Using a Modified ELISA Assay to Demonstrate the Transmission of a Simulated Disease

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VIII. Student Prior Knowledge and Skills; Students must know:
   a. How to use a dichotomous key
   b. How to correctly use a microscope
   c. General characteristics of microorganisms
   d. How to follow a lab protocol

IX. Student Expectations
   a. Formal laboratory report including:
      i. Background information/introduction
      ii. Experimental design/materials/procedure
      iii. Data and analysis
      iv. Conclusion
   b. Presentations on various pathogenic organisms

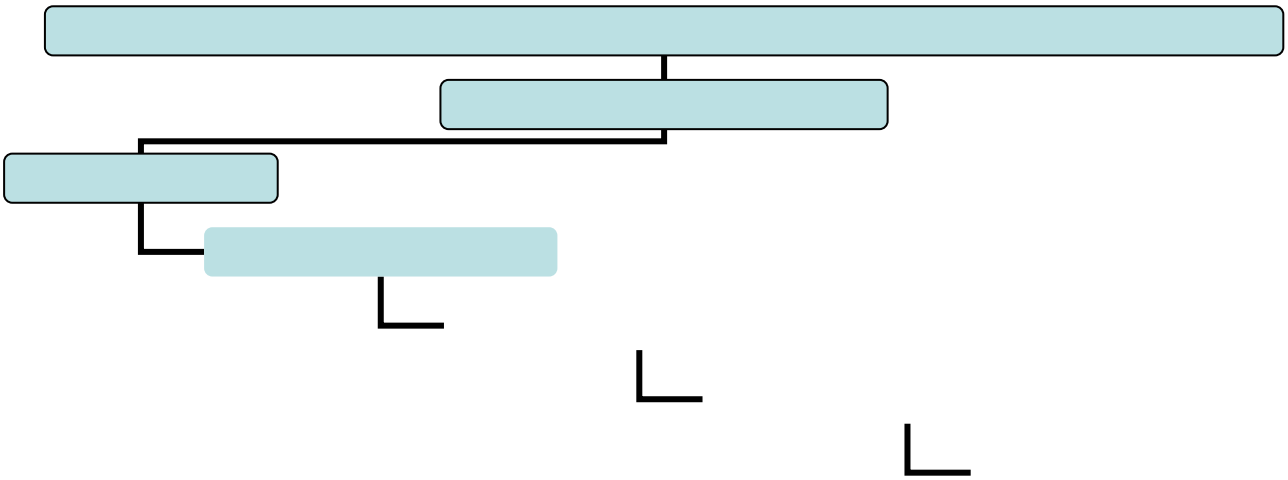
X. Anticipated Results
   a. Results will vary based upon simulated ‘fluids’ and bacterial strains available to each teacher
   b. Results are dependent upon initial number of infected students

XI. Classroom Discussion
   a. “Stop and Think”
      i. What are some different disease-causing agents?
      ii. How does disease spread?
      iii. If one person in this room were infected with an airborne disease, what do you think the chances are that you will be exposed or infected with this disease?
      iv. How quickly do you think disease spreads? Does the rate of transmission vary? Why or why not?
   b. Analysis questions
      i. What percent of the class became infected?
      ii. What characteristics make a bacterium either Gram positive or Gram negative
      iii. See analysis questions for “ELISA Protocol”
   c. Discussion questions
      i. Are you surprised by the results obtained from this exercise? Why or why not?
      ii. How can you apply this knowledge to help you maintain a healthy, disease-free lifestyle?

STUDENT SECTION:

I. Rationale
   a. The purpose of this unit is to familiarize students with the caus

Experimental Overview (Flow Chart)



Teacher Guide Pre-Lab Preparation

The recipes for the pre-lab preparation are from The University of Arizona Biotech Project. http://biotech.biology.arizona.edu/labs/ELISA_assay_teacher.html

You will need to prepare the following materials for the disease transmission and ELISA test:

- Washing Solution
- 1X Na

Preparation of Materials:

Bacteria to identify for positive tes

Positive Antigen Solution (biotinylated albumin)

1. Dissolve 10 mg biotinylated bovine albumin in 20 mL 1X Na_2CO_3 buffer for a final concentration of 0.5 mg/mL. Store as 1 mL aliquots in freezer.

