

## **Using a Modified ELISA Assay to Demonstrate the Transmission of a Simulated Disease and the Subsequent Identification of the Bacterial Agent**

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### Abstract:

Students simulate disease transmission by sharing “body fluids”, then use an ELISA assay to determine the rate of transmission of the simulated disease. Through the ELISA assay, students determine if they are carriers of the disease. If students are carriers for the disease, then they swab, grow, and identify the type(s) of bacteria that caused the disease using microbiology techniques. *Note: All fluids are simulated! Real body fluids are not shared. Specific inoculations will be made for “carriers”.*

Prior to the above simulation, a brief mini-unit will be taught using a PowerPoint presentation. The purpose of this will be to demonstrate lab safety techniques, use of lab equipment (ELISA, microbiology techniques), and general safety precautions necessary to perform the simulation.

Objectives: The objectives for this unit are multifaceted. Through research, laboratory exercises, and presentations, students will apply their knowledge to real life epidemiology study.

- To demonstrate proper lab safety precautions
- To illustrate how disease spreads through a population/rate of transmission
- To research the spread of communicable diseases in the United States
- To learn how an ELISA is performed and its applications (forensics, research, and diagnostic tests)
- To understand the antibody-antigen interaction
- To learn a sterile technique to plate and identify bacteria
- To learn disease characteristics caused by specific bacterial strains
- To identify various strains of bacteria in a sample

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- I. Overview
  - a. Concepts covered in unit
    - i. Lab safety
    - ii. Scientific method
    - iii. Disease transmission
    - iv. Antibody-antigen interaction
    - v. How an ELISA (*Enzyme Linked-Immuno-Sorbent Assay*) is performed and applications of the ELISA test
    - vi. Significance of a sterile technique
    - vii. How to plate and identify bacterial strains
  - b. General goals of lab exercises
    - i. To teach importance of lab safety and use of sterile technique
    - ii. To successfully perform an ELISA and analyze results
    - iii. How to culture bacteria using sterile technique
    - iv. How to identify various bacterial strains
  - c. Recommended placement for the unit within a biology course
    - i. Placement will vary depending on school district curricula
    - ii. Possible placements include, but are not limited to:
      - 1. Classification of living things, specifically Kingdom Eubacteria (Monera)
      - 2. Human anatomy, physiology, and health
      - 3. Microbiology/Immunology
  - d. Technical skills acquired by students
    - i. Pipetting into microwells
    - ii. Making and pouring of agar plates
    - iii. Isolating bacterial strains using sterile technique
    - iv. Plating bacterial strains using sterile technique
    - v. Identification of bacterial strains
  - e. Relevance to students' lives
    - i. Knowledge of disease transmission
      - 1. How quickly disease can spread under everyday conditions
      - 2. How easy it can be to contract a disease caused by bacteria on a daily basis
    - ii. Knowledge of diagnostic procedures
  - f. Relation to other scientific concepts
    - i. Classification and use of a dichotomous key
    - ii. Human anatomy and physiology
    - iii. Human health
    - iv. Microbiology
    - v. Immunology
- II. Science Background
  - a. Elisa
  - b. Agar/ID

- III. Student Outcomes
  - a. General Description
    - i. Elisa
    - ii. agar/ID
  - b. Student outcomes
    - i. To demonstrate proper lab safety precautions
    - ii. To illustrate how disease spreads through a population/rate of transmission
    - iii. To research the spread of communicable diseases in the United States
    - iv. To learn how an ELISA is performed and its applications (forensics, research, and diagnostic tests)
    - v. To understand the antibody-antigen interaction
    - vi. To learn a sterile technique to plate and identify bacteria
    - vii. To learn disease characteristics caused by specific bacterial strains
    - viii. To identify various strains of bacteria in a sample
  - c. Relationship between technical procedures and science concepts
    - i. All procedures are performed to gain a better understanding of the above concepts
    - ii. Technical procedures are a practical application of said concepts
- IV. Learning Objectives
  - a. Qualitative and quantitative learning objectives
    - i. To demonstrate proper lab safety precautions; qualitative and quantitative
    - ii. To illustrate how disease spreads through a population/rate of transmission; qualitative and quantitative
    - iii. To research the spread of communicable diseases in the United States; quantitative (if extension is completed)
    - iv. To learn how an ELISA is performed and its applications (forensics, research, and diagnostic tests); qualitative
    - v. To understand the antibody-antigen interaction in the ELISA; qualitative and quantitative
    - vi. To learn a sterile technique to plate and identify bacteria; qualitative
    - vii. To learn disease characteristics caused by specific bacterial strains; qualitative (if extension is completed)
    - viii. To identify various strains of bacteria in a sample; qualitative and quantitative
  - b. Demonstration of student knowledge; Students will:
    - i. Safely follow various lab protocols
    - ii. Determine the rate of disease transmission
    - iii. Perform and apply ELISA
    - iv. Model antibody-antigen interactions
    - v. Perform a bacterial culture using sterile technique
    - vi. Identify various bacterial strains
- V. Time requirements
  - a. Single class periods needed for general instruction
  - b. Double class periods needed for exchange of fluids and performance of ELISA, preparing and plating of bacteria, and identification of bacteria

