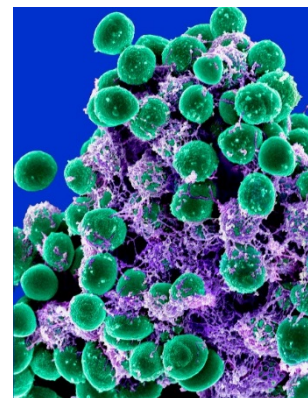


Identification of Pathogenic Bacteria-Case Studies



Teacher's Guide

Materials

24 well culture plates

ABTS Peroxidase Substrate System (2-Compound), KPL Part Number 50-62-00

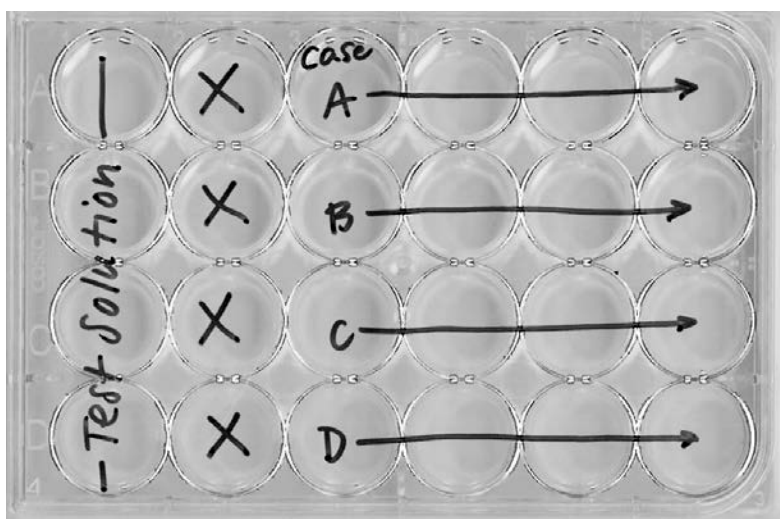
HRP Goat anti-Rabbit IgG antibody

Protocol

Instructor:

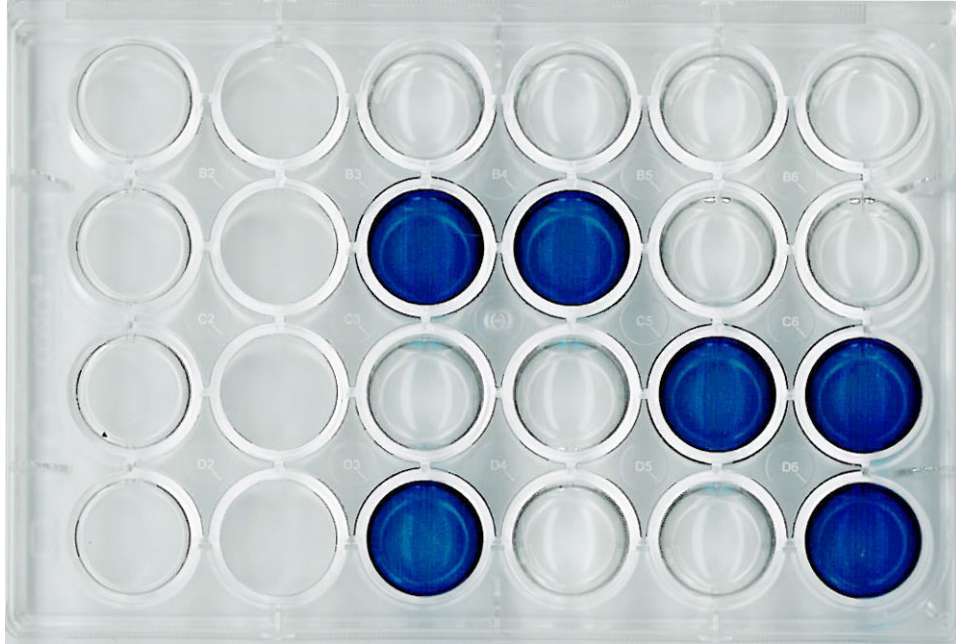
Prepare the 24 well plates as outlined below for each group of students. For each positive reaction well, 500 uL of HRP substrate will be added. To make the HRP substrate, mix equal parts of Solution A and Solution B from the KPL Peroxidase Substrate System Kit. For each negative reaction well, 500 uL of water will be added. Column 1 will contain 1 mL of "Test Solution" in each well which is HRP Goat anti-Rabbit IgG diluted 1:5000 in water.