2. The positive antigen solution contains two types of bovine albumin, biotinylated bovine albumin and normal, non-biotinylated bovine albumin. To prepare positive antigen solution, mix 0.1 mL of the 0.5 mg/mL biotinylated bovine albumin solution with 9.85 mL 1X Na_2CO_3 solution and 50 ul of 10 mg/ml **normal, non-biotinylated** bovine albumin (for recipe, see "Antibody Solution"). This solution can be stored for up to one week in the refrigerator.

3. This solution is given to 1-3 students in a class (based on class size) as the infected samples. These students would be the original infected carriers in the class.

4. Use the remaining solution as positive control samples for the ELISA test. Put 1 ml of positive antigen solution into a centrifuge tube (1.5 ml size) for each lab group.

Antibody Solution (Streptavidin peroxidase)

1. Add 1 mL of a 50% glycerol solution to the 0.5 mg of streptavidin peroxidase in the container. Store in refrigerator; this concentrate should be stable for several years.

2. Prepare a 10 mg/ml bovine albumin solution by mixing 0.5 g bovine albumin (this is normal, non-biotinylated albumin) in 50 ml deionized or distilled water. The 10 mg/ml solution can be stored in 10 ml aliquots in the freezer.

3. To prepare antibody solution, mix 0.5 mL 20X PBS, 50 ul of 10 mg/ml normal bovine albumin and 9.45 mL deionized or distilled water. To this mixture, add 1 uL streptavidin peroxidase solution. Store in refrigerator and use within one week after dilution. Distribute in 3 ml aliquots to each student group.

Color Reagent Solution (TMB)

1. Dissolve 1.46 Na_2HPO_4 and 1.02 g citric acid in water to final volume of 200 mL. This is citrate phosphate solution (0.05 M). This solution can be stored indefinitely in refrigerator.

2. To prepare color reagent solution, add 1 mg TMB or 1 TMB tablet to 10 mL citrate phosphate solution. Next, add 2 uL of hydrogen peroxide (30%) to this solution.

3. Use this solution on the same day and store in the refrigerator.

Sources of Laboratory Materials

Below is a table that contains an example of sources for the materials necessary to perform this experiment. Most materials can be purchased through a variety of scientific supply companies.

Item	Source	Item #	Quantity	m	n

Lab Protocol for Simulated Disease Transmission

Purpose:

The purpose of the disease transmission activity is to share simulated body fluids with three classmates of your selection. This is in preparation for the following sections that will determine "infected" individuals, culture samples of "pathogen", and identify the type of bacteria that caused the infection. Th

Simulated Disease Transmission Protocol Teacher Section

Procedure:

1. Using the marker provided, label both of your tubes with your initials.
2. Label one tube with S (sharing) and the other with NS (non-sharing).
3. Use the transfer pipette to transfer half of the simulated body to the empty tube. (now each tube should have an equal volume of the simulated body fluid in it)
4. Put your NS tube with your initials aside in the designated rack. (set rack aside to avoid confusion)

Note: use the S (sharing) tube for the directions below

5. When your instructor tells you, find one member of your class to share the simulated body fluid with. (wait until all students are ready)
6. Use a transfer pipette to combine both fluids in one of the S tubes (either yours or your partners).
7. **Gently** mix the contents by using the pipette to siphon some of the fluid mix and then to expel it back into the tube. Complete this for approximately three to five times to ensure complete mixing of the fluids.
8. Once the fluid has been completely mixed, use the transfer pipette to transfer one-half of it into the other S tube (either yours of your partners dependant upon which tube is empty).
9. Record the name of the student that you made your first exchange with in the table provided. (wait until all students have had ample time to share fluid and record their exchange partner)
10. When your instructor gives the direction to complete the second exchange, move about the room and find a second person to exchange the simulated fluid with.
11. Complete the procedure to share a

Record of Sample Sharing

Simulated Disease Transmission Protocol Teacher Section

Lab Protocol for Simulated Disease Transmission (alternative if materials for ELISA are not available)

Purpose:
The purpose of the disease transmission activity is to share simulated body fluids with three classmates of your selection. This is in preparation for the following sections that will determine "infected" individuals, culture samples of "pathogen", and identify the type of bacteria that caused the infection. The way in which a communicable disease is spread can be represented by sharing simulated body fluids.

