beneficial bacteria than there are harmful bacteria. For example, bacteria are used in human industries, such as in the production of dairy products.

Bacteria come in three basic shapes:

Spheres (coccus) - such as *streptococcus* (causes strep throat)
Rods (bacillus) - such as *E. coli (*causes food poisoning)
Spirals (spirillum)

Materials Needed
Microscope slides & coverslips
Plain yogurt
Saline water
Bacteria cultures
Microscope

1. Obtain a slide and a coverslip. Scoop up a tiny bit of yogurt and spread it thinly over a small area of the slide. Add a drop of water to the yogurt smear and mix it well with a toothpick. Place a coverslip over this mixture.

2. Carefully observe the yogurt smear under the microscope in an area where the smear is thinnest. Draw the bacteria that you see. These bacteria secrete digestive enzymes that chemically change the milk protein and sugars. These chemical changes result in the characteristic texture and flavor of yogurt.

3. Observe the prepared slides that show the three main shapes of bacteria - coccus (spherical), bacillus (rod-shaped), and spirillum (spiral). These cell shapes are used in the classification of bacteria and commonly occur in their names, such as *Staphylococcus aureus* or *Bacillus subtillus*. (The scientific name for each species of bacteria is commonly written in *italics*.)

4. Now make your own slides using bacteria cultures from your instructors. Scrape a tiny bit of bacteria from the petri dish (careful not to dig into the Jello-like agar that the bacteria grow on.). Transfer the bacteria onto a clean slide. Add a drop of saline water onto the slide and mix with a toothpick. (Alternatively, you can transfer your scraped bacteria into a test tube of saline water - this will spread out the bacteria. Then add a drop of this bacteria/water mixture onto the glass slide). Add a cover slip on top. This is called a "wet mount".

5. Observe the slide under the microscope and draw what you see. Repeat this procedure with the different bacteria cultures.

6. Scrape another bit of bacteria and mix it into a test tube of saline water. Add a drop of stain. Place a drop of stained bacteria onto a clean microscope slide. Add a coverslip, and observe under the microscope. Can you see things in better detail now that the bacteria are stained? Draw what you see.

Do Anti-bacterial Soaps Work?

Proper handwashing performed by employees of hospitals or medical centers is the most effective method of controlling infections. This first line of defense has become even more important due to the many antibiotic resistant organisms that inhabit most hospitals. Hand washing, before eating or preparing meals, is also an essential method to prevent bacteria from being transmitted onto what we eat and drink.

Simple hand washing, using bath soap and water, is not effective against many microorganisms. A layer of oil as well as the structure of the skin prevent the removal of microorganisms by simple handwashing. A number of different "anti-bacterial" soaps are sold in our stores which, presumably, will rid us of unwanted bacteria on our hands.

You will test various soaps and disinfecting agents to explore their effectiveness in removing microorganisms from your skin.

Materials Needed
Agar plate
Soap
Test tubes of saline water
Microscope slides & coverslips
Microscope

1. Take an agar plate and mark the bottom into 2 sections. Write your initials on the bottom of the plate.

2. Place two fingers on one section of the plate.

3. Each lab pair will choose a different item to wash their hands with. One person of the pair will wash their hands for 10 seconds, and the other person will wash their hands for one minute.

4. Being careful not to re-contaminate your hands, place the same two fingers on the other section of the agar plate.

5. Your instructor will place the plates in the incubator overnight.

6. **The next day:** Compare the growth of the agar plate of washed and unwashed fingers. Were there more or less bacteria growing after washing? Was 1 minute of washing more effective than 10 seconds of washing? Record your results in the table and determine the most effective item and time of washing for disinfecting your hands.

7. Pick several colonies from the "after washing" section of the plate and transfer them into a test tube with saline water. Place a drop of the bacteria solution onto a microscope slide to observe what type of bacteria was still growing on your hands after you washed them. Draw a picture of what you see.

PLATE GROWTH AFTER WASHING

Type of Soap	**10 sec.**	**1 min.**

Surface Microorganisms

Microorganisms can be found on almost all surfaces. In this experiment, you will test different areas around the Medical Center for the presence of microorganisms. You will obtain swabs of various surfaces, and streak these on agar plates. After growing your plates overnight you will try to determine what types of bacteria are found on your swab sample.

Materials Needed
Agar plate
Sterile cotton swabs
Sterile inoculating loops
Saline water
Microscope slides & coverslips
Microscope

Day 1:

1. Mark an agar plate on the bottom into two halves, using a marker pen. Write your initials on the bottom of the plate.

2. Use sterile swabs to wipe hard surfaces from various locations around the Medical Center that you think may have surface microorganism growth (Examples: telephone mouthpiece, doorknobs, vending machine, sink faucet).