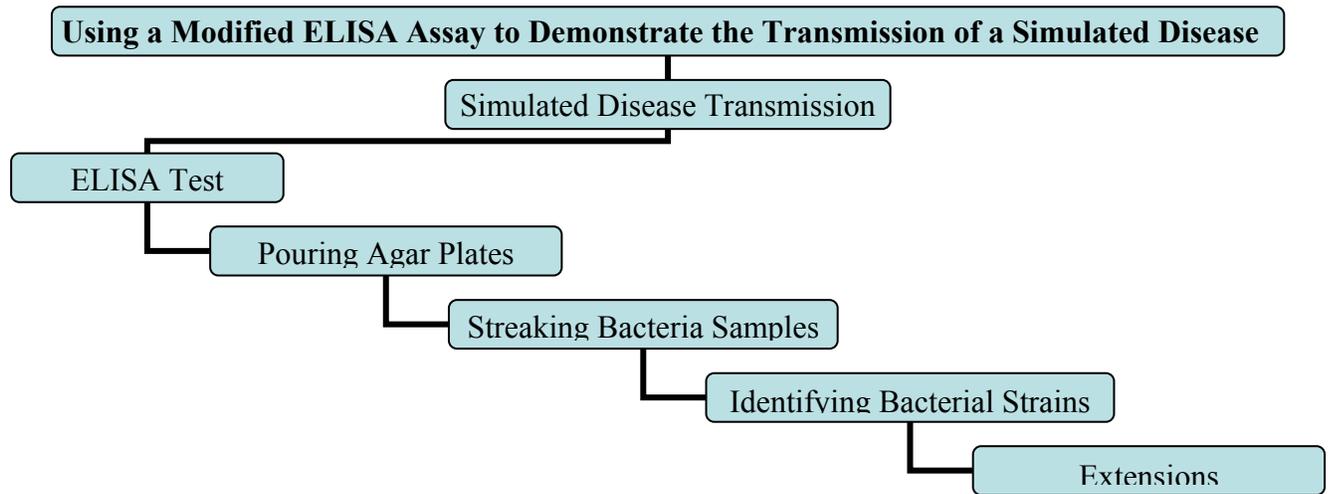
- VI. Advance preparation
  - a. Simulated Disease Transmission see “Teacher Section”
  - b. ELISA test see “Teacher Section”
  - c. Pouring Agar Plates see “Teacher Section”
  - d. Streaking Bacterial Samples see “Teacher Section”
  - e. Identifying Bacterial Strains see “Teacher Section”
- VII. Materials and Equipment
  - a. Simulated Disease Transmission see “Teacher Section”
  - b. ELISA test see “Teacher Section”
  - c. Pouring Agar Plates see “Teacher Section”
  - d. Streaking Bacterial Samples see “Teacher Section”
  - e. Identifying Bacterial Strains see “Teacher Section”
- 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?

- iii. How can you apply this knowledge to prevent the spread of disease?
- XII. Assessment
- a. Assessment of laboratory reports and presentations vary due to individual teacher's formats
  - b. Sample rubric for a laboratory report:
  - c. Sample rubric for a presentation:

## STUDENT SECTION:

- I. Rationale
  - a. The purpose of this unit is to familiarize students with the cause, diagnosis, and spread of disease in a community.
  - b. Introduction
    - i. Students will simulate disease transmission by sharing “body fluids”, then use an ELISA (*Enzyme Linked-Immuno-Sorbent Assay*) to determine the rate of transmission of the simulated disease. Through the ELISA assay, students determine if they are carriers of the disease. If students are carriers for the disease, then they swab, grow, and identify the type(s) of bacteria that caused the disease using microbiology techniques. *Note: All fluids are simulated! Real body fluids are not shared. Specific inoculations will be made for “carriers*
    - ii. Overview of appropriate science background
      1. How to use a dichotomous key
      2. Characteristics of microorganisms
      3. How to follow lab protocol
    - iii. Procedures
      1. ELISA(*Enzyme Linked-Immuno-Sorbent Assay*)
      2. Isolation bacterial strains
      3. Pouring agar plates
      4. Plating bacterial strains
      5. Identifying bacterial strains
    - iv. Explanation of Equipment Use
      1. How to use a micropipette
      2. Streaking and isolation of bacteria
- II. Materials
  - i. Per group (Please refer to separate lab protocols for list of materials for class- materials per group to be determined after lab is taught)
  - b. Safety Equipment
    - i. Safety goggles
    - ii. Lab aprons
    - iii. Emergency eye wash station
- III. Procedure (Please refer to separate lab protocols for each procedure)
- IV. Data Collection (Please refer to “Data Collection” section within each lab protocol)
- V. Discussion/Analysis (Please refer to “Analysis and Conclusion” section within each lab protocol)

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](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<sub>2</sub>CO<sub>3</sub> Buffer Solution
- Positive Antigen Solution
- Antibody Solution
- Color Reagent Solution