Shared Materials:
Permanent Marker
Micro centrifuge racks (there must be enough to hold two tubes per student)
4% NaOH

Materials per Student:
1 empty micro centrifuge tube
1 micro centrifuge tube containing simulated body fluid
(Water if negative, phenopthalein if positive)
1 transfer pipet 1 Tf0 Tc 0 Tw 4 0 12 108 pBT/TT3 1 Tf0 T 417.7802 Tm(e))Tj 0 a

Simulated Disease Transmission Protocol Teacher Section

Procedure:

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3. Use the transfer pipette to transfer half of the simulated body to the empty tube. (now each tube should have an equal volume of the simulated body fluid in it)
4. Put your NS tube with your initials aside in the designated rack. (set rack aside to avoid confusion)

Simulated Disease Transmission Protocol Teacher Section

Record of Sample Sharing

Your Name	
First Student Shared With	
Second Student Shared With	
Third Student Shared With	

Observation Table

Micro Centrifuge Tube Label	Color before addition of NaOH	Color after addition of NaOH	Positive (+) or Negative (-) based on color after NaOH
S			
NS			

14. Complete the procedure for sharing simulated body fluids (#6-8).
15. Record the name of your third contact in the record of sample sharing below.
16. Place your tube in the rack provided to complete the simulated EILSA on to determine the # of infected individuals from the activity.

Record of Sample Sharing

Your Name	
First Student Shared With	
Second Student Shared With	
Third Student Shared With	

10. When your instructor gives the direction to comp

ELISA Protocol Teacher Section

9. Add **washing solution** to the wells as done in step 7, and repeat washing a total of three times as described in #6.

10. Add three drops of the **color reagent solution** to each well.

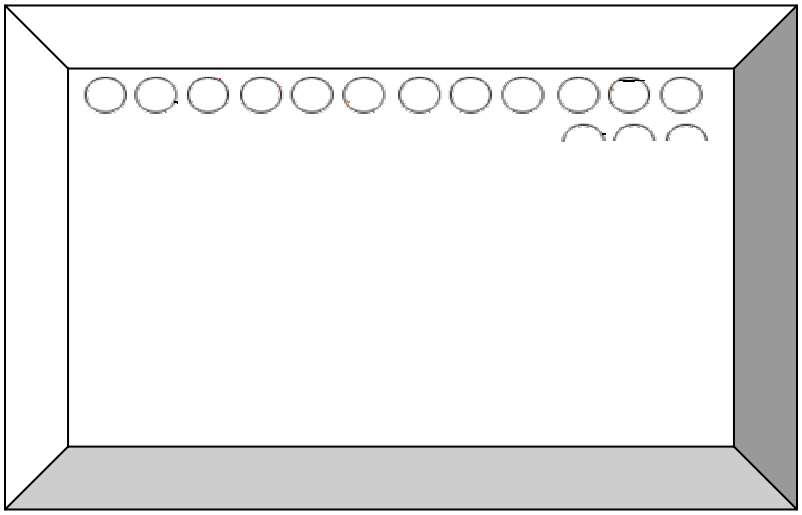
11. Record your observations of the color after five minutes of all of your wells as well as the positive and negative control wells.

12. Each group will record the results of the tests in the data table. Be sure to record whether you are infected. You will also record your test results and list of partners on a overhead to be shown to the class.

13. Given the classroom data, determine the original infected carriers. After identifying potential sources of the disease, you can confirm your analysis by performing another ELISA assay on the non-shared samples.

Data and Observations:

Mark off the sections that are for each group member and the positive and negative controls in the sample ELISA plate drawn below.



Analysis Questions:

1. What is the purpose of an ELISA i2 362.0092 742pb0 10. 678.84 T293.0987

ELISA Protocol Stude

Materials per Group:

- ELISA test plate
- Positive and negative control solutions (in dropper bottle)
- Paper towels
- Antibody solution (in dropper bottle)
- Washing solution (in wash bottle)
- Color reagent solution (in dropper bottle)

Materials per Student:

- S (shared) micro centrifuge tube from disease transmission activity
- NS (not shared) micro centrifuge tube from disease transmission activity

Procedure for ELISA Test:

1. Using your transfer pipette, add three drops of your sharing tube fluid into each of three wells. Record which wells contain your fluid to avoid confusing your wells with another student's on the ELISA plate provided in the data/observation section of this lab.

2. Using the dropper in the bottle, add three drops of a positive control solution into three wells and three drops of a negative control solution into a different set of three wells. (This is to do a positive control- the results if the test is positive and a negative control- the results if the test is negative)

3. Leave the plate on the lab table undisturbed for five minutes after all members of the group have added their solution and the positive and negative control have been added. This will allow time for the antigen to bind to the antibody if the fluid is positive for the pathogen.