Case	1-Test Sol'n	2-Empty	3-Test 1	4-Test 2	5-Test 3	6-Test 4
A	α -HRP		Water	Water	Water	Water
B	α -HRP		HRP	HRP	Water	Water
C	α -HRP		Water	Water	HRP	HRP
D	α -HRP		HRP	Water	Water	HRP



Students:

Add 5 drops of “Test Solution” to each well in the test rows for Cases A, B, C and D. A positive result is if the solution turns blue-green. Record your findings. Using the charts provided for identifying gram-positive and gram-negative bacteria, determine the bacteria causing the symptoms noted in each case study.



Gram-Positive Bacteria

Organism	Gram Stain	Cellular Morphology	Capsule	Motility	Hemolytic Activity	Catalase Activity	Coagulase Activity	PYR Activity	Hippuricase Activity
<i>Clostridium tetani</i>	Positive	Bacilli-rods	Positive	Positive	Positive	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Positive	Cocci-clusters	Positive	Negative	Positive	Positive	Positive	Negative	Negative
<i>Staphylococcus epidermidis</i>	Positive	Cocci-clusters	Positive	Negative	Negative	Positive	Negative	Negative	Negative
<i>Streptococcus agalactiae</i>	Positive	Cocci-chains	Positive	Negative	Positive	Negative	Negative	Negative	Positive
<i>Streptococcus pneumoniae</i>	Positive	Diplococci	Positive	Negative	Positive	Negative	Negative	Negative	Negative
<i>Streptococcus pyogenes</i>	Positive	Cocci-chains	Negative	Negative	Positive	Negative	Negative	Positive	Negative

Gram-Negative Bacteria

Organism	Gram Stain	Cellular Morphology	Capsule	Motility	H ₂ S Production	Urea Production	Lactose Metabolism	Lysine Metabolism
<i>Escherichia coli</i>	Negative	Bacilli-rods	Negative	Positive	Negative	Negative	Positive	Positive
<i>Shigella sonnei</i>	Negative	Bacilli-rods	Negative	Negative	Negative	Negative	Negative	Negative
<i>Salmonella enteritidis</i>	Negative	Bacilli-rods	Negative	Positive	Positive	Negative	Negative	Positive
<i>Salmonella typhi</i>	Negative	Bacilli-rods	Positive	Positive	Positive	Negative	Negative	Positive
<i>Yersinia enterocolitica</i>	Negative	Bacilli-rods	Negative	Positive	Negative	Positive	Negative	Negative
<i>Yersinia pseudotuberculosis</i>	Negative	Bacilli-rods	Negative	Positive	Negative	Positive	Negative	Negative

Case A

A 54 year old woman presents with sudden onset of chills, cough, fever, and severe chest pain. She is feverish and coughing up sputum. Wheezing sounds are heard in her lungs.

Preliminary Lab Results:

Gram positive diplococci
Capsule positive
Hemolytic activity positive
Motility negative

					Test 1	Test 2	Test 3	Test 4
Gram Stain	Cellular Morphology	Capsule	Motility	Hemolytic Activity	Catalase Activity	Coagulase Activity	PYR Activity	Hippuricase Activity
Pos	Diplococci	Pos	Neg	Pos	Neg	Neg	Neg	Neg

Identification: *Streptococcus pneumoniae*

Case B

An 8 year old girl comes into the doctor office for a skin infection on her face. Areas of skin can be easily removed and she has a small boil in her nose.

Preliminary Lab Results:

Gram positive cocci-clusters
Capsule positive
Hemolytic activity positive
Motility negative

					Test 1	Test 2	Test 3	Test 4
Gram Stain	Cellular Morphology	Capsule	Motility	Hemolytic Activity	Catalase Activity	Coagulase Activity	PYR Activity	Hippuricase Activity
Pos	Cocci-clusters	Pos	Neg	Pos	Pos	Pos	Neg	Neg

Identification: *Staphylococcus aureus*

Case C

A 40 year old flight attendant comes into the hospital with complaints of bloody diarrhea, abdominal pain, fatigue and fever. The gastrointestinal symptoms had appeared quickly. Physical examination revealed a skin rash.

Preliminary Lab Results:

Gram negative bacilli-rods

Capsule negative

Motility positive

				Test 1	Test 2	Test 3	Test 4
Gram Stain	Cellular Morphology	Capsule	Motility	H ₂ S Production	Urea Production	Lactose Metabolism	Lysine Metabolism
Neg	Bacilli-rods	Neg	Pos	Neg	Neg	Pos	Pos

Identification: *Escherichia coli*

Case D

A group of fraternity brothers show up at the student health clinic with complaints of abdominal cramping and diarrhea. There is no history of recent travel. A few days ago, the fraternity hosted a picnic with one of the sororities on campus. The main dish was barbecue chicken.