**Setting up the classroom for this lab:**

Shared Materials:

Permanent Marker

Micro centrifuge racks (there must be enough to hold two tubes per student)

Materials per Student:

1 empty micro centrifuge tube

1 micro centrifuge tube containing 1 ml simulated body fluid

1X Na<sub>2</sub>CO<sub>3</sub> Negative

Positive antigen solution

1 transfer pipette (plastic disposable)

**During the lab:**

Materials per Group of Four Students: (after all three exchange of fluid takes place)

- ELISA plate (a quarter section of a full ELISA tray)
- positive control solution (in dropper bottle or with clean transfer pipette)
- negative control solution (in dropper bottle or with clean transfer pipette)
- Paper towels to dry ELISA tray after washing ELISA tray
- Antibody solution (in dropper bottle or with clean transfer pipette or with clean transfer pipette)
- Washing solution (~ 100 ml in wash bottle)
- TMB Color reagent solution (in dropper bottle or with clean transfer pipette)

Note: it is important to dispose of all transfer pipettes immediately after use to decrease the change of contamination. During the exchange of the simulated body fluids is the only procedure in which the transfer pipettes can be reused!

## Preparation of Materials:

### Bacteria to identify for positive tests:

1. Culture in nutrient broth any Gram negative or Gram positive bacteria that are available per the instructions of the specific bacteria. A good choice would be a Gram positive and a Gram negative bacterium that is relatively safe to work with.
2. Add 1ml of bacteria broth culture to student tubes that tested positive for the pathogen upon completion of the ELISA test so they can identify the strain of bacteria that caused the simulated disease.

### Washing Solution: 1X PBS with 0.1% Tween 20

1. Dissolve 160 g NaCl, 4g KCl, 22.4 g Na<sub>2</sub>HPO<sub>4</sub>, and 4g KH<sub>2</sub>PO<sub>4</sub> in deionized or distilled water to give final volume of 1000 ml buffer. This is 20X PBS.
2. Dilute 50 ml of 20X PBS to 950 mL with deionized or distilled water for each class. Add 10 ml 10% Tween 20 to buffer. This buffer (1X PBS, 0.1% Tween 20) can be stored indefinitely at room temperature.

This buffer is used to wash plates after antigen addition and after antibody addition.

### Sodium Carbonate: 1X Na<sub>2</sub>CO<sub>3</sub> Buffer Solution

1. Dissolve 3.2g Na<sub>2</sub>CO<sub>3</sub> and 5.86 g NaHCO<sub>3</sub> in deionized or distilled water to final volume of 200 mL. This is a 10X Na<sub>2</sub>CO<sub>3</sub> concentrate. This solution can be stored indefinitely in the refrigerator.
2. To make a 1X Na<sub>2</sub>CO<sub>3</sub> buffer solution for classroom use, add 20 ml 10X concentrate to 180 ml deionized or distilled water. This solution can be stored indefinitely in the refrigerator.
3. Give 1 ml of the 1X Na<sub>2</sub>CO<sub>3</sub> buffer solution to each student as simulated body fluid. Distribute 1 ml aliquots in micro centrifuge tubes as a negative control for the ELISA test.

### **Positive Antigen Solution (biotinylated albumin)**

1. Dissolve 10 mg biotinylated bovine albumin in 20 mL 1X Na<sub>2</sub>CO<sub>3</sub> 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<sub>2</sub>CO<sub>3</sub> 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<sub>2</sub>HPO<sub>4</sub> 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	Price
Biotinylated albumin	Sigma	Co. A8549	10 mg	
TMB (3, 3', 5, 5'-Tetramethylbenzidine) tablets	Sigma	T3405	50 tablets	
TMB (3, 3', 5, 5'-Tetramethylbenzidine) powder	Sigma	T8767	1 g	
Streptavidin peroxidase	Kirkegaard and Perry	14-30-00	0.5 mg	
Bovine albumin	Sigma	A4503	??????	
Tween 20	Sigma	P1379	100 mL	
Flexible assay plates-U bottom	VWR	62406-220	50 per pack (lids optional)	
Fine tip transfer pipets, Samco	VWR	14670-330	5 ml	
Microfuge tubes, 1.7 ml	VWR	20170-331	500 per pack	
Sodium chloride	Sigma	S9888	500 g	
Potassium chloride	Sigma	P3911	500 g	
Disodium phosphate	Sigma	S9390	100 g	
Potassium phosphate	Sigma	P0662	500 g	
Dibasic sodium phosphate	Sigma	S0876	100 g	
Citric acid	Sigma	C7129	100 g	
Sodium carbonate	Sigma	S1641	500 g	
Sodium bicarbonate	Sigma	S6014	500 g	
Glycerol	Sigma	G7893	500 mL	
Hydrogen peroxide (30%)	Sigma	H1009	100 mL	
Any Gram Positive and Gram Negative	Any supply catalogue			

## 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. 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)

### Materials per Student:

1 empty micro centrifuge tube

1 micro centrifuge tube containing simulated body fluid (1ml)

1 transfer pipette (plastic disposable)

Most simulated body fluids will initially be negative for the pathogen. One will be positive (for classes of less than 20 students, for each increment of 10 students add an additional positive tube initially. This allows one student out of 20 to have a tube that contains the pathogen before sharing occurs. (Choose an outgoing student for the initial positive tube this will ensure it gets shared widely- DO NOT allow students to know who has the positive solution!).

