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Personal Behavior and Partner's Sexual History: a Simulation of the Spread of HIV

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Abstract: In this hands-on simulation students evaluate factors associated with the spread of HIV. Each student begins the simulation with an "uninfected" solution. They sequentially mix their solution with a variable number of solutions representing potential mates, some of which contain an antigen used to represent HIV. Students evaluate each individual's final solution with a modified ELISA procedure to screen for the antigen simulating HIV. They then use these results to reconstruct the spread of "HIV" through the population. The results are evaluated in light of the impact of two risk factors: number of mates, and mates' sexual history.

Keywords: ELISA, HIV, AIDS, hands-on simulation, epidemiology

Contents:

Introduction

This simulation experiment aims to help students develop an understanding of the impacts of personal, as well as partners', sexual history on the probability of becoming infected with a sexually transmitted disease. It also introduces to the ELISA method for screening for antibodies made in response to infection, and it introduces students to reconstructing the spread of a disease through a population. We use this exercise in a freshman level non-majors biology course that surveys central biological concepts from an interdisciplinary perspective, emphasizing interactions between biology and society. It takes an experienced graduate student about four hours to set up this exercise; additionally, each instructor has 10 to 15 minutes of prep work to do before they begin teaching their section. The exercise takes approximately two hours with our students. A version of this exercise using a simpler method that does not include the ELISA process is described in Dickey (1989); the simulated ELISA used in our exercise is derived from a laboratory exercise posted on the University of Arizona website by Grimes *et al.* (available at http://www.blc.arizona.edu/aids/ as of October 2005). Having read hundreds of reports students have written after conducting this simulation, we feel that the biggest impact this simulation has on students is opening their eyes to the impact of their partner's sexual history on their risk of infection.

The first section of this chapter includes the description of the experiment that students are expected to read prior to coming to class, and then refer to as they work their way through the experiment in class.

The last section is the Instructor's Guide that we hand out to our Graduate Teaching Assistants and to the assistant who sets up the experiment prior to our laboratory meeting. In the laboratory meeting we discuss and run through the experiment with the instructors. In addition to some notes regarding teaching the experiment, the instructor's guide contains sample data, the lists of materials, equipment, and methods for setting up the experiment.

Student Outline

Diseases range from minor inconveniences that simply slow us down for a few days, to conditions that alter our way of life or even end our life. For centuries, people have attempted to understand disease in the hope that this understanding will lead to the ability to alleviate suffering and premature death. Scientific study of disease has led to a tremendous increase in understanding of factors that cause many diseases and factors that are associated with the spread of diseases. This understanding of the basis of disease has contributed to partial, but far from complete, prevention of disease.

Scientific study of disease relies on a variety of methods. The effectiveness of potential medicines and vaccines cannot be fully understood until clinical trials are conducted with carefully monitored human subjects. These clinical trials are typically preceded by extensive experiments with animal subjects. Some characteristics of the spread of disease can even be investigated by simulating the spread of disease in a model system. This is the approach that we will use in this laboratory experiment. We will use a substance in a solution to represent a disease and we will investigate how alternative behaviors influence the spread of this simulated disease. Notice that in simulation experiments, the actual experiment units (e.g. vials of salt and protein solutions) are being used to represent the behavior of the actual units of interest (e.g. vials of human bodily fluids).

There is tremendous variation between diseases in how, or if, they are spread from one individual to another, and in the nature of the factors or conditions that cause the disease. A few diseases are purely a result of inherited information passed from parents to offspring. Cancers, a second category of disease, are caused by damage to an individual's genetic information. This damage is caused by a wide variety of carcinogens, cancer-causing substances, such as tobacco, UV light, and certain viruses. Although carcinogens are environmental, rather than genetic, there is genetic variation among individuals which influences their ability to resist and repair damage to genetic information caused by environmental carcinogens.

A third important category of disease is infectious diseases. These diseases primarily are due to infection by pathogens. Pathogens are disease-causing substances (such as viruses or prions) and disease-causing organisms (such as certain bacteria, fungi, protozoans, and parasitic invertebrates). Infectious diseases are fundamentally environmental, rather than genetic. However, just as we saw with cancer, the line between environmental and genetic disease blurs because there is genetic variation among individuals in their ability to resist infectious diseases.

