Those Pesky Pathogens!

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Abstract:

Those Pesky Pathogens is designed to introduce Bioscience I students to the various types of pathogens, the diseases caused by those pathogens, the vectors responsible for the transmission of the pathogens, possible ways to control the pathogens (or the vectors), and the social and economic implications of these pathogenic diseases. This unit incorporates laboratory activities, web-based activities, simulations, role-playing along with traditional means of instruction. Students will take a pre- and post-test to measure learning.

Rationale:

Infectious diseases are the leading cause of death worldwide and the third leading cause of death in the United States. Emerging infectious diseases are newly identified diseases that cause public health problems either locally or globally. Emerging diseases are ones that have not occurred in people before or have affected only a small number of people in isolated locations. Not all infectious diseases that pose a risk to human populations are unknown. There is a number of re-emerging infectious diseases which were once thought to be under control, but have once again become public health concerns (for example, malaria and tuberculosis). The purpose of the unit is to teach students basic biotechnology skills and to learn about infectious diseases and the pathogens that cause those diseases. Additionally, students will learn simple common biotechnology techniques/protocols and research the career opportunities in the field.

Key Questions/Essential Ideas

- What are emerging and re-emerging diseases?
- How are infectious diseases transmitted?
- What are the social and economic impact of infectious diseases?
- How can infectious disease be controlled?
- What is a virus and how does it use a host cell to replicate itself?
- Why do scientists and public health professionals need to monitor the spread and evolution of viruses?
- What are some common characteristics of HIV and HPV?
- What type of scientific information can you find in the news?
- How are the processes and techniques that are used in identifying a suspect at a crime scene similar to those used to identify a virus?
- What processes and techniques are used to identify a disease-causing virus?
- What is PCR and what are the steps involved?
- How is traditional PCR different than Real-Time PCR?
- What is reverse transcription and what is its function?

- What are some common biotechnology careers?
- What are the qualifications and salaries for careers in biotechnology?

Target Audience: Honors Bioscience I students, Grades 10-12

Science Concepts:

- Infectious diseases
- Pathogens
- Vectors
- Koch's postulate
- Scientific method
- Controlled experiments
- Antigen switching
- Disease and civilization
- Microscopy
- DNA structure and function
- Differences between DNA and RNA
- DNA extraction
- Polymerase Chain Reaction (PCR)
- Reverse transcription
- HIV and HPV viruses
- Careers in Biotechnology

Overall Time Estimate: 21 class periods (50 minutes each)

Module 1: Pathogens and Infectious Disease (11 class periods)

Student Learning Objectives:

The student will be able to:

1. Understand the scientific principles related to emerging and re-emerging infectious diseases.
2. Identify the pathogens responsible for causing infectious diseases.
3. Describe how vectors transmit pathogens.
4. Participate in a simulation to understand factors in the transmission of disease.
5. Demonstrate the proper use of compound light microscope.
6. Use microscopes, agar plates, and powers of observation to identify the bacteria used to produce yogurt and to provide proof for their hypothesized identification.
7. Utilize Koch's postulates to find a causative agent for disease.
8. Practice microbial techniques.
9. Design a controlled experiment.

Florida Sunshine State Standards:

SC.912.N.1.1 Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following:

1. pose questions about the natural world,
2. conduct systematic observations,

3. examine books and other sources of information to see what is already known,
4. review what is known in light of empirical evidence,
5. plan investigations,
6. use tools to gather, analyze, and interpret data (this includes the use of measurement in metric and other systems, and also the generation and interpretation of graphical representations of data, including data tables and graphs),
7. pose answers, explanations, or descriptions of events,
8. generate explanations that explicate or describe natural phenomena (inferences),
9. use appropriate evidence and reasoning to justify these explanations to others,
10. communicate results of scientific investigations, and
11. evaluate the merits of the explanations produced by others.

SC.912.N.1.7 Recognize the role of creativity in constructing scientific questions, methods and explanations.

SC.912.L.14.4 Compare and contrast structure and function of various types of microscopes.

SC.912.L.14.6 Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.

SC.912.L.14.52 Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics.

SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues

Lesson 1. Infectious Diseases-It's Catching! (4 class periods)

Infectious diseases are the leading cause of death worldwide and the third leading cause of death in the United States. Emerging infectious diseases are newly identified diseases that cause public health problems either locally or globally. Emerging diseases are ones that have not occurred in people before or have affected only a small number of people in isolated locations. Not all infectious diseases that pose a risk to human populations are unknown. There is a number of re-emerging infectious diseases which were once thought to be under control, but have once again become public health concerns (for

example, malaria and tuberculosis). The purpose of the unit is to teach students basic biotechnology skills and to learn about infectious diseases and the pathogens that cause those diseases.

Lesson 1. Anticipatory Set

Each student is given a stuffed giant microbe and also a question card. Students have to find the microbe that answers the question card.

Materials:
CPET Equipment Locker: Giant Microbes with Question/Answer cards.