3. Rub each swab on one half of the agar plate. Use a sterile inoculating loop to streak out an area of the culture (see diagram). This technique will hopefully assure that the culture will show isolated colonies, instead of just a big smear of many colonies. Carefully label the bottom of the plate with the location of the sample.

4. Your instructor will incubate the plate overnight at 37º C.

Day 2:

1. Examine your culture. Note and draw the different types of colonies. Your bacterial colonies may have different colors, sizes, and shapes.

3. Use a sterile inoculating loop to pick up a bit of a colony and mix it into a test tube of saline water. Take a drop of this mixture and place it on a microscope slide. You may want to also add a drop of stain to the bacteria on the slide. Repeat this step for all of your different types of colonies

4. Observe the slides under the microscope and draw what you see. Can you identify any of the types of bacteria on your slides?

Blood Cells

Blood consists of water (50%), dissolved salts, plasma proteins, and three types of blood cells (red blood cells, white blood cells, and platelets). Red blood cells are the most numerous. Their main function is oxygen transport. White blood cells are important in fighting infection and producing immunity against disease. Platelets, which are cell fragments, function in blood clotting.

Materials Needed
Blood cell slides:
normal human blood, Sickle Cell Disease blood, frog or fish blood
Microscope

1. Examine the prepared blood smear slide under low and high power. The numerous cells throughout the slide are red blood cells, or erythrocytes, that carry oxygen. Erythrocytes contain hemoglobin, a red pigmented protein that chemically binds with oxygen, and transports it through the circulatory system.

2. Focus carefully on an individual erythrocytes. The nucleus is absent, having been lost during development in the bone marrow where erythrocytes are produced. Notice that erythrocytes are slightly concave on each side, and somewhat flattened. This shape increases the surface area of erythrocytes, making them more efficient in transporting oxygen. Draw a picture of an erythrocyte below.

3. Return to low power and search the slide for larger, less common cells with large, darkly stained nuclei. These are white blood cells, generally known as leukocytes. When compared to erythrocytes, leukocytes are present at a ratio of only 1:700. There are various kinds of leukocytes, but they all function in some aspect of the immune system, such as producing antibodies or engulfing foreign bacteria or viruses. Draw a picture of a leukocyte below.

4. Platelets, another component of blood, are not visible on your slide. Platelets are cell fragments of cytoplasm (the inside portion of cells) that pinch off from large cells in the bone marrow. Platelets are essential for the production of a blood clot that prevents excessive blood loss from a wound. Plasma, also not present on your slide, is the liquid portion of blood in which the cells are suspended. Plasma is mostly water and contains a complex mixture of dissolved salts, gases, wastes, nutrients, hormones, and various proteins.

5. Observe the slide of a blood smear from a person with Sickle Cell Disease (also known as Sickle Cell Anemia). Sickle Cell Disease is caused by a genetic mutation of the gene that codes for the production of hemoglobin. A person with Sickle Cell Disease produces an abnormal hemoglobin in their red blood cells (erythrocytes). This causes the erythrocytes of a person with Sickle Cell Disease form strange-looking shapes. Some look like crescent-shaped sickles. Because of the strange shapes of these cells, they do not travel through the small blood capillaries efficiently, and tend to get stuck. This causes a host of various medical problems, including joint pain, swelling, and fatigue. There is no known cure yet for Sickle Cell Disease. Draw a picture of some of the cells that you observe on this slide.

6. Now observe the slide of frog (or fish) blood. That the erythrocytes (red blood cells) are very large in comparison to human red blood cells. You should also notice that the erythrocytes of frog and fish blood have a nucleus. Draw a picture of what you see.

Blood Typing
Introduction

Around 1900, Karl Landsteiner discovered that on the surface of red blood cells (erythrocytes), there are 2 different types of protein molecules called agglutinogens (or agglutinating antigens). These antigens are called A and B. A person with A antigens on his red blood cells has blood type A; a person with B antigens has blood type B; a person with both A and B antigens has blood type AB; a person with neither A or B antigens has blood type O.

Blood type O is the most common in the United States (45% of the population), followed by blood type A (39%), B (12%), then AB (4%).

Antibodies against antigens A and B begin to be produced in the blood plasma shortly after birth. These antibody levels peak at about 8-10 years of age, and the antibodies remain present throughout life. The reason for the initiation of antibody production is not clear. It has been proposed that antibody production is initiated by minute amounts of A and B antigens that may enter the body through food, bacteria, or by other means.

A person normally produces antibodies against antigens that are not present on his red blood cells. Thus, a person with antigen A on his blood cells (type A)will produce anti-B antibodies; a person with B antigens (type B) will produce Anti-A antibodies; a person with neither A or B antigens (type O) will produce both Anti-A and Anti-B antibodies, a person with both antigens A and B (type AB) has neither Anti-A nor Anti-B antibodies. The person's blood type is based on the antigens, not the antibodies, that he has.