In this simulation experiment, you will investigate the transmission of an infectious disease that is spread through exchange of body fluids. Acquired Immune Deficiency Syndrome (AIDS) is caused by a virus, Human Immunodeficiency Virus (HIV), which is spread through direct exchange of bodily fluids: blood, semen, vaginal secretions, and mother's milk. Unlike the pathogens that cause many other diseases, including colds and flu viruses, HIV is *not* spread through contact with shared inanimate objects, such as phones, toilet seats, eating utensils, or other objects that are free of blood; nor can HIV be spread through tears, sweat, saliva, coughing, sneezing, or insect bites. The spread of HIV is limited to direct contact with the specific body fluids listed above. Currently in the U.S., this is typically due to using contaminated needles, homosexual and heterosexual intercourse, mother-to-fetus transmission, and mother-to-infant transmission through breast-feeding (Stine 1997).

While HIV does not spread as readily as many pathogens, it has spread throughout the world and as of 2001 is the 4th leading cause of death in the world and the 1st leading cause of death in Africa (CDC 2001). A December 2002 report, produced by the Joint United Nations Programme on HIV/AIDS and by the World Health Organization (UNAIDS/WHO 2002) estimated that 3.1 million people died from AIDS in 2002, and that approximately 42 million people were infected by the end of the year. Currently, there is no cure for AIDS or proven vaccine to protect against infection by HIV, although vaccine tests are underway. However, there are antiretroviral drugs that delay, or possibly prevent, the progression from infection with HIV to having a highly compromised immune system (AIDS); and although there is no proven vaccine, there are behaviors which greatly reduce the chance of transmission and drugs that reduce mother-to-fetus transmission. The combination of drugs to reduce progression from infection with HIV to AIDS and behaviors that reduce transmission of HIV have reduced the number of new infections per year in some countries, including the U.S. Although progress has been made, 5 million new infections occurred in 2002 (UNAIDS/WHO 2002).

Currently, our best tool available to reduce suffering associated with HIV/AIDS is education about the avoidance of high risk behaviors. This simulation experiment, derived from exercises described by Dickey (1989) and Grimes *et al*. (1998) aims to illustrate the spread of a pathogen, such as the virus which causes AIDS, through a population engaged in a high-risk behavior. This version of the simulation also illustrates the influence of the unknown sexual history of a sexual partner on an individual's risk of being exposed to HIV. Your risk of exposure to sexually transmitted diseases, in addition to your personal sexual history, is the sum of all the previous unprotected sexual experiences of

your partner and the experiences of their partners. This risk is greatly reduced, but not totally prevented, by the use of a latex condom with a spermicide.

The Simulation Experiment

The first stage of this experiment will simulate the spread of HIV through the exchange of bodily fluids during sexual contact. At the start of the experiment, each student will be given a tube containing a solution representing their uninfected bodily fluid. Additional solutions, representing potential sexual partners, will be set up in vials on a side table. Approximately 10% of these solutions will contain a protein that we will use as an indication of infection. In reality, none of these solutions contain any human bodily fluids: the "uninfected" solutions contain a buffered salt solution, and the "infected" solutions also contain bovine serum albumin, a protein isolated from blood of cattle. As in people and HIV, you will not be able to visually distinguish between infected and uninfected solutions. In this simulation, we will refer to the buffered solution that you will be given as your bodily fluid; we will refer to the vials of solutions on the side table as potential sexual partners.

Each student will exchange a portion of their bodily fluid with that of from 1 to 3 partners. Each student's risk behavior (1, 2, or 3 exchanges) will be randomly assigned. In addition to varying student's number of interactions, some students will happen to exchange fluids with sexual partners who have no prior sexual experience; other students will happen to exchange fluids with partners who have already engaged in a number of unprotected sexual encounters. Individuals will not know the history of each partner until we tabulate and analyze the sequence of exchanges at the end of the experiment. This simulation will allow the class to vary two factors (independent variables): 1) an individual's behavior, and 2) their partner's sexual history. The dependent variable will be the infection status of each class member's bodily fluids after the simulation. You will use the class results from the simulation to evaluate the following hypothesis.