Lesson 2. PowerPoint Notes: What are Pathogens?

Lesson 3. Activity: Spread of an Infection

Using simple reagents, students will simulate the spread of a simple imaginary disease in order to explore the factors that affect the rate of infection, the challenges of epidemiology, and measures which can help prevent the spread of disease.

Materials:

- test tube and dropper for each student
- distilled water
- 0.1 molar NaOH
- Phenolphthalein solution

Procedure, Part 1. Simulating an Epidemic:

1. Let students know they are going to model the transmission of a disease by exchanging some of their test tubes' contents with that of other students. Mention that one of the test tubes is "infected" with an imaginary infectious disease. (Prepare the test tubes prior to class: Fill one tube halfway with 0.1 molar NaOH; fill the rest of the tubes halfway with distilled water.)

2. Distribute prepared test tubes and droppers randomly to the class. Make a mental note of who receives the test tube containing NaOH.

3. Have students walk around the room with the test tubes. When you say "Stop!" each student should use a dropper to trade a drop of fluid with the person nearest them. Repeat until at least three trades have occurred.

4. Now it's time to test for the imaginary infection. Put a drop of phenolphthalein in each test tube. If the fluid turns pink, the test tube is "infected" with NaOH. How many students are now "infected"?

CAUTION: Sodium hydroxide (NaOH) and phenolphthalein can irritate the eyes and skin. Alert students to avoid spilling.

Procedure, Part 2. Tracing the source of Infection.

1. Now that a portion of the class has been "infected" put students in the role of epidemiologists. Their challenge is to collect data that will help them trace the path of the epidemic and locate the original carriers.

2. As a class, use the data to try to deduce which individual was the original carrier of the disease.

Discussion Questions:

1. Why might it be important to locate the source of infection?
2. What difficulties arise in trying to collect and interpret data?
3. Note that the simulated disease has a 100% rate of infection that appears immediately under testing. Some infections, such as AIDS and chicken pox, can remain dormant in the body for a long time. Others, such as Ebola, kill the host rapidly. How might each of these factors affect the spread of disease and the ability to identify carriers?

Results:

The final number of "infected" test tubes will vary depending on (1) the number of trades and (2) how many trades occur between two already infected tubes.

A possible method to find "patient zero" is to have each student write his or her name on the board and underneath it the names of students with whom he or she exchanged fluids in the order in which the exchanges occurred. Then, as a class, highlight the names of the currently "infected" people.

	Ann	Bob	Chris	David	Ed	Fran	Greg	Hal
Exchange 1	Fran	Chris	Bob	Ed	David	Ann	Hal	Greg
Exchange 2	Jo	Fran	Greg	Hal	Ilana	Bob	Chris	David
Exchange 3	Greg	David	Ed	Bob	Chris	Jo	Ann	Lynn

This visual representation can help clarify which students may have infected one another, and in what order. Students who "test positive" and find that everyone with whom they traded also tested positive may be original carriers of the disease. It is likely that there will be several candidates for "patient zero." Cross-checking the history of each contact can narrow the field, but probably not to less than two candidates. If students are unable to reach a clear conclusion, the exercise will raise useful questions about the challenges facing real epidemiologists as they try to trace the sources of an infection.

Lesson 4. Outbreak!

Simulates an investigation to determine the strain of pathogen by comparing the banding pattern of known and unknown samples.

Materials: CPET Equipment Locker

Lesson 2. Microscope Mania. (3 class periods)

Microscopes are very important tools to biologists, microbiologists, and biotechnicians. The term microscope can be translated as “to view the tiny,” because microscopes are used to study things that are too small to be easily observed by other methods. The type of microscope that we will use in this class is a compound light microscope. Light microscopes magnify the image of the specimen using light

and lenses. Successful use of a microscope depends on a variety of factors including the quality of the slide preparation, proper focusing, and adjustment for optimal illumination.

A **simple microscope** is nothing more than a single convex lens, also called a magnifying glass. A **convex lens** is one in which the center of the piece of glass curves out. The other major type of lens is a **concave lens**. It is a piece of glass that is thickest on the edges and curves inward in the middle. Looking at it on its side, it forms two "caves". Concave and convex lenses can be used in combination to produce various optical effects. A **compound microscope** is one that uses two or more lenses together.

Compound microscopes were invented around the 1590s by Zaccharias Janssen and his son Hans (from Holland). Robert Hooke (England) built the first useable British compound microscope around 1655. Using his microscopes to look at a slice of cork, he described "cells", like the tiny rooms used by monks, coining the term that we still use today. Although he worked after the beginning of the compound microscopes, Anton van Leewenhoek (Holland) built many superior simple microscopes (having only one lens) that magnified items over 200 times, starting about 1670. His tiny lenses had incredibly good resolution, and allowed for many new discoveries. Because of his many discoveries, he is often known as the "Father of Microscopy". In 1674, he described the green alga Spirogyra, in 1683 living bacteria, in 1702 the ciliate Vorticella. He also was the first to describe microscopic sperm, foraminifera, nematodes, and rotifers.