Solution in the positive tubes will be positive antigen solution (see pre-lab preparation)

Solution in the negative tubes will be 1X  $\text{Na}_2\text{CO}_3$  (see pre-lab preparation)

Please see preparation for instructions on solution preparation.

Simulated Disease Transmission Protocol Teacher Section
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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 or 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 the simulated body fluids (#6-8).
12. Record the name of your second contact in the table provided. (wait until all students have had ample time to share fluid and record their exchange partner)
13. When your instructor gives the direction to complete the third exchange, move about the room and find a third person to exchange the simulated body fluid with.
14. Complete the procedure for sharing simulated body fluids (#6-8).
15. Record the name of your third contact in the table provided.
16. Place your tube in the rack provided to complete the simulated EILSA on to determine the # of infected individuals from the activity.

Solutions should be stored in the refrigerator for later use in the ELISA for up to one week.

*Note: there is an alternative available to show the spread of diseases if the teacher elects not to do the ELISA due to limited time or funds.*

## Record of Sample Sharing

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

## 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, phenolphthalein if positive)

1 transfer pipette (plastic disposable)

Name of Product	Amount Needed Per Class	ID# from Carolina Supply Company	Price

Most simulated body fluids will initially be negative for the pathogen. One will be positive (for classes of less than 20 students, for each increment of 10 students add an additional positive tube initially). Choose an outgoing student for the initial positive tube this will ensure it gets shared widely).

Simulated Disease Transmission Protocol Teacher Section
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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 or 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 the simulated body fluids (#6-8).
12. Record the name of your second contact in the table provided. (wait until all students have had ample time to share fluid and record their exchange partner)
13. When your instructor gives the direction to complete the third exchange, move about the room and find a third person to exchange the simulated body fluid with.
14. Complete the procedure for sharing simulated body fluids (#6-8).
15. Record the name of your third contact in the table provided.
16. To determine if you are infected, use a clean transfer pipette to add 10 drops of 4% NaOH to your S micro centrifuge tube. If it turns pink, you have been infected. If not, you have not been infected.
17. To determine if you were the original infected carrier, obtain your NS micro centrifuge tube and add ten drops of 4% NaOH to it. If it turns pink, you were an originally infected individual if not, you were not infected originally.

## 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
<b>S</b>			
<b>NS</b>			

## 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. The way in which a communicable disease is spread can be represented by sharing simulated body fluids.

### Shared Materials:

Permanent Marker

Micro centrifuge racks

### Materials per Student:

1 empty micro centrifuge tube

1 micro centrifuge tube containing simulated body fluid

1 transfer pipette (plastic disposable)

### 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.

*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.
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 or your partners dependant upon which tube is empty).
9. Record the name of the student that you made your first exchange with in the record of sample sharing below.
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 the simulated body fluids (#6-8).
12. Record the name of your second contact in the record of sample sharing below.
13. When your instructor gives the direction to complete the third exchange, move about the room and find a third person to exchange the simulated body fluid with.

Protocol for Simulated Disease Transmission Student Section

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	

## 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, phenolphthalein if positive)

1 transfer pipette (plastic disposable)

### 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 or 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 the simulated body fluids (#6-8).
12. Record the name of your second contact in the table provided. (wait until all students have had ample time to share fluid and record their exchange partner)
13. When your instructor gives the direction to complete the third exchange, move about the room and find a third person to exchange the simulated body fluid with.
14. Complete the procedure for sharing simulated body fluids (#6-8).
15. Record the name of your third contact in the table provided.
16. To determine if you are infected, use a clean transfer pipette to add 10 drops of 4% NaOH to your S micro centrifuge tube. If it turns pink, you have been infected. If not, you have not been infected. Record the color of your sample in the S tube in the observation table below.
17. To determine if you were the original infected carrier, obtain your NS micro centrifuge tube and add ten drops of 4% NaOH to it. If it turns pink, you were an originally infected individual if not, you were not infected originally. Record the color of your sample in the NS tube in the observation table below.