> HYPOTHESIS The risk of infection by a sexually-transmitted pathogen increases as an individual has more unprotected sexual encounters, and if the individual's partners have had more unprotected sexual relationships.

After exchanging fluids you will use a modified ELISA process to measure the dependent variable and determine which individuals are infected. This process, with different sets of chemicals, is used in a number of applications from home-pregnancy tests to screens for HIV. It relies on the biological properties of antibodies and antigens. When an individual is infected by a pathogen containing surface molecules that the immune system can recognize as "non-self," something that would not normally be living in that particular individual, the immune system responds by producing antibodies. Antibodies are proteins which can bind to and disable the infecting organism or cells. The molecules on the surface of the infecting organism that the immune system recognizes as non-self, and responds to, are called antigens, antibody generating molecules.

Antibodies and antigens are specific; a particular antibody often will only bind to a particular type of antigen. It is this property of specificity, or complementarity, in the binding of antibodies and antigens which allows us to use them in ELISA tests to screen for specific substances or organisms. However, antibody-antigen interactions are not completely specific—occasional non-specific interactions during ELISA tests results in false positives. Two to three percent of people who are *not infected* with HIV will generate a false positive in the ELISA test, incorrectly suggesting they *are infected* (Minkoff and Baker 2001). The strength of the ELISA test is that it has high sensitivity; it seldom (less than 1%) generates

false negatives (Minkoff and Baker 2001). This means that if someone infected with HIV takes this test, it has a high probably of correctly recognizing that they are infected. Because of its high sensitivity (few false negatives) and low specificity (many false positives), and its relatively low cost, the ELISA test is best used as an initial screen. If the ELISA test generates a positive result, it should be followed by a more specific, and more expensive, Western Blot Assay. Both of these tests look for antibodies in an individual's blood serum (the non-cellular component of blood) that have been produced in response to HIV; even the combination of ELISA and Western Blot occasionally produces erroneous results that can devastate an individual's life. The proportion of positive results that correctly indicate HIV infection will be much higher when testing individuals with high-risk behaviors in populations with a high frequency of HIV infection. Other tests are being developed which directly identify the presence of the virus' genetic information.

First we will consider the ELISA test for HIV, and later in the Methods section, we will discuss the modified version of this test that you will use after the simulation. In the ELISA test for HIV, disrupted components of HIV are bound to the bottom of small wells in a microtiter plate. An individual's blood serum is then added to the wells and, if antibodies made in response to HIV are present, they will bind to the HIV at the bottom of the well; if these antibodies are not present, no binding will occur. After thoroughly rinsing out unbound serum, an antibody solution is added which binds to all human antibodies and so will bind to the plate if the human HIV antibodies are present. This second antibody is linked to an enzyme. After another series of rinses, a substrate molecule is added which will bind to the enzyme and change colors, if it is present; if the enzyme is not present, no color change occurs.

After simulating the exchange of body fluids during sex, and testing these body fluids with a modified version of the ELISA test, you will be ready to analyze the class results. Epidemiologists study the distribution of diseases in order to determine how they are transmitted in the hope that this information will provide keys to prevention. This approach has been central to identifying risk behaviors associated with the spread of HIV and in determining that AIDS is caused by HIV. As you study the pattern of infection identified by the modified ELISA test, and consider the sequence of simulated sexual contacts, you will attempt to determine the source of the original infection and how it spread through the population. You will also use your analysis to evaluate the hypothesis about risk factors associated with the spread of HIV.

Methods

The class will work in large groups (approximately 12 students in each group) during the simulation stage of the experiment. You will then divide into smaller groups to conduct the modified ELISA test, and finally, come back together into the large group to share your results.

Simulated Exchange of Bodily Fluids Associated with Sexual Contact

Your instructor has set out two sets of labeled tubes at a side table to represent potential sexual partners; one or two of these tubes in each set is infected with the protein that we will use to represent HIV. Each of you will be given a solution in a microcentrifuge tube which represents your initial uninfected body fluid. While you are waiting as other students exchange fluids, you will analyze a practice set of epidemiological results representing the spread of a disease. When the person before you is exchanging fluids, you will move to a table where you can practice using a micropipette. You will then move to the table to exchange fluids; and after exchanging fluids return to your group and resume work on the epidemiological data.