1. Introduction:
   Watch the Discovery Education video, *How to use a Microscope* (20:33 minutes)
2. Virtual Microscope Lab (http://www.udel.edu/biology/ketcham/microscope/scope.html)
   Students test their skills and practice using a compound microscope . The virtual microscope has the same controls as a real microscope.
   Microscope controls:
   - Turn knobs (click and hold on upper or lower portion of knob)
   - Throw switches (click and drag)
   - Turn dials (click and drag)
   - Move levers (click and drag)
   - Change lenses (click on a slide)
   - Select a specimen (click on a slide)
   - Adjust oculars (in "through" view, with the light on, click and drag to move oculars closer or further apart)

3. Lab: *Using a Compound Microscope*

   Objectives:
   In this lab the student will:
   1. Identify the parts of a compound light microscope and their functions.
   2. Calculate the magnification.

3. Make a wet mount microscope slide.
4. Understand how the orientation and movement of the specimen's image changes when viewed through the compound light microscope.
5. Properly use the low and high power objectives lenses.
6. Properly use the coarse and fine adjustments for focusing.

Materials

- microscope
- slides
- cover slips
- lens paper
- paper towels
- newspaper pages
- water
- pipette
- scissors
- magazine picture with various colors
- hairs of different colors
- other objects for viewing

General Procedure:

1. Students will name the parts and functions of the different parts of a compound microscope.
2. Learn how to calculate the total magnification.
3. Prepare and use a wet mount.
4. Compare and contrast how different magnification and diaphragm settings affect the image.

Lesson 3.What causes "Yogurtness"? (4 class periods)

This lab sets up a scenario in which students discover the cause of a new disease, "Yogurtness"-an affliction of "healthy" milk that causes it to become acidic and thick. Students will join Robert Koch, Louis Pasteur and the founders of modern microbiology to find the bacterial cause of this new disease. Using microscopes, agar plates, and their powers of observation, students identify the bacteria used to produce yogurt.

Objectives:

- To utilize Koch's postulates to find a causative agent for disease
- To practice microbial techniques
- To design a controlled experiment
- To reach a scientific conclusion from data and defend that conclusion

Timeline:

Pre-Lesson. Prepare agar plates 3-7 days ahead (30-60 minutes instructor preparation). Purchase milk (400 mL/class) and yogurt (100mL/class).

Lesson 1. Comparison of milk and yogurt. Inoculation of agar plates with yogurt followed by incubation at 37°C for 1-2 days (50 minute class, 30-60 minute instructor preparation).

Lesson 2. Identification of bacteria and inoculation of milk followed by incubation at 37°C for 1–2 days (50 minute class, 30–60 minute instructor preparation).

Lesson 3: Identification of bacteria from newly made yogurt (50 minute class, 15 minute instructor preparation).

Bacteria Are Everywhere

Bacteria are the single most successful form of life on the Earth. There are probably more bacteria, more species, and more total biomass of bacteria than any other lifeforms. Bacteria are found in soil, water, in and on animals, in and on plants, in and on humans, and even miles below the ground. There is speculation that bacteria or similar forms of life may exist on Mars or other planets.

Bacteria as Pathogens

When we think of bacteria we usually think of disease. In fact, only a tiny minority of bacteria are capable of causing disease. Bacteria that do cause disease have played an enormous role in the history of humanity—cholera, typhus, the bubonic plague, tuberculosis, and other bacteria have sickened and killed millions. The development of antibiotics has greatly reduced the dangers of bacterial diseases. However, due to the overuse of antibiotics some bacterial strains (such as methicillin-resistant Staphylococcus aureus or MRSA) have developed antibiotic resistance leaving humanity exposed to the reemergence of old bacterial threats.
Bacteria can also spoil food such as milk. Milk is an ideal growth medium for bacteria and may contain both spoilage bacteria capable of souring milk, and pathogenic bacteria which might cause disease in humans, such as brucellosis, bovine tuberculosis, and scarlet fever. Milk is pasteurized by heating to 62.9°C for 30 min, or 71.6°C for 15 sec, and then cooling rapidly. Pasteurization destroys all pathogenic bacteria, and most but not all, spoilage bacteria. Thus milk still needs to be kept cool when stored. Grade A milk should contain less than 30,000 bacteria per milliliter.

History of Bacteriology

Anton van Leeuwenhoek of the Netherlands first saw bacteria through a microscope in 1676 and called them animalcules (tiny animals). Later Christian Gottfried Ehrenberg coined the term “bacterium” (meaning “small staff” in Greek) in 1828. In 1835 Agostino Bassi proposed the “germ theory of disease” which connected the spread of disease to unseen microorganisms, as previously bacteria were thought to arise spontaneously in suitable environments. Louis Pasteur and John Tyndall showed that boiled broth grew bacteria only when exposed to the air thus disproving the theory of spontaneous generation. In 1875 Robert Koch was able to offer convincing proof of the germ theory by proving that anthrax was caused by bacteria. Koch’s set of rules (Koch’s postulates) for proving the cause of anthrax are the basis for assigning the cause of disease to a particular microbe. The postulates are also the basis for the experiments in this lab.