In 1940 another group of antigens on the surface of red blood cells, called Rh factors, was discovered. They are called Rh factors because they were first found on the surface of rhesus monkey blood cells. A person who has these antigens on their red blood cells is Rh+; a person who does not have these antigens on their red blood cells is Rh-. About 85% of Caucasians, 94% of Blacks, and 99% of Orientals have Rh factors on their red blood cells. Unlike the ABO system, antibodies to the Rh factors are not normally present in the blood plasma, but are produced upon exposure to Rh factors, which can occur during a blood transfusion, if Rh+ blood is transfused into an Rh- recipient. This can also occur when an Rh- mother carries a fetus who is Rh+.

Who Dunnit
Simulated Blood Typing

Crime Scenario:

Crime investigators were called to the scene of a burglary. Mr. Smith had come home, and found someone robbing his apartment. As the criminal rushed to leave the apartment, he ran into a glass door, cutting his arm and tearing his shirt. The crime investigators were able to remove small pieces of clothing that appeared to be blood stained from the broken glass door. The blood samples from the crime scene, along with the victim's blood, were sent to the forensic lab to be analyzed. After the crime investigators carefully reviewed all of the evidence, they apprehended four suspects. The last remaining piece of evidence needed to solve the crime is to match the blood type found at the scene of the crime with one of the suspects'. You have been chosen to provide this last piece of evidence and determine which of the suspects is the burglar.

Objective:

In this investigation, you will assume the role of a forensic scientist as you attempt to solve the crime, using as evidence blood samples found at the scene of the crime. First you will confirm that the evidence found at the crime scene is blood, and then you will determine its blood type and match it to one of the four suspect's and the victim's blood type.

Materials Needed (per team):

4 cloth squares stained with simulated blood
6 blood typing trays
stirring sticks
microscope slide & microscope
simulated blood samples (Victim, and Suspects #1, #2, #3, and #4)
Typing serums (Anti-A, Anti-B, and Anti-Rh)

Procedure:

A. Microscopic Investigation

The first step in this investigation is to distinguish the blood stains from other similar-looking compounds such as fruit juice, jam, paint, etc.

1. Your instructor will provide you with a piece of stained cloth found at the scene of the crime. Place the stained cloth flat on a microscope slide and put a drop of water on it.

2. View the cloth under low and high power for any clues that would lead you to prove that the stain on the cloth is indeed blood.

Describe what you see under the microscope:

B. Blood Typing

Each team will determine the blood type of the victim, the four suspects, and the blood found at the crime scene.

1. Use a wax pencil or marker to label each of your 6 blood typing trays as follows:
   Tray 1: Crime Scene
   Tray 2: Victim
   Tray 3: Suspect #1
   Tray 4: Suspect #2
   Tray 5: Suspect #3
   Tray 6: Suspect #4

2. To determine the type of blood found at the crime scene, place a piece of the blood-stained cloth in each of the 3 wells of you blood typing tray labeled "Tray 1: Crime Scene" Note that the 3 well are labeled "A", "B" and "Rh"

3. Add 3 drops of Anti-A serum onto the cloth that is in the "A" well of the tray.

4. Add 3 drops of Anti-B serum onto the cloth that is in the "B" well of the tray.

5. Add 3 drops of Anti-Rh serum onto the cloth that is in the "Rh" well of the tray.

6. Use 3 different stirring sticks to stir each sample of anti-serum into the cloth sample in the well. Record your observation in the Data Table below.

Once you have determined the blood type of the crime scene evidence, you will then type the blood of the victim and the 4 suspects.

7. Place 3 drops of the victim's blood in each well of "Tray 2: Victim".

8. Place 3 drops of the Suspect #1 blood in each well of "Tray 3: Suspect #1"

9. Place 3 drops of the Suspect #2 blood in each well of "Tray 4: Suspect #2"

10. Place 3 drops of the Suspect #3 blood in each well of "Tray 5: Suspect #3"

11. Place 3 drops of the Suspect #4 blood in each well of "tray 3: Suspect #4"

12. Add 3 drops of Anti-A serum into each "A" well in the 5 trays.

13. Add 3 drops of Anti-B serum into each "B" well in the 5 trays.

14. Add 3 drops of Anti-Rh serum into each "Rh" well in the 5 trays.

15. Use different stirring sticks to stir each sample of serum and blood. Record your observations and results in the Data Table below.

Data Table
Agglutination Reactions

Blood Source	Anti-A Serum	Anti-B Serum	Anti-Rh Serum	Blood Type
Crime Scene				
Victim				
Suspect #1				
Suspect #2				
Suspect #3				
Suspect #4				