Your instructor will hand you a card indicating your group (A or B) and your turn in the simulation sequence (1-12, if there are 12 members in your group). Use a fine-tipped permanent marker to label the rough, less glossy, surface of your microfuge tube with your group letter and turn number. If each student does not exchange fluids in their group, at their designated turn, everyone's results will be erroneous and difficult to interpret in your lab reports. After studying the *Practice Pipetting*, and *Fluid Exchange* sections so that you will know what to do when it is your turn, work through the *Epidemiological Practice Data* section while you wait.

Practice Pipetting

Micropipettes, when used correctly, allow scientists to accurately measure small amounts of fluids. While the student before you is exchanging body fluids, use the following methods to practice using a micropipette.

1. Open the practice micro-centrifuge tube (not your sample!) that contains food coloring, and open an empty tube. Set the tubes back in the block.
2. Open the box holding pipet tips, and without touching the tips, firmly press the pipet into a tip and remove it from the box. Close the tip box. *Do not let the end of the pipet tip touch anything as this might lead to contamination of your sample*.
3. Holding the pipet tip above the sample of food coloring, depress the push button down to the first stop and hold it in this position as you lower the pipet into the solution for 2 to 3 seconds; release the push button slowly to its initial position and give the solution a few seconds to be drawn up into the pipet tip.
4. Remove the tip from the solution and continue to hold the pipet in a vertical position as you transfer the solution into the empty micro-centrifuge tube. Place the tip close to the inner wall of the empty tube. Press the push button to the first stop. Then press the push button continuously to the second stop. Still holding the push button all the way down, wipe the tip against the inner wall of the tube to remove any drops clinging to the end of the tip.
5. Remove the tip from the tube and then release the push button. Hold the pipet over the garbage container and push the tip ejector button.

Fluid Exchange

When the student in front of you, from your group, is done move to the fluid exchange table.

1. Check in by setting your card in the sequence box. Make sure the card under yours is for your group, and is the number immediately before yours.
2. Print your name on the Exchange Record at the station behind the screen so that we can keep track of the sequence of exchanges for your eventual epidemiological analysis.
3. Randomly select a note from the assignment box which will indicate the number of exchanges you will conduct (1, 2, or 3). Set the note in the Used Notes box.
4. Select a partner (tube of fluid) and record their name (number code) in Table 1, and on the exchange record.

Table 1. Record of Exchanges.

Your Name	Identify of Partners		
	First	Second	Third

5. Open your microfuge tube and set it in a block. Place a clean tip on a pipet and use the pipet to remove half the solution from your tube; while still holding the pipet with half of your solution in one hand, use your other hand to use a second pipet to remove half the solution from its partner tube. Dispense your solution from the pipet into the partner tube, and the partner's solution into your tube.

6. Repeat steps 4 and 5 as many times as indicated on your assignment card.

Epidemiological Practice Data

Our goal is to use the data summarized in Table 2, which includes the results of the ELISA tests, and the sexual history of the group, in order to track the path of the epidemic. This data will allow you to uncover some, but not all steps in the spread of the infection.

1. Look at the negative results to make a list of exchange partners whom you know were not initially infected.
2. Now consider the ELISA results of student A3. What does this tell you about one source of the epidemic? Draw a circle around one source of the infection, and squares around partners subsequently infected from this source.
3. How many partners did Student A1 and Student A10 individually have sex with and what was the identity of these partner(s)?

   Individual Behavior:
   A1:
   A10:

4. Exposure to sexually transmitted diseases is a result of the individual's sexual history, as well as the history of all their partners, and their partner's partners. Compare the exposure to STDs of Student A1 in contrast to A10. List the number of individuals that each has been directly and indirectly exposed to.

   Exposure Risk:
   A1:
   A10:

Table 2. Epidemiological Practice Data

Student ID	Exchange Partners				ELISA Result
	1st Partner	2nd Partner	3rd Partner	4th Partner	
A1	3				Neg.
A2	6	4	1		Neg.
A3	7	3			Pos.
A4	4	5	3		Pos.
A5	10	7	4	5	Pos.
A6	2				Neg.
A7	7	2			Pos.
A8	8	9			Neg.
A9	10	2	5	7	Pos.
A10	3				Pos.
A11	9	4			Pos.
A12	4	6			Pos.