## 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
<b>S</b>			
<b>NS</b>			

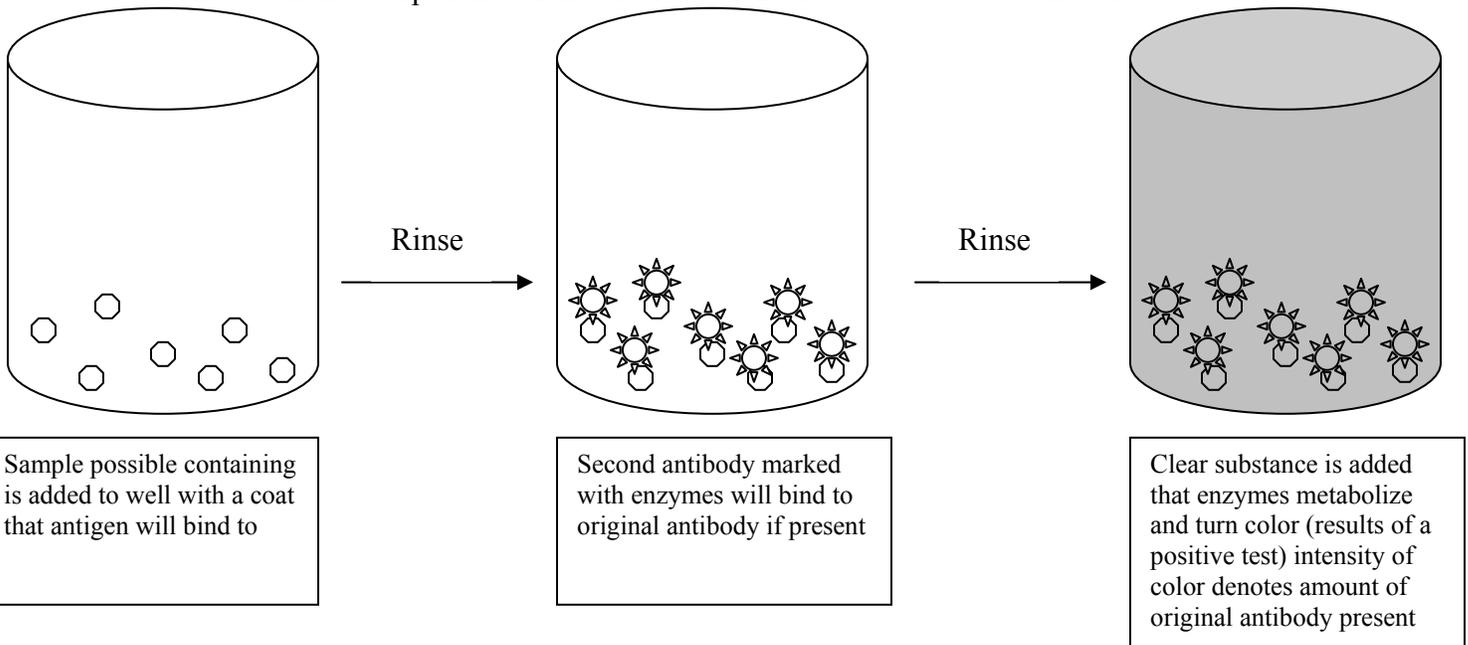
## ELISA Protocol using Shared Simulated Body Fluids

### Purpose:

The purpose of an ELISA is to determine if a particular protein is present in a sample. Using a plate reader, the quantity of protein can also be determined based upon optical densities- the amount of colored product. The amount of color (intensity) is directly proportional to the amount of original antibodies in the serum. There are two main variations on this method: you can determine if and how much antibody is in a sample, or you can determine if and how much protein is bound by an antibody. In this procedure you determine if there is antibody in the sample of simulated body fluid that was exchanged previously.

ELISAs are performed in 96-well plates- see diagram on following pages. The bottom of each well is coated with a protein to which will bind a certain antibody that is being tested for. In medical tests, the serum from blood is placed into the wells. In this lab, we are using the simulated body fluids from the previous activity- since some have the positive antigen, only some will test positive using the ELISA.

A positive control and a negative control are also added to the 96-well plate to compare the results of the unknown samples to. After a given amount of time (determined by the specific antibody tested for), the liquid is removed from the plate and a second antibody is added to the well (this will bind or stick to the first antibody if present in the sample- it is generally produced from a rodent in a lab. The second antibody (the one from the rodent) has an enzyme that is capable of breaking down colorless substances into colored waste products. After the colorless substance is added, it is clear which wells had the second antibody in them that must be bound to the antibody in the human serum. If it is colorless it is said to be negative if it is metabolized and now has a color, it is said to be positive. The negative control should remain clear since there is no original human antibody in it and the positive control should turn color (usually a shade of yellow) since there is the original human antibody in it that bound to the second antibody that broke down the components of the clear solution and released wastes that are colored.



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

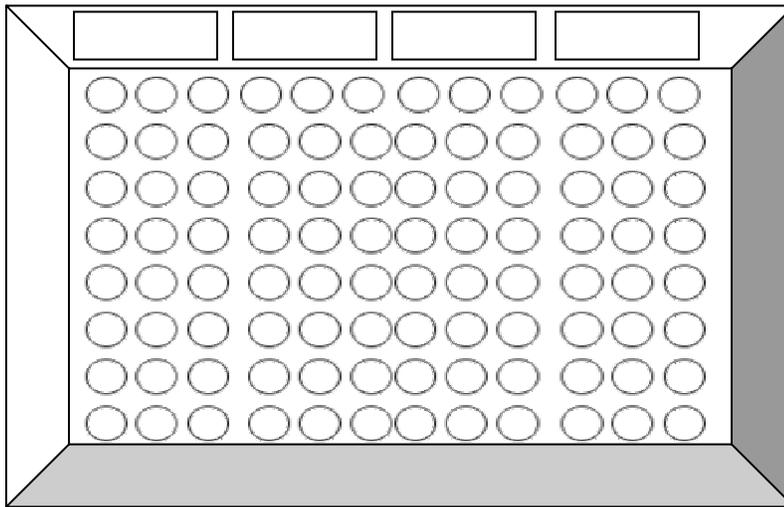
Procedure for ELISA Test:

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.