4. Shake off the fluid into a nearby sink or designated container, making sure that the fluid has emptied from each well. To do this, **SECURELY** hold the ELISA plate in the palm of your hand flat then pretend that you have a fly swatter and quickly snap your arm propelling your hand towards the sink. Complete this three times to remove most liquids from the wells.

5. Tap the plate upside down onto the paper towel to remove any excess liquid or bubbles. Complete five times to ensure it is as dry as possible.

6. Add **washing solution** to the wells by gently filling all wells from the bottle of washing solution, and shake off fluid as in #4. Repeat the washing procedure a total of three times.

7. Add three drops of the **antibody** solution to each well.

8. Allow five minutes incubation time on the lab table and then shake off the fluid as described in #4.

9. Add **washing solution** to the wells as done in step 7, and repeat washing a total of three times as described in #6.

10. Add three drops of the **color reagent solution** to each well.

11. Record your observations of the color after five minutes of all of your wells as well as the positive and negative control wells.

12. Each group will record the results of the tests in the data table. Be sure to record whether you are infected. You will also record your test results and list of partners on a overhead to be shown to the class.

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Data and Observations:

Mark off the sections that are for each group member and the positive and negative controls in the sample ELISA plate drawn below.

Lab Protocol for Pouring Agar Plates

Purpose:

The purpose of creating agar plates is to have a medium (plural = *media*) to culture, or grow, bacteria on. The first microbiologists, in the 1800s, would use fresh, thinly sliced potatoes to culture bacteria on! We, however, are going to use agar. It looks like Jello™, but don't be fooled! An agar–filled petri dish provides a large surface area in which you can streak bacterial samples on in order to isolate clones of individual cells. However, this same surface is easily contaminated and easily dries out. You must be very careful when preparing your agar!

MATERIALS:

hot plate (must be able to heat AND stir)		distilled or deionized water	
electric balance	weighing dish	1 Liter beaker	nutrient agar
scoopula	magnetic stirrer	petri dishes	thermal gloves

Name of Product	Amount Needed Per Class	ID# from Carolina Supply Company	Price
Nutrient Agar	100 grams (?)	ER-78-5300	$27.95/100 gram package
Petri Dishes	24	ER-19-9278	$14.80/20 dishes
Sterile Swabs	24	ER-70-3032	$19.70/200 swabs

Procedure:

1. Using a scoopula and a weighing dish, mass out 24 g of nutrient agar. Be sure to *tare* the balance prior to adding nutrient agar to the weighing dish.
2. Carefully empty the agar into a 1 L beaker.
3. Fill the beaker with 1 L of either distilled or deionized water.
4. Place the beaker on the hot plate and add the magnetic stirrer to the beaker.
5. Heat the agar solution until it boils.
6. Allow the solution to boil until all powdered agar is dissolved and the liquid is transparent.
7. Using thermal gloves, remove the beaker from the hot plate.
8. Carefully fill each petri dish 2/3 of the way with agar.
9. Cover the petri dishes to avoid contamination from the air. Let the agar cool and solidify, then invert to refrigerate (this will lessen the amount of condensation that accumulates on the surface of the agar.
10. Clean up all materials thoroughly!

Lab Protocol for Streaking Bacterial Samples

Background:

To obtain colonies of bacteria from your sample, you need a smooth surface and nutrients to allow the bacteria to proliferate. This is why you made nutrient agar plates. Once the plates have solidified, you should have a very smooth surface upon which you can spread your bacterial sample.

You will be performing a *quadrant streak.* This method enables you to dilute the bacteria in the sample by dragging them (literally!) across the agar surface. This process will leave microscopic 'clumps' of bacteria as you streak, until only a few cells are left on the swab. These few cells will be left to grow singly here and there. It is assumed each single cell deposited on the surface of the agar plate will produce a progeny of clones that will eventually grow into a bacterial colony. In today's lab, you will use a sterile swab to inoculate your agar plates.

MATERIALS:

prepared agar plates	Positive ELISA samples	test tube rack
sterile swabs	latex or nitrile gloves	safety goggles
laboratory apron	permanent marker	

Procedure: TEACHERS- Remember to add bacteria to student samples that tested positive in the ELISA!

NOTE: You must wear gloves, goggles, and an apron at all times!