Modified ELISA Assay

After everyone in your large simulation group has exchanged fluids with the sexual partners at the station, your instructor will assign you to a smaller group which will use a modified ELISA protocol, developed by Grimes et al. (1998), to determine the infection status of your bodily fluids.

In this modified ELISA protocol, you will add your fluids to wells in a microtiter plate. If the fluid is infected (contains the protein that we are using to represent HIV), the protein will adhere to the sides of the well. After rinsing, you will add a molecule which binds to the protein, if it is present. This molecule is linked to an enzyme which will turn from clear to blue after the addition of a substrate. Your group will run the test on a positive control, a solution known to contain the antigen that should produce a positive result if the methods have worked correctly; on a negative control, a solution known not to contain the antigen that should produce a negative result if the methods have worked correctly; and on each student's bodily fluid of unknown infection status.

1. Your group will add the negative control to 2 wells in the top row of the microtiter plate; 2 wells in the second row will contain the positive control; and each student will add their bodily fluid to a set of 2 wells. Record the locations of the sets of 2 wells that each student in your group will use; do not assume that you will remember.
2. Add 4 drops of the negative control solution into each of the 2 wells in the first row. Use a clean pipet to add 4 drops of the positive solution into each of 2 wells in the second row. Then each student in your group will use a clean pipet to add 4 drops of their bodily solution into their 2 designated wells.
3. After everyone has added their fluids to their wells, let the wells sit and incubate for 5 minutes.
4. Slap the plate, face down, on a paper towel to quickly remove the fluid without cross-contamination between wells.
5. Immerse the plate in a washing solution at an angle so that air pockets do not get trapped in the wells and prevent the solution from rinsing the wells. Dump out the excess washing fluid and rinse the plate again, for a total of 3 rinses. Remove the last of the washing solution out of the wells by tapping the plate, face down, on a paper towel.

6. Add 4 drops of the antibody solution to each well. Let it sit in the wells and incubate for 5 minutes.
7. Rinse the plate, as in step 5.
8. Add 4 drops of TMB, the colorizing substrate; let it sit for 5 minutes.
9. Record the infection status of each solution (far right row of Table 3).

Results

In order to conduct your epidemiological analysis—to determine which tube at the sexual partner station was originally infected, to determine the path of the infection as it spread to other individuals, and to evaluate the original hypothesis—you will need to consider the sequence of exchanges, and the results of your entire simulation group. Your instructor has drawn a copy of Table 4-3 on the board and recorded the sequence of names from the Exchange Record. In the correct row of the table on the board, record the sequence of your exchange partners and whether your ELISA results were positive or negative for infection. Once the table on the board is complete, copy the results into the blank table below.

Table 3. Record of Simulation Sequence and Results.

Sequence / Names Group A	Potential Sexual Partners												Infection
	1	2	3	4	5	6	7	8	9	10	11	12	+ or -
A1													
A2													
A3													
A4													
A5													
A6													
A7													
A8													
A9													
A10													
A11													
A12													

Sequence / Names Group B	Potential Sexual Partners												Infection
	1	2	3	4	5	6	7	8	9	10	11	12	+ or -
B1													
B2													
B3													
B4													
B5													
B6													
B7													
B8													
B9													
B10													
B11													
B12													

Discussion Questions

The first set of questions is designed to help you determine the results predicted by the hypothesis. Remember, you are evaluating the following hypothesis.

> HYPOTHESIS The risk of infection by a sexually-transmitted pathogen increases as an individual has more unprotected sexual encounters, and if the individual's partners have had more unprotected sexual relationships.

Predicted Results

The first step in determining the predicted results of the hypothesis is to determine its predictions relative to individual's personal behavior. Secondly, we will consider predictions associated with their partners' behaviors.

1. Notice that Table 3 clearly shows each individual's level of risk, on a scale of one to four, based on their individual behavior.
2. Assign each individual in your simulation group to a risk level represented by the average number of prior sexual encounters of their partners and all prior partners linked to their partners.