Types of Bacteria and Bacterial Colonies

There are several distinct morphologies or shapes of bacteria. The three major shapes are

coccus (spherical), bacillus (rod-shaped), and spirillum (spiral). Cocci and bacilli can exist singly, in pairs (diplococci or diplobacilli), attached in long strings (streptococci or streptobacilli), or connected in other arrangements (staphylococci or staphylobacilli). There are various forms of spiral bacteria too, such as comma-shaped (Bdellovibrio), helical (Helicobacter pylori), or long twisted spirochete forms. It is best to examine fresh cultures as older bacteria are occasionally oddly shaped and may have lost motility.

Bacteria increase in number by binary fission (splitting in half). Some bacteria can divide every 15–20 minutes! A single bacterium on a solid medium, such as an agar plate, increases logarithmically so that overnight a single bacterium becomes millions or billions. These millions or billions of bacteria form a visible "colony" on an agar plate. A colony of bacteria can itself have a distinct form and be large or small. Some bacterial colonies are so small that they cannot be seen with the unaided eye. Colonies may be circular, irregular, or branching. The edge of the colony may be smooth, wavy or serrated. The colony may be flat, raised or raised only in the center.

Bacteria are also differentiated by their cell walls. Some have thick cell walls made of peptidoglycan molecules. The cell walls of these bacteria take up a dye called Gram stain and thus are called Gram-positive bacteria. Other bacteria have thinner cell walls that do not absorb Gram stain and thus are called Gram-negative bacteria. The lactic acid bacteria found in yogurt are Gram-positive bacteria. The HB101 K-12 E. coli bacteria provided in this kit are Gram-negative bacteria.

Bacterial Metabolism

Like all living things bacteria require food, often in the form of sugars, to gain energy. Bacteria break down sugars chemically into other molecules using enzymes. Enzymes are large proteins that speed up chemical reactions. This process of bacterial metabolism is often called fermentation.

Some bacteria require oxygen from the air to grow and are called aerobes. Other bacteria grow only in the absence of oxygen and are called anaerobes. Some bacteria can grow either with or without oxygen and are referred to as facultative anaerobes. Aerobic bacteria use oxygen to break sugar into intermediate products and then finally into carbon dioxide and water. Lacking oxygen, anaerobic or facultative anaerobic bacteria usually do not reduce sugars completely to carbon dioxide and water. Often these bacteria convert sugar into pyruvic acid and then convert the pyruvic acid into other by-products.

Yogurt forming bacteria are anaerobes and break down milk sugar (lactose) into pyruvic acid and then into lactic acid using enzymes. Lactic acid is the by-product or waste product made by lactic acid bacteria. Lactic acid also lowers the pH of milk making it acidic. The acidic conditions cause casein (a common protein in milk) to denature (or curdle) and become more solid. In addition the acidic conditions inhibit the growth of other microorganisms that might spoil the yogurt. Thus lactic acid causes the yogurt to stay fresh, while at the same time remaining digestible by people who can break lactic acid down for additional energy. Other bacteria can break down sugars and pyruvic acid and make other by-products. The E. coli bacteria break sugar down into succinic acid, ethanol, acetic acid, formic acid, and lactic acid.

Koch's Postulates

By the mid-19th century, the famous French scientist Louis Pasteur had conducted extensive studies on the role of bacteria in fermentation, and had shown conclusively that germs did not spontaneously appear in susceptible hosts (spontaneous generation), but rather needed to come in contact with the host first. There was already a prevailing assumption at the time that microbes were in some way connected with disease, but whether their presence was the cause or just a result of disease was unclear. Furthermore, many infected tissues contained more than one type of microorganism. This made it difficult to define with certainty the role any particular type of bacterium played in disease. The work of Pasteur and others, along with improved techniques in microscopy and the discovery of semi-solid culture media, all paved the way for the work of Robert Koch.

Koch had been studying anthrax, a deadly disease that infects both humans and animals, and he noticed that certain rod-shaped bacteria and their spores were characteristically found in the tissues of sick sheep. He meticulously isolated these bacteria, which he named Bacillus anthracis, and grew pure cultures in a medium consisting of the aqueous humor of cattle or rabbit eyeballs. Next, he introduced the bacteria from the cultures into healthy rabbits. When the rabbits subsequently developed symptoms of anthrax, Koch again isolated the bacteria from the rabbit tissue and observed them under the microscope to confirm that they were indeed the same ones he had seen in his original culture.

The steps he used are now known as "Koch's postulates." Meeting the criteria laid down by Koch is referred to as "satisfying Koch's postulates" and is considered the standard evidence required to show that a microorganism causes a particular disease.