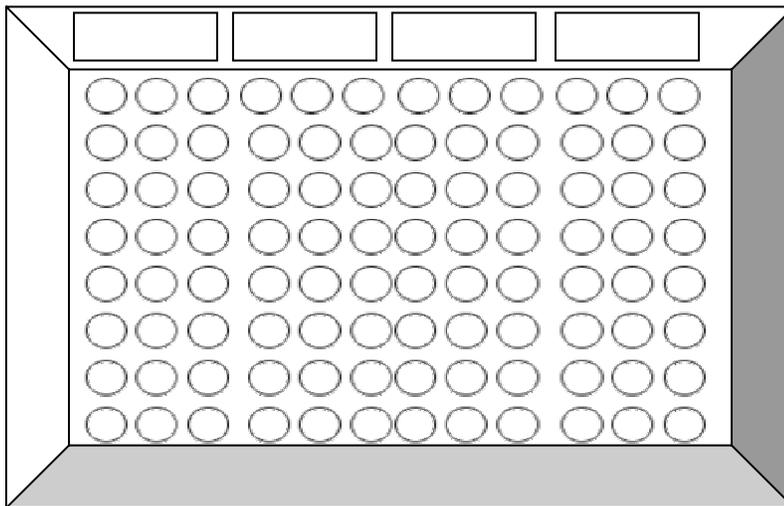
9. Add **washing solution** to the wells as done in step 7, and repeat washing a total of three times as described in #6.
10. Add three drops of the **color reagent solution** to each well.
11. Record your observations of the color after five minutes of all of your wells as well as the positive and negative 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.
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.**



**Use a colored pencil to indicate the color of each well after the ELISA is complete. Then use a black pen to write a “+” (positive) or a “-” (negative) on each well on the drawn ELISA plate below.**



**Analysis Questions:**

1. What is the purpose of an ELISA test?
2. What are the medical applications of an ELISA test?
3. Compare your results with the group that tested fluids from the same potential carriers. Did you have identical results?
4. How could you explain a reason why two groups from #2 may have differing results?
5. Why was it necessary to run positive and negative controls?
6. What was the purpose of washing the plates before a new solution was added?
7. In medical diagnostics, sometimes a false positive result is given. What does that mean?
8. In the experiment that you did what is one error that could result in a false positive test.
9. In medical diagnostics, sometimes a false negative result is given. What does that mean?
10. In the experiment that you did what is one error that could result in a false negative test?
11. How is the presence of an antibody detected in a sample during an ELISA?
12. After doing the lab, would you agree or disagree with the following statement: " When you kiss someone, you are also kissing everyone that they have previously kissed." Explain your answer.