Discussion of the Conclusion

3. Evaluate the hypothesis by comparing the results predicted by the hypothesis to your actual observed results.

Discussion of the Implications

4. What have you learned from this simulation? What factors not included in the simulation experiment could influence an individual's level of risk? If you were teaching a group of high school students about HIV and AIDS, what are the most important points that you would like to make?

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Instructor's Guide

Concepts

Before you orient students to types of diseases, stop and consider some of the difficulties that we run into as we try to categorize diseases as genetic, cancerous, or infectious. The interactions between environmental and genetic effects are complex and varied. Most environmental (infectious) diseases have a genetic contribution to resistance; genetic diseases often are also influenced by the individual's general health and environment; and cancers result from environmental and genetic effects on genetic material. While students often struggle with conditional reasoning, the importance of these relationships motivates students to work through their complexity.

While diseases can be used to provide us with compelling and thought-provoking examples of a wide variety of biological principles, remember that in some respects, these are not hypothetical examples. Diseases commonly mentioned to illustrate biological concepts are the same diseases that have affected students themselves, their family members, or their friends. As you discuss these diseases in the classroom, be sensitive to the feelings of these students. Also remind students that you are not a medical doctor and are not qualified to answer some of their questions. In spite of the challenges associated with using the study of disease to teach biological principles, students are engaged in these topics and this topic compels students (and instructors) to apply and synthesize concepts from genetics, cell biology, physiology, ecology, and evolution.

In this experiment, students start the simulation with uninfected fluid. Students will be randomly assigned to sequentially exchange fluids with from 1 to 4 partner tubes (some of which are infected). In addition to varying the number of partner tubes, the history of these partner tubes varies through the simulation. After simulating the spread of a sexually-transmitted disease, the epidemiological analyses will reconstruct the spread of the infection. Individuals' infection status will also be compared to their number of sexual partners as well as their partners' history. Simulating the effects of high-risk behaviors has an impact on individuals' understanding of these relationships that is much greater than that derived from simply discussing or reading about these relationships (see student responses reported in Grines et al. 1998). It is important for students to consider the strengths and weaknesses of different types of scientific investigations. In this experiment, students are introduced to the value, and the limitations, of simulations. Exposure to different types of scientific investigations will help them interpret the results of such studies as they are reported in the secondary literature, and it also will allow them to make more informed contributions to debates about the ethics of different types of investigations. This exercise will also introduce your students to the ELISA (Enzyme-Linked ImmonoSorbant Assay) process. These assays are used in a variety of applications from home pregnancy tests to screens for HIV.

Methods

Each individual in the laboratory class will start the simulation as an uninfected individual, which means that their microcentrifuge tube will contain a buffer solution without the antigen. Full laboratory sections will run the simulation in two large groups with 12 students in each. In addition to each individual student having their own solution, each large group will exchange portions of their solutions with a set of 10 solutions, representing sexual partners of unknown infection status. Two of these partner tubes, in addition to containing buffer, will contain an antigen which will simulate an infection. If you have smaller groups, less than 8 students, start with only one infected individual. Ideally, the disease begins to spread through the population midway through the simulation. However, this is a

stochastic process influenced, but not solely determined, by the number of initially infected tubes (1 or 2) and the number of exchanges of individual students (1 to 3). If you are monitoring the exchanges and notice that no one has happened to pick the infected tube(s) half way through the simulation, you can stack the exchange rate cards with a higher number of threes to increase the chance of spread. Once the disease begins to spread, it tends to move rapidly through the population. Throughout the simulation, as students exchange fluids with these partner tubes, the infection (antigen) will spread through the population.

After the simulation, students will work in smaller groups as they use the modified ELISA process to determine the infection status of each individual's tube. Students will be able to track the path of the infection by considering the record of each exchange in light of the final infection status of each individual's tube. For example, if a student named Mathew exchanged fluids with Tubes C, F, and G, and if Mathew had a negative ELISA result (non-infected), then we can deduce that tubes C, F, and G began the simulation as uninfected potential partners and that every individual who exchanged fluids with C, F, and G prior to Mathew was, at that time, also uninfected. This type of epidemiological reasoning will allow students, after they have combined the large group's results, to develop a map of the path of the infection. Lastly, students will compare final infection status with the number of exchanges and with the prior number of exchanges of each partner.