Preliminary Lab Results:

Gram negative bacilli-rods

Capsule negative

Motility positive

				Test 1	Test 2	Test 3	Test 4
Gram Stain	Cellular Morphology	Capsule	Motility	H ₂ S Production	Urea Production	Lactose Metabolism	Lysine Metabolism
Neg	Bacilli-rods	Neg	Pos	Pos	Neg	Neg	Pos

Identification: *Salmonella enteritidis*

Simulation of Transmission

Teacher's Guide

Purpose

Working as a team of BRASS scientists, we will use our epidemiological skills to trace a disease back to “Patient Zero”.

Materials

- 15 mL blue capped polypropylene conical tubes-“Stock Tube”, one per student
- 15 mL orange capped polypropylene conical tubes-“Working Tube”, one per student
- 5 mL tubes “Reagent Tube”, 2 per student
- Distilled white vinegar
- Phenol Red-Hanks Solution
- Disposable plastic transfer pipets, 3 per student
- Test tube racks (one per student that can hold 4 tubes- broken apart Styrofoam racks for 15 mL conical tubes work well)

Methods

Instructor:

1. Prepare the Stock Tubes: Number the blue capped conical tubes 1-XX. The number of tubes depends on the number of students. Fill all tubes with 10 mL of DI water EXCEPT FOR ONE tube. Fill this remaining tube with undiluted distilled vinegar (acetic acid). **Make a side note for your record of the tube # containing the acetic acid, as this tube represents “Patient Zero”.** The tubes now contain the Stock Solution.
2. Prepare one rack for each student with 1 empty Working Tube, 1 numbered Stock Tube containing Stock Solution, and 1 pipet.
3. Have the students record the number that they have on their tube. Then have each student use their pipet to transfer about 5 mL of the Stock Solution to the Working Tube. Remind them to cap their Stock Solution so that it does not become contaminated.
4. Facilitate the exchange by having one side of the room donate about 1 ml from their Working Tube to another student's Working Tube from the other side of the room using their pipet. Remind them to work over diapers or work stations and to not touch the other person's Working Solution with their pipet as that will contaminate it and they will need a new pipet. **It works best if you know where the acetic acid tube is and make sure that person donates on the first round.** Record keeping is very important for this experiment so have students return to their stations and record their interaction before the next round.

5. For the second interaction, students that previously donated should receive from students that received the first round. At the end of this round each student should have donated once and received once and should be back to 5 mL in their working tube. **Remind them to record results and not to interact with the same person more than once.**
6. Repeat the first two rounds so each student will donate twice and receive twice.
7. After the 4 rounds are completed have students return to their work station. Distribute one Reagent Tube (phenol red solution) and a new pipet to each student.
8. Have the students test their working solution to see if it is infectious by instructing students to add a few (5-10) drops of working solution to the test reagent. If the test reagent turns yellow then they record their sample as infected and if it remains pink/red they are not infected.
9. Have the students ask the people they exchanged fluids with if they are also infectious and record this on the handout.
10. Students can return to their seats and then the instructor uses the white board to map out the infection. Start with someone who is infected, mapping their interaction and who is infected. Continue until everyone who is infected is on the board. Ask students to explain who is patient zero and why. Have them make guesses and explain their reasoning.
11. To confirm who is patient zero, pass out a second Reagent Tube and new pipet to each student. Have them test their Stock Solution and confirm who has the Patient Zero tube.

Students:

Write down the number of your unknown solution (Blue cap). I have TUBE #_____.

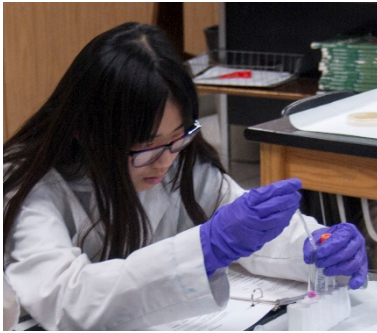
Take the BLUE capped tube containing the **Stock Solution** and transfer 5 ml into the empty orange capped tube using the graduations marked on the tube. Return the BLUE capped tube to your test tube rack for later testing. The orange capped tube is now your **Working Solution**. Handle the solutions carefully. Remember, you may be handling an infected sample!