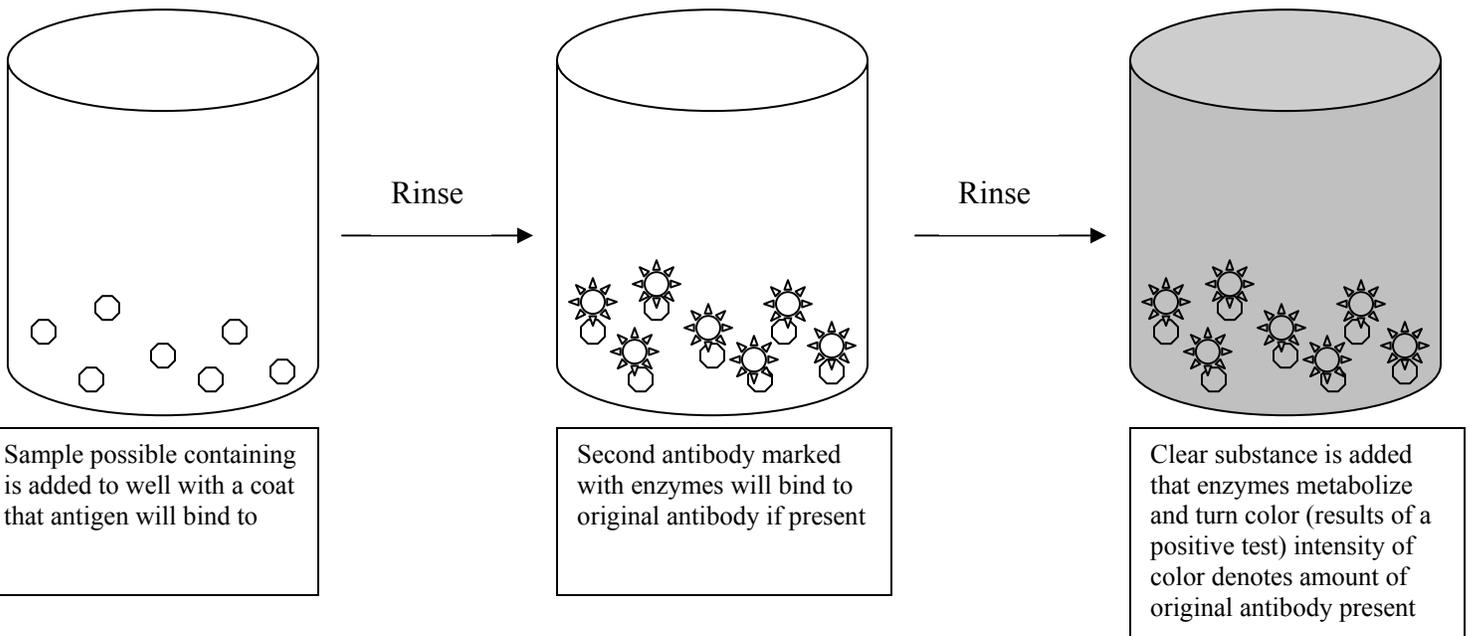
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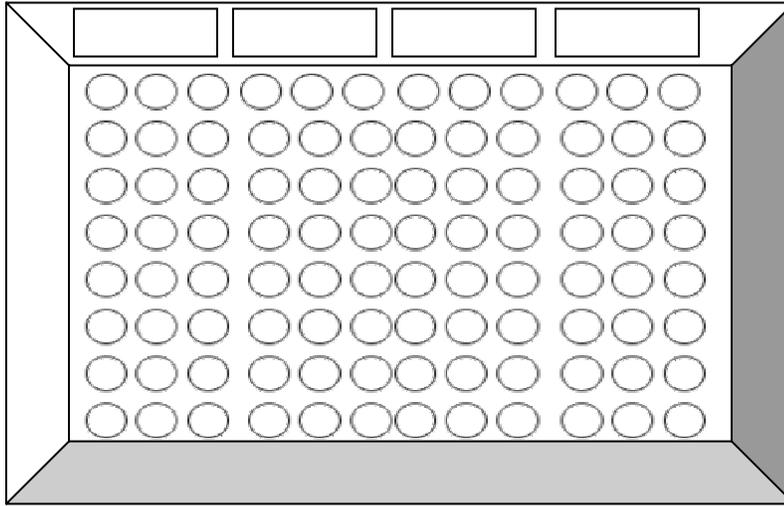
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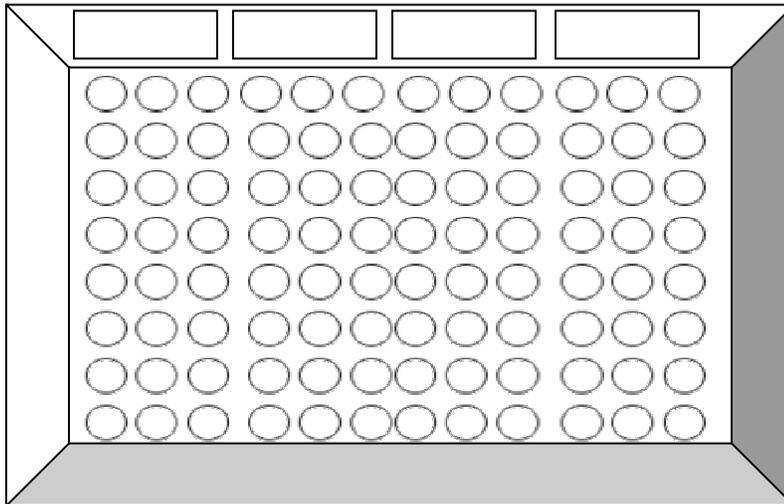
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## Lab Protocol for Pouring Agar Plates

### 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
scoopula	magnetic stirrer
	1 Liter beaker
	nutrient agar
	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:

- 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.
- Carefully empty the agar into a 1 L beaker.
- Fill the beaker with 1 L of either distilled or deionized water.
- Place the beaker on the hot plate and add the magnetic stirrer to the beaker.
- Heat the agar solution until it boils.
- Allow the solution to boil until all powdered agar is dissolved and the liquid is transparent.
- Using thermal gloves, remove the beaker from the hot plate.
- Carefully fill each petri dish 2/3 of the way with agar.
- 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).
- Clean up all materials thoroughly!

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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.