Table 4 and Figure 1 provide sample data from a disease simulation experiment. Ten students exchanged "bodily fluids" with one, two, or three partners. One of the partners (A through H) was infected from the start and they are referred to as the original carrier. The students, partners, and the order in which the exchanges occurred are shown in the table. The last column of the table indicates two wells of the ELISA plate in which the students "bodily fluids" were tested for the infection.

Table 4. Sample Data for Practice Simulation

	Exchange Partners			
Student	1st Partner	2nd Partner	3rd Partner	Wells of ELISA Plate
1	F	E		E1, E2
2	C	G		E4, E5
3	B	D		E6, E7
4	A			E8, E9
5	D	A	B	F6, F7
6	C	B		F11, F12
7	A	C	E	G1, G2
8	G	E		G6, G7
9	H			G8, G9
10	C	F		H6, H7

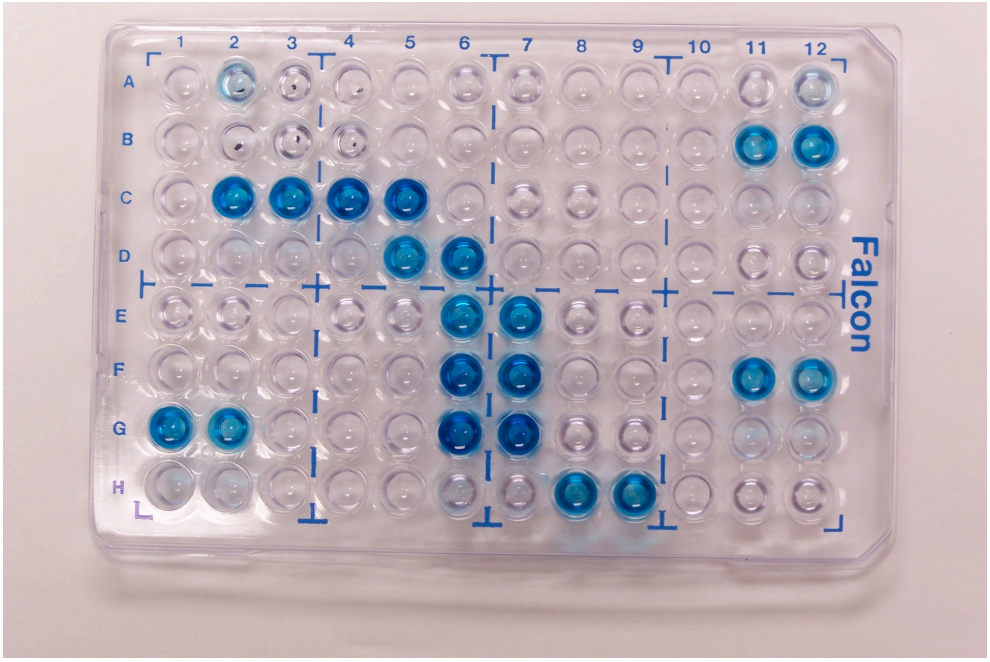

Figure 1. Example ELISA Plate. Wells H8, H9 are positive controls, and wells H11, H12 are negative controls.

Prep Done by Lab Instructors Prior to Class: Preparing the individual and partner body fluids

Dilute 10 ml of the 10X sodium carbonate buffer solution with 90 ml of water. Pipet 1 ml of this buffer into a microcentrifuge tube for each student and prepare a 2 sets of 10 uninfected partner tubes. This solution will also be used as the negative control.

Pipet 1 ml of sodium carbonate buffer with biotinylate albumin (this stock solution should be kept in the refrigerator) into each of the infected partner tubes (we will probably use 2 per large group).

Prep Methods for the Laboratory Assistant

Each lab section will need enough buffer for 24 individual's tubes and for 16 partner tubes (2 of which will contain the antigen). Each tube will contain 1 ml of a carbonate buffer; infected tubes will also contain biotinylated albumin (the antigen).