To demonstrate Koch's postulates, students must do the following:

- Describe and record the symptoms shown
- Isolate and identify the suspected pathogen from the infected material and establish a pure culture
- Use the pure culture to infect new material. Describe and record the symptoms shown by the material. Check that these are the same as their original observations
- Again isolate and identify the organism

Beneficial Bacteria and Yogurt

Despite our longstanding association of bacteria with disease, most bacteria are essentially harmless. In fact, many bacteria are beneficial. Bacteria break down waste organic material. Rhizobium bacteria take nitrogen from the air and convert it into a usable form (fixation). Intestinal bacteria break down indigestible material and synthesize nutrients. Some types of bacteria are necessary for the manufacture of certain food products, such as cheese, kimchi, sour cream, pickles, and yogurt.

Yogurt is made by adding specific strains of bacteria into milk, which is then fermented under controlled temperatures and environmental conditions. The bacteria ingest natural milk sugars and release lactic acid as a waste product thus making the milk acidic. The increased acidity causes casein (the most common milk protein) to tangle into a solid mass (called curd) in a process called denaturation. The increased acidity (the usual pH of yogurt is 4–5) also inhibits the growth of other dangerous bacteria. To be classified and sold as yogurt in the United States it is required that yogurt must contain the bacteria strains Streptococcus thermophilus (Streptococcus salivarius subsp. thermophilus) and Lactobacillus bulgaricus (Lactobacillus

delbruecki subsp. bulgaricus). Often these two are cocultured with other lactic acid bacteria for taste or health effects including Lactobacillus acidophilus, Lactobacillus casei, or Bifidobacterium bifidum. In most countries, a product may be called yogurt only if live bacteria are present in the final product. A small amount of live yogurt can be used to inoculate a new batch of yogurt, as the bacteria reproduce and multiply during fermentation. Pasteurized products, which have no living bacteria, are called fermented milk. In the United States yogurt must contain at least a billion viable bacteria per gram at the time of manufacture and at least a million viable bacteria per gram at the expiration date.

Yogurt has nutritional benefits beyond those of milk—people who are lactose intolerant often enjoy yogurt without ill effects, apparently because live yogurt cultures contain enzymes which help break down lactose inside the intestine. Yogurt also has medical uses, in particular for a variety of gastrointestinal conditions, such as preventing antibiotic-associated diarrhea.
In this lab students will isolate the bacterial strains found in a yogurt sample on agar in a petri dish, then use those same strains to inoculate fresh milk to find out if they can reproduce the same yogurt. Students should be able to conclude that the acidic, solidified nature of yogurt is caused by bacteria acting upon milk.

Antibiotics
Early attempts to treat bacterial infections sometimes employed substances, such as arsenic or strychnine, that were nearly as toxic to humans as to bacteria. In 1928 Alexander Fleming discovered penicillin, a compound produced by mold, that inhibited the growth of bacteria without serious harmful effects upon humans. Many different types of antibacterial antibiotics have been discovered since that time. These antibiotics have vastly reduced the incidence of bacterial disease. Modern society has almost forgotten how great the dangers of bacterial disease once were. Careless misuse of antibiotics now threatens a return of potent bacterial diseases.
Massive amounts of antibiotics are used in animal feed inadvertently selecting for the growth of bacteria resistant to many classes of antibiotics. People often needlessly take antibiotics for viral infections – again selecting for the growth of antibiotic resistant bacteria. In addition patients often discontinue use of antibiotics as soon as they feel better leaving the most resistant bacteria in place to start a new round of infection.

Antibacterial antibiotics are either bactericidal (kill bacteria) or bacteriostatic (prevent bacteria from dividing). There are many different modes of action for antibiotics. The antibiotic ampicillin is included in this kit both as an additional control and as a tool to allow further experimentation. Ampicillin is a beta-lactam antibiotic similar to penicillin and amoxicillin. It inhibits Gram-positive bacteria and some Gram-negative bacteria, such as E. coli, and it acts by preventing the synthesis of bacterial cell walls eventually leading to the death of the bacteria. Ampicillin is widely used in molecular biology as a selective agent since the gene for resistance to ampicillin (encoding for the beta-lactamase enzyme) can be inserted into bacteria on plasmids that may also carry genes of interest to scientists. Those bacteria that survive on ampicillin containing media will also have the gene of interest.

Sterile Technique
When culturing bacteria it is important to avoid contamination. Contaminating bacteria and molds are found everywhere, including on hands and lab benchtops, so it is important to avoid these surfaces. The round circle at the end of inoculating loops and the surfaces of agar plates should not be touched or placed onto potential contaminating surfaces. Wipe down lab benches with 70% alcohol or a 10% bleach solution wearing appropriate safety equipment.

Materials:
This section lists the equipment and reagents necessary to conduct the microbes and health experiment in your classroom or teaching laboratory. Biorad recommend student teams of 2–4 students per workstation. The kit contains reagents for 32 students working at 8 workstations made up of 4 students each.