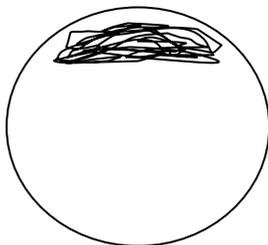
### **MATERIALS:**

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!**

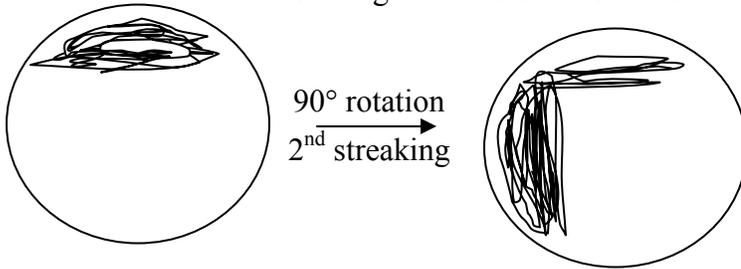
1. Hold the tube from which you will obtain the inoculum (bacterial sample) in one hand. You want to hold the tube at an angle so that nothing falls into the tube to contaminate it.
2. Remove the cap from the tube using your other hand.
3. Remove the sterile swab from its packaging. Do NOT let it touch anything, or it will no longer be sterile!
4. Submerge the 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:



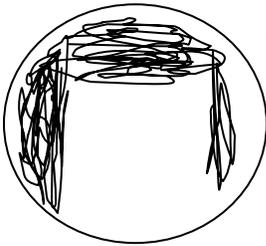
1. Start at one edge of the plate, moving the swab back and forth to streak about  $\frac{1}{4}$  of the plate. Do not press too hard; you don't want to puncture that agar. Try to maximize the number of streaks you make by keeping your streaks close together.

Streaking Bacterial Samples Teacher Section

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.



8. When you are through, immediately dispose of your swab in the waste container and cover your agar plate.
9. Using a permanent marker, label the cover of your agar plate with your initials.
10. Bring your agar plate to your instructor for proper storage.

## 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.

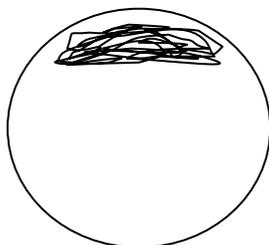
### **MATERIALS:**

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

### **Procedure:**

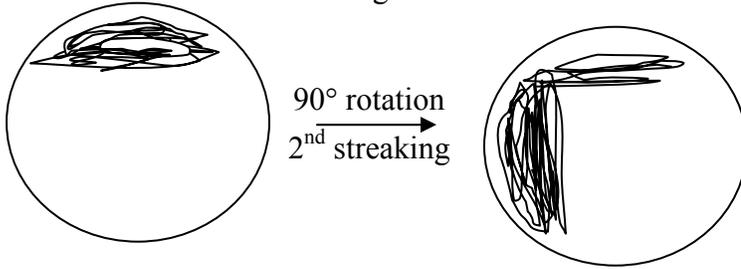
**NOTE: You must wear gloves, goggles, and an apron at all times!**

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!
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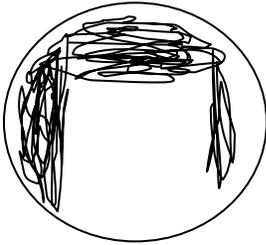


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2. Rotate the dish 90° and streak ½ of the remaining surface, starting with two streaks that overlap the first one.



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



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8. When you are through, immediately dispose of your swab in the waste container and cover your agar plate.
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## Lab Protocol for Identifying Bacterial Strains

### **Purpose:**

The purpose of this lab is to identify the type of bacterium in your fluid sample by using the single most commonly used staining procedure in microbiology...

THE GRAM STAIN!!!

This staining procedure can identify Gram positive bacteria or Gram negative bacteria. Gram positive bacteria are characterized by having very thick cell walls that include a **peptidoglycan structure as well as polysaccharides and/or teichoic acids** whereas Gram negative bacteria have very thin walls that lack the peptidoglycan structure. This procedure involves applying a basic dye, such as crystal violet, to your bacterial isolates, staining ALL of the bacteria present. A *mordant*, such as iodine, is used to improve the binding between the dye and the bacteria. The stain is then decolorized using alcohol. This decolorization removes the crystal violet-iodine combination from Gram negative cells. Gram positive cells retain the crystal violet-iodine combination throughout the decolorization process. A red counter-stain, safranin, is added after the decolorization. Gram negative cells will then show up as pink, where Gram positive show up as purple.

Prior to the staining procedure, you must create a bacterial smear. This allows you to select an isolated bacterial colony to stain and view under the microscope. Bacteria are applied to a microscope slide and are ‘heat-fixed’, meaning that the slide is held over a flame. The heat-fixation kills the bacteria and makes them stick to the slide so that they are not washed off during the staining procedure.

### **MATERIALS:**

crystal violet	95% alcohol	microscope
Gram’s iodine	glass microscope slide	latex or nitrile gloves
safranin	sterile swabs	lab aprons
safety goggles	Bunsen burner	water dropper
distilled/deionized water		inoculated agar plates with colonies

## Procedure A: The Bacterial Smear

**NOTE: You must wear gloves, goggles, and aprons at all times!**

1. Place one drop of water in the center of your microscope slide.
2. Using the cotton tip of the sterile swab, remove an isolated colony from your agar plate.
3. Gently ‘smear’ the tip of the swab in the drop of water on the slide. Limit the extent of your smear to the center to the slide. Be careful to stay on the slide; do not slip off of the edges!
4. Spread the sample evenly over the center of the slide.
5. Let the slide air-dry.
6. Give the slide to instructor to heat-fix the sample for you.
7. Be sure to clean up all materials thoroughly.

## ***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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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.

## 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