Sodium Carbonate Buffer (uninfected body fluid)

- Add 3.2g Na_2CO_3 and 5.86g $NaHCO_3$ to 200 ml of water to prepare a 10X solution of the buffer.

Biotinylated albumin solution (infected body fluid)

Each section will need 10 microliters of biotinylated albumin diluted to 10 ml using 1X sodium carbonate buffer. Each infected partner tube (1 or 2 per large group) will contain this solution. The remaining 6 ml can be diluted up to 10 ml and used as the positive controls for each section.

- PBS with Tween 20 (washing solution for ELISA after antigen addition and after simulated antibody addition)

- Stock Solution (20X PBS with 2% Tween 20): Add 320g NaCL, 8g KCl, 44.8g Na_2HPO_4, and 8g KH2PO4 to 2L water. Add 20 ml Tween 20.
- Working Solution (1X PBS with 0.1% Tween 20): For each class, dilute 50 ml of stock solution with 1 Liter of water.
  This buffer is stable at room temperature.

Streptavidin Peroxidase (enzyme-simulated antibody solution for ELISA)

- Stock Solution: Add 1 ml of 50% glycerol solution to 0.5mg of streptavidin peroxidase in the purchased vial. This concentrate, stock solution, is stable in the refrigerator for years.
- Working Solution: One micro liter of the stock solution added to 10 ml of 1X PBS/1.0% albumin will simulate the antibody in the experiment. This diluted solution can be stored in the refrigerator for up to 1 week.

Color reagent (TMB) in 0.05M Citric Phosphate Solution

- Stock 0.05M Citric Phosphate Solution: Add 25.7 ml of 0.2M dibasic sodium phosphate and 24.3 ml of 0.1M citric acid to 50 ml of de-ionized water. Adjust pH to 5.0, if necessary.
- Working solution: This solution needs to be made the day it will be used and stored in the refrigerator protected from the light. For each section, add 2 mg TMB to 20 ml of the citric phosphate solution. Then add 4 micro liters of 3% hydrogen peroxide.

Materials

Equipment

- micro-pipets (for prep)
- pH meter (for prep)
- barrier screens
- microcentrifuge tube blocks

Supplies

- microcentrifuge tubes
- fine-tipped permanent markers
- transfer pipets
- used solutions disposal beakers
- ELISA wash beakers
- micro-titer plates
- exchange record form
- exchange assignment box / random cards
- $Na_2 CO_3$ Sodium carbonate (Sigma S1641)
- Na H CO_3 sodium bicarbonate (Sigma S6014)
- biotinylated albumin (Sigma A8549)
- Bovine albumin (Sigma A4503)
- NaCl sodium chloride
- KCl potassium chloride
- Na_2 HPO_4 Disodium Phosphate (sodium phosphate dibasic) (Sigma S9390)
- KH_2 PO_4 potassium phosphate (Sigma P0662)
- tween 20 (Sigma P1379)
- glycerol (Sigma 7893)
- streptavidin peroxidase (KPL 14-30-00)
- TMB tetramethylbenzidine (Sigma T8768)
- citric acid (Sigma C7129)
- hydrogen peroxide (3%) (Grocery Store)

About the Authors

Ralph Preszler earned his B.S. at Southern Oregon State College (now Southern Oregon University) where he was given the opportunity to work as an undergraduate teaching assistant in a botany laboratory. While he earned his M.S. and Ph.D. at Northern Arizona University he taught and coordinated botany laboratories, and worked as a lecturer. In his graduate and postgraduate research, he investigated interactions between plants, the endophygous fungi that live in their leaves, and the herbivores attempting to eat the leaves. He worked at New Mexico State University for a number of years as coordinator of lower-division laboratory courses. He is currently an assistant professor of biology education in the New Mexico State University Department of Biology.

Amy Marion earned her B.S. at Marywood College (now Marywood University) where she conducted her senior thesis on the transformation of *Bacillus*. At University of Vermont, she earned her Ph.D. conducting research in fungal molecular genetics. After moving to New Mexico, Dr. Marion taught at the Albuquerque Technical Vocational Institute and at the University of New Mexico. She now works in the New Mexico State University Department of Biology as Laboratory Coordinator and Director the Biology Advising Center.