Kit Components:

- Ampicillin (2 vials)
- LB nutrient agar powder (to make 500 ml) 1 pouch
- Petri dishes, sterile, bags of 20 2 bags
- Culture tubes, sterile, bags of 25 3 pks
- Inoculation loops, sterile, 10 µl, packs of 10 loops 8 pks
- E. coli HB101 K-12, lyophilized 1 vial
- LB broth capsules, bags of 12 (to make 50 ml each)* 1 bag
- Disposable plastic transfer pipets, packs of 10 pipets 1 pack

* LB broth capsules are included to extend the activity by using liquid cultures, if desired.

Required Accessories Number/Kit:

- Microwave or autoclave 1
- Incubator at 37°C (Catalog #166-0501EDU) 1
- 500 ml graduated cylinder 1
- 1 L flask or bottle 1
- Microscopes 1/workstation
- Microscope slides and cover slips 40 slides/80 cover slips
- pH paper (pH range 4–7 or wider) 48 pieces
- Permanent marker pens 8
- Table sugar (sucrose) 10 grams
- Toothpicks or micropipet tips box
- Distilled water 1 L
- Milk 400 ml
- Plain cow's milk yogurt (2–4 brands, must be 100 ml labeled as containing live and active cultures – the latest available expiration date is preferred)

Optional Accessories

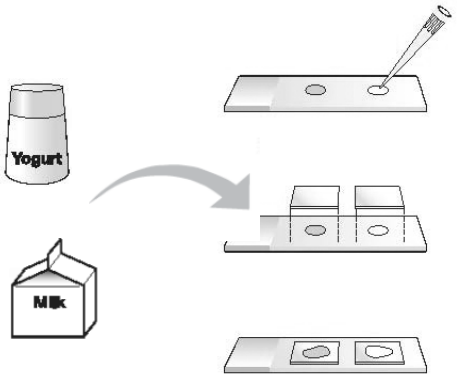
- Magnifying glasses (to view bacterial colony morphology)

Quick Guide Procedure:

Lesson 1.

Postulate 1. Identify possible pathogens.

1. Compare yogurt and milk with respect to appearance, smell, and pH. Record observations.
2. Label left hand edge of slide "yogurt" and right hand edge "milk".
3. Dip toothpick in yogurt, mix with a drop of water on left hand side of slide, and cover with cover slip.
4. Add drop of milk to right hand side of slide and cover with cover slip.
5. Observe yogurt and milk under the microscope. Describe and draw what you see.
6. Repeat steps 1–5 with a different brand of yogurt.



Postulate 2: Isolate and culture suspected pathogens

7. Label 3 LB sugar agar plates on the bottom(not the lid) with your initials and one as "milk", one as "yogurt", and the third as "E. coli".

8. Streak milk onto milk plate for single colonies. Streak yogurt onto yogurt plate for single colonies as above. Streak E. coli onto E. coli plate for single colonies as above.

   A) Streak for single colonies by gently rubbing the loop back and forth in the top left corner of the plate about 10 times. Stay in the top left quadrant of the plate and do not break the surface of the agar.

   B) Rotate the plate 45° and using the same loop draw the loop through one end of the first streak. **Do not dip the loop back into the starting material**. Then rub the loop back and forth in the second quadrant about 10 times. Avoid passing the loop into the first streak.

   C) Rotate the plate 45° and using the same loop draw the loop through one end of the second streak and rub the loop back and forth in the third quadrant about 10 times. Avoid passing the loop into the first and second streaks.

   D) Rotate the plate 45° and using the same loop draw the loop through one end of the third streak and rub the loop back and forth in the fourth quadrant about 10 times avoiding all previous streaks.

9. Invert the plates and place in incubator at 37°C for 24–48 h.

Lesson 2

Postulate 2 continued: Isolate and culture suspected pathogens

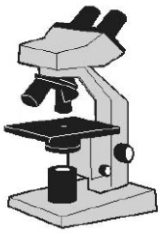
1. Obtain plates from previous lesson. Count the individual colonies on each plate. Record results.

2. Observe colonies. Use a magnifying glass if available. Record how many different types of colonies you have on each plate. Use a marker to circle one of each type of colony and label with a number on the bottom of the plate.

3. Describe the appearance of each numbered colony.

4. Label some slides according to your colony numbers. Use one slide for two samples as in the first lesson.

5. Pick a numbered colony from the yogurt plate, mix with a drop of water on right hand side of the appropriately numbered slide, and cover with a cover slip.



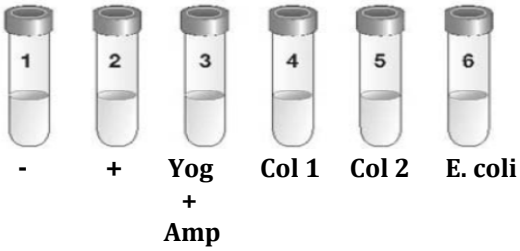
6. Repeat with the other numbered colonies from the yogurt, milk, and E. coli plates.