1. Hold the tube from which you will obtain the inoculum (bacterial sample) in one hand. You want to hold the tube at an angle so that nothing falls into the tube to contaminate it.
2. Remove the cap from the tube using your other hand.
3. Remove the sterile swab from its packaging. Do NOT let it touch anything, or it will no longer be sterile!
4. Submerge the cot(Hold t sterile swab fr/p s Tm(fr/p s T2m(e752/TT4 1 Tf0.00rom)T 12 407.68.9251 26

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sterile swabs	latex or nitrile gloves	safety goggles
laboratory apron	permanent marker	

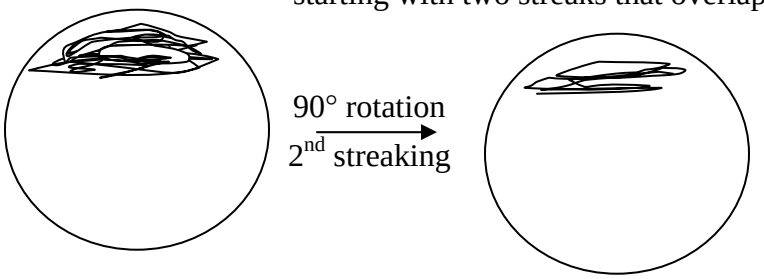
Procedure:

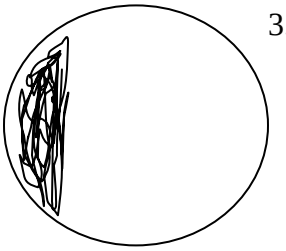
NOTE: You must wear gloves, goggles, and an apron at all times!

1. Hold the tube from which you will obtain the inoculum (bacterial sample) in one hand. You want to hold the tube at an angle so that nothing falls into the tube to contaminate it.
2. Remove the cap from the tube using your other hand.
3. Remove the sterile swab from its packaging. Do NOT let it touch anything, or it will no longer be sterile!
4. Submerge the cotton tip of the swab into the culture and then remove the swab from the tube. Again, do NOT touch anything except the culture with your swab!
5. Cap the tube and place it in the rack.
6. Remove the cover from your agar plate.
7. Perform a quadrant streak as shown below:

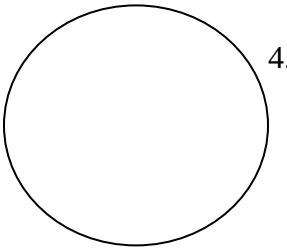
1. Start at one edge of the plate, moving the swab back and forth to streak about ¼ of the plate. Do not press too hard; you don't want to puncture that agar. Try to maximize the number of streaks you make by keeping your streaks close together.

2. Rotate the dish 90° and streak ½ of the remaining surface, starting with two streaks that overlap the first one.





3. Repeat for the third section. Remember – you do NOT want to overlap either previously inoculated section after the first two.



4. Repeat for the fourth section, completing the quadrant. This is the isolation streak.

Identifying Bacterial Strains Teacher Section

Procedure B: The Gram Stain

NOTE: You must wear gloves, goggles, and aprons at all times! The staining procedure must be performed over a sink.

1. Obtain your cooled microscope slide from your instructor.
2. Gently flood the sample with crystal violet by steadily dropping the stain over the sample. Once the sample is covered, let the stain sit for 1 minute.
3. Rinse off the excess stain with a gentle stream of distilled or deionized water. Be sure to hold the slide at an angle towards the sink.
4. Add Gram's iodine to the smear while it is still moist. Again, flood the smear and allow the iodine to sit for 1 minute.
5. Rinse off the excess iodine with a gentle stream of distilled or deionized water. Be sure to hold the slide at an angle towards the sink.
6. Decolorize the smear using 95% acetone alcohol. Gently flood the smear, while holding the slide at an angle towards the sink, until no more color comes off.
7. Rinse off the excess alcohol with a gentle stream of distilled or deionized water. Be sure to hold the slide at an angle towards the sink.
8. While still moist, flood the smear with safranin. Allow the safranin to sit for 30 seconds.
9. Rinse off the excess safranin with a gentle stream of distilled or deionized water. Be sure to hold the slide at an angle towards the sink.
10. Allow the slide to air dry.
11. Once the slide is dry, you can look at it under the microscope.
12. Identify whether your sample is Gram positive or Gram negative.

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Extensions

These extensions may be done at the discretion of individual teachers. They are designed to give students a more in-depth understanding of concepts introduced in this unit. They would best be used in higher level classes where students were able to do independent research.

- Research the spread of communicable diseases in the United States
- Research the antibody-antigen interaction
- Research disease characteristics caused by specific bacterial strains