Spreading the disease

Each person can **transmit (donor) or receive (recipient)** the “disease”. You will have a chance to interact with a **total of four people**. You should try to donate twice and receive twice. To transmit, transfer **1 ml** of your **Working Solution** into another person’s Working Solution tube, trying not to touch their liquid. Mix the solutions after each interaction by shaking gently. **Write down the name of the person with whom you interacted on the table and put a check mark next to the appropriate statement. It is very important that you keep an accurate record of your interactions.** We will do one round of interactions at a time. Leave your desk and interact with one person. Return to your desk and record the interaction on the table. When everyone has recorded the data on their table, the instructors will then allow you to start the subsequent rounds of interactions.



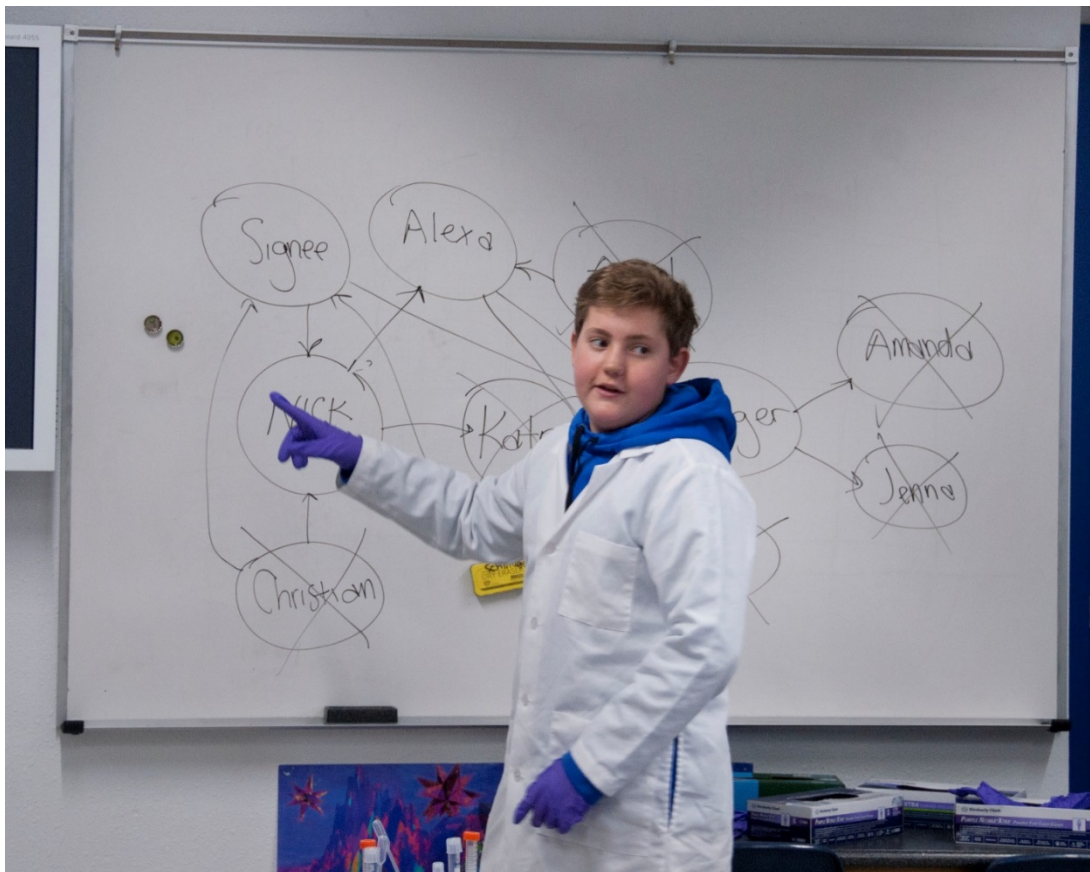
Identification of the infected, but asymptomatic persons



Transfer 5 drops of your Working Solution (Orange capped tube) into one tube of the test reagent. The instructors will hand out the test reagent tubes when required. Mix gently and note the color change, if any. **Red – uninfected; Yellow – infected.** If the persons you have interacted with are infected, write YES in the column labeled “Infected??”, otherwise, write NO.

Identification of patient zero

The class will use their logic to identify the first person that was infected and transmitted the disease.



Verification of the result

Retrieve the Blue capped tube containing your remaining 5 mL of **Stock Solution**, a new tube of test reagent, and a new pipet. Transfer **5 drops** of the **Stock Solution** into the new tube of test reagent using the new transfer pipet. Note color change. Did the class guess the correct person as being patient zero?