7. Observe colonies under the microscope. Describe and draw what you see.

8. Compare the bacteria with your descriptions of those observed in the yogurt in the first lesson.

Postulate 3: Inoculate healthy individual with pure culture of suspected pathogen

9. Label 6 tubes of milk as follows:
   Tube 1 Negative control
   Tube 2 Yogurt (positive control)
   Tube 3 Yogurt + amp
   Tube 4 Yogurt Colony #1
   Tube 5 Yogurt Colony #2
   Tube 6 E. coli



10. Add 10 µl or 1 drop of ampicillin to tube "Yogurt + amp".

11. Dip a fresh inoculation loop into the yogurtand swirl the loop into tube "positive control".

12. Use the same loop to dip into the yogurt again and swirl into the "Yogurt + amp" tube.

13. Identify two colonies on the yogurt agar plate that you investigated in the previous lesson of different types, if possible. Number the colonies 1 and 2 on the bottom of the plateand record which is which. If there is only one type of colony on your yogurt plate then number two similar colonies.

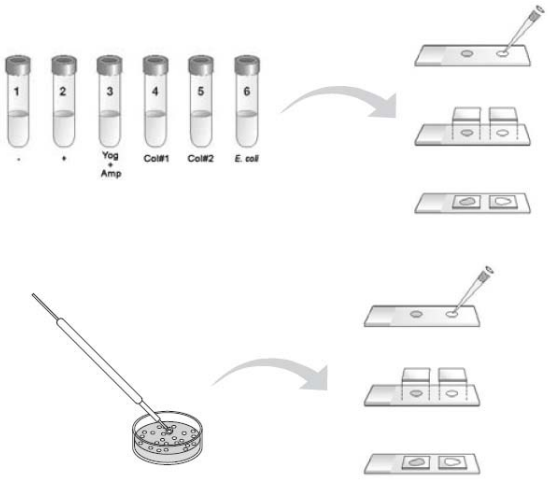
14. Using a fresh inoculation loop, pick colony #1 and transfer it to the tube " yogurt colony #1".

15. Using a fresh inoculation loop, pick colony #2 and transfer it to the tube "yogurt colony #2".
16. Using a fresh inoculation loop, pick an E. coli colony and transfer it to the tube "E. coli".
17. Place the tubes in an incubator or water bath at 37°C for 24-48 h.

Lesson 3

Postulate 4: Isolate and identify suspected pathogen from newly diseased individual

1. Obtain milk tubes and yogurt agar plate from previous lesson. Describe each milk culture with respect to appearance, smell, and pH.
2. Label 3 slides according your milk tube labels. Use one slide for two samples onthe right and the left as in the first lesson.
3. Label a fourth slide yogurt colony #1 on the right and yogurt colony #2 on the left.
4. Prepare slide samples of each milk culture for viewing under microscope as in previous lessons. For solid cultures, dip toothpick in culture and mix with a drop of water. For liquid cultures, add a drop to the slide. Cover with cover slip.



5. Pick a colony from the yogurt plate similar to that used to start the yogurt cultures in Tube 4 (i.e. the same colony type as yogurt colony #1). Mix colony with a drop of water on right hand side of the appropriately numbered slide and cover with cover slip. Repeat with yogurt colony #2 on the left of the slide.
6. Observe slides under the microscope. Describe and draw what you see.
7. Using the microscope compare any bacteria in the newly infected cultures in milk tubes 4 and 5 with the pure bacteria used to inoculate these cultures. Are they the same?

Post Lab Analysis:

1. From your results, what can you conclude about what causes milk to turn into yogurt?
2. What evidence do you have to support your conclusions?
3. Can any bacteria turn milk into yogurt? What evidence do you have to support your answer?
4. Can yogurt-making bacteria be prevented from making yogurt? What evidence do you have to support your answer?
5. If you had just added yogurt to the milk and found that it made yogurt, what would that show and what would it fail to show?
6. Why is it important to inoculate milk with bacteria from a single colony rather than from multiple bacterial colonies?
7. Some bacteria will only grow when they have access to specific types of nutrients. If some bacteria in the yogurt would only grow in milk, and would not grow in agar, how would this affect your investigation?

Module 2: Viral Quest Curriculum (2 weeks)

Student Learning Objectives:

The student will be able to:

1. Demonstrate understanding of core concepts in biotechnology including (but not limited to) cellular biology, DNA, RNA, and viruses.
2. Identify and follow common protocols used in a biotechnology laboratory (i.e. DNA extraction, PCR, reverse transcription).
3. Identify a variety of viruses, their characteristics, and symptoms caused in humans.
4. Analyze Real-Time PCR results to identify unknown viruses.
5. Identify careers in biotechnology.
6. Communicate with other individuals in a classroom environment.

Florida Sunshine State Standards:

SC.912.L.14.52 Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics.

SC.912.L.16.3 Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.

SC.912.L.16.4 Explain how mutations in the DNA sequence may or may not result in phenotypic change. Explain how mutations in gametes may result in phenotypic changes in offspring.

SC.912.L.16.5 Explain the basic processes of transcription and translation, and how they result in the expression of genes.

SC.912.L.16.7 Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.

SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.

SC.912.L.16.11 Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism(RFLP) analysis.

SC.912.L.16.12 Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).

SC.912.L.18.4 Describe the structures of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and function of enzymes.

SC.912.L.18.11 Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity.

SC.912.N.1.3 Recognize that the strength or usefulness of a scientific claim is evaluated through scientific argumentation, which depends on critical and logical thinking, and the active consideration of alternative scientific explanations to explain the data presented.

SC.912.N.1.4 Identify sources of information and assess their reliability according to the strict standards of scientific investigation.

Lesson 1: Outbreak!
An introduction to the Viral Quest curriculum and materials. Students are introduced to the scenario used during the curriculum.

Lesson 2: Viruses
Covers the structure and function of viruses and viral replication.

Lesson 3: Viral News
Students explore and analyze the available media resources about HIV and HPV. One purpose of this section is develop the ability to critically analyze media sources of information.

Lesson 4: Viral Whodunit
Students explore a fictional crime scene to explore the processes and techniques involved in identify a disease-causing virus.

Lesson 5: DNA Extraction (modified)
In this lesson, students extract DNA from a strawberry and compare the process to DNA extraction in a biotechnology laboratory.

Lesson 6: Polymerase Chain Reaction (modified)
Using the University of Utah website, Learn Genetics, students will learn the basics of PCR by performing a virtual polymerase chain reaction.

Lesson 7: Polymerase Chain Reaction PCR
Students will simulate the copying of DNA through the PCR Dash activity.

Lesson 8: Reverse Transcription
Students will learn about the processes and function of reverse transcription in converting RNA into DNA in preparation for subsequent identification through Polymerase Chain Reaction (PCR).

Lesson 9: Real-Time PCR
Students learn how to analyze Real-Time PCR.

Lesson 10: Biotechnology Careers
Students will explore the various careers in the field of biotechnology.

Budget

Unit	Item	Company	Cost	Total Cost
Microbes and Health	What Causes Yogurtness?	Bio-Rad	$118 Refills $55 x2	$228
The Power of Germs	Guns, Germs, and Steel DVD	PBS	$39.95	$39.95
			Total	$267.95

Resources

1. **Action Proposal Rational/Background Information**
"Diseases - Understanding Infectious Diseases, Page 3." *Emerging and Re-Emerging Infectious Diseases*. National Institutes of Health, 1999. Web. 07 Sept. 2011. <http://science.education.nih.gov/supplements/nih1/diseases/guide/understanding3.htm>

2. **Spread of an Infectious Disease Simulation**
"A Science Odyssey: Resources: Educator's Guide: Medicine." *Disease Detectives*. PBS: Public Broadcasting Service, 1998. Web. 07 Sept. 2011. <http://www.pbs.org/wgbh/aso/resources/guide/medact4index.html>.

3. **Microscopy Background**
Introduction and Microscopy I: Compound Microscope and Micropipette Lab." *Bio 101 Laboratories*. University of Rhode Island. Web. 11 Sept. 2011. <http://www.uri.edu/cels/bio/wetherbee/bio101/lab1>.

4. **Discovery Education video**
How to Use a Microscope. Prod. Colgren Communications. Colgren Communications, 1989. *Discovery Education*. Web. 7 September 2011. <http://www.discoveryeducation.com/>.

5. **Virtual Microscope**
Barrett, Margie, Joelle Cona, Paul Hyde, Bob Ketcham, Becky Kinney, and Justin Schakelman. "Virtual Microscope." *Welcome to the University of Delaware*. Department of Biological Sciences, 16 Feb. 2004. Web. 05 Sept. 2011. <http://www.udel.edu/biology/ketcham/microscope/>

6. **Lab: How to use a compound microscope**
Wanamaker, Jim R. "Lab: Using a Compound Microscope." *Lew-Port's Biology Place*. Lewiston Porter High School, 17 Nov. 2008. Web. 24 June 2011. <http://lpscience.fatcow.com/jwanamaker/teacherresources.htm>.

7. **Viral Quest Curriculum**
Klosterman, Michelle L., Troy D. Sadler, and Julie C. Brown. *Viral Quest*. *OUTBREAK (Opportunities to Use Innovative Technologies to Explore Biotechnology Resources, Career Education, And Knowledge)*. University of Florida, 2011. Web. 15 June 2011.

8. **University of Utah, Learn Genetics, Virtual PCR**
Genetic Science Learning Center. "PCR Virtual Lab." Learn.Genetics 7 December 2010 <http://learn.genetics.utah.edu/content/labs/pcr/

9. **PCR Dash**
"Polymerase Chain Reaction (PCR)." *Bush-to-Base Bio-Informatics | Virginia Tech*. Web. 07 Sept. 2011. <http://www.bush2base.vt.edu/readit/DNA/DNA_files/Page799.htm>.