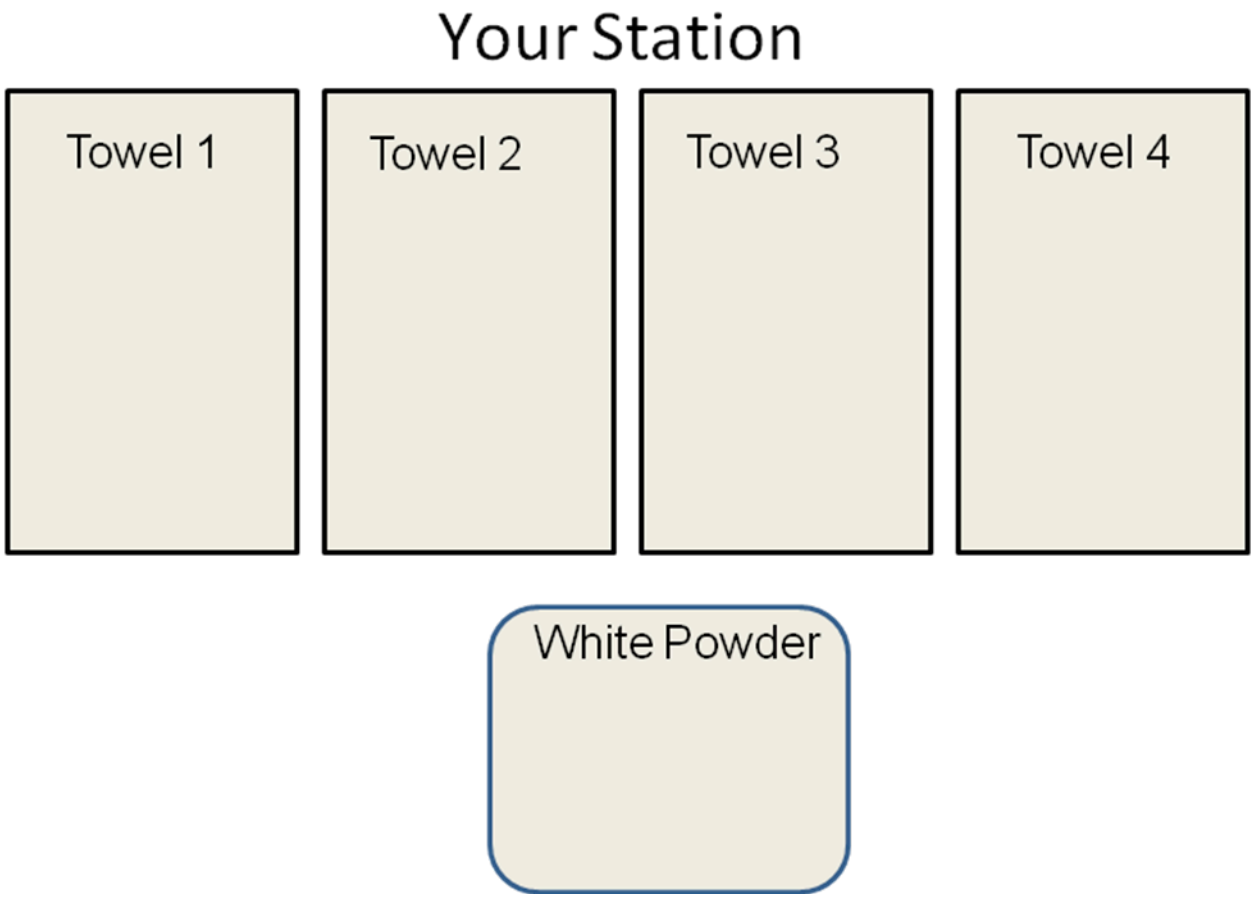
Experiment Number: 1
Title: Synthetic Epidemic
Purpose: To simulate the transmission of a bacterial infection amongst a population. Students will not only model the disease but will attempt to identify who patient zero was.

Materials:

Initial Table Setup: Lab safety/Synthetic Epidemic Model Table		
Item	Session	Total
Paper towels "Bounty Select A Sheet" 4 Papers/ station labeled: I.D. # and trial number (initial, round 1, round 2, round 3)	1 roll	3-4?
Paper Plates (1/student)	27	~100
Baby powder covered with foil	¼ container	1 container
Glow germ	2 g of glow germ (enough to cover plate)	1 bottle of glow germ
Black light (BIORAD Key chain)	~10	~10

Pre-lab Setup:

1. THE NUMBER OF STUDENTS PARTICIPATING SHOULD BE KNOWN BEFORE HAND
2. Gloves, lab coats should be placed at each table
3. Plates with either baby powder or glowgerm
4. 4 paper towels per station.
5. Each station should look like it does below.



Protocol

1. Students will receive a number when they arrive at their bench.
    i. It is important for them to know this is their ID number for this exercise.
2. Students should put gloves on.
3. Then place their hand in the powder.
    i. Stress that it is important to get as much powder as possible on their RIGHT HAND.
4. Students will then do a self test, where they place their right hand onto the paper towel 1.
    i. They should press and hold their hand on the paper towel for 5 seconds.
5. Rounds of Handshaking:
    i. They will then be given the number of the person they will shake hands with in each round. (see below for example.)

Your #	Shakee's #
1	24
2	23
3	22
4	21
5	20
6	19
7	18
8	17
9	16
10	15
11	14
12	13
13	12
14	11
15	10

Your #	Shakee's #
16	9
17	8
18	7
19	6
20	5
21	4
22	3
23	2
24	1

ii. Students will then shake hands.
    1. Right hand only
    2. Hold for 5 seconds

iii. Then return to their station to blot their hand on the paper towel with the corresponding round.
    After all the rounds the papers should be analyzed with UV light to see who has been infected.

6. Analysis
    i. Use black like pens to visualize glowgerm.
    ii. Start with the last round of handshaking
        1. If the paper glows the student is "infected"
        2. Ask the students who are "infected" to stand up
        3. Circle the numbers of students that were "infected"
            a. You can write them on the board
            b. Or use the power point presentation and highlight them on there.

Experiment Number: 2
Title: Gram Staining
Purpose: Students will use microscopy and gram staining to identify the bacteria's morphological and structural characteristics.

Materials:

Gram Staining		
Item	Session	Total
Cultures 1 18 hour broth culture of each organism/day *S. epidermis* (B, D, U) *E. coli* (C) *B. megatarium* (A)	3	12
5 slides/ table (smears will be prepped before the lab)	25	100
3 Gram Staining Kits per table Kit includes: Crystal violet, Iodine, ethanol, Safranin, sterile dH2O, triangle to hold slide over sink, immersion oil	12	12
Additional Materials		
1 microscope per two students	12	12
Microscope video camera hooked up to TV (optional)	1	1

Pre-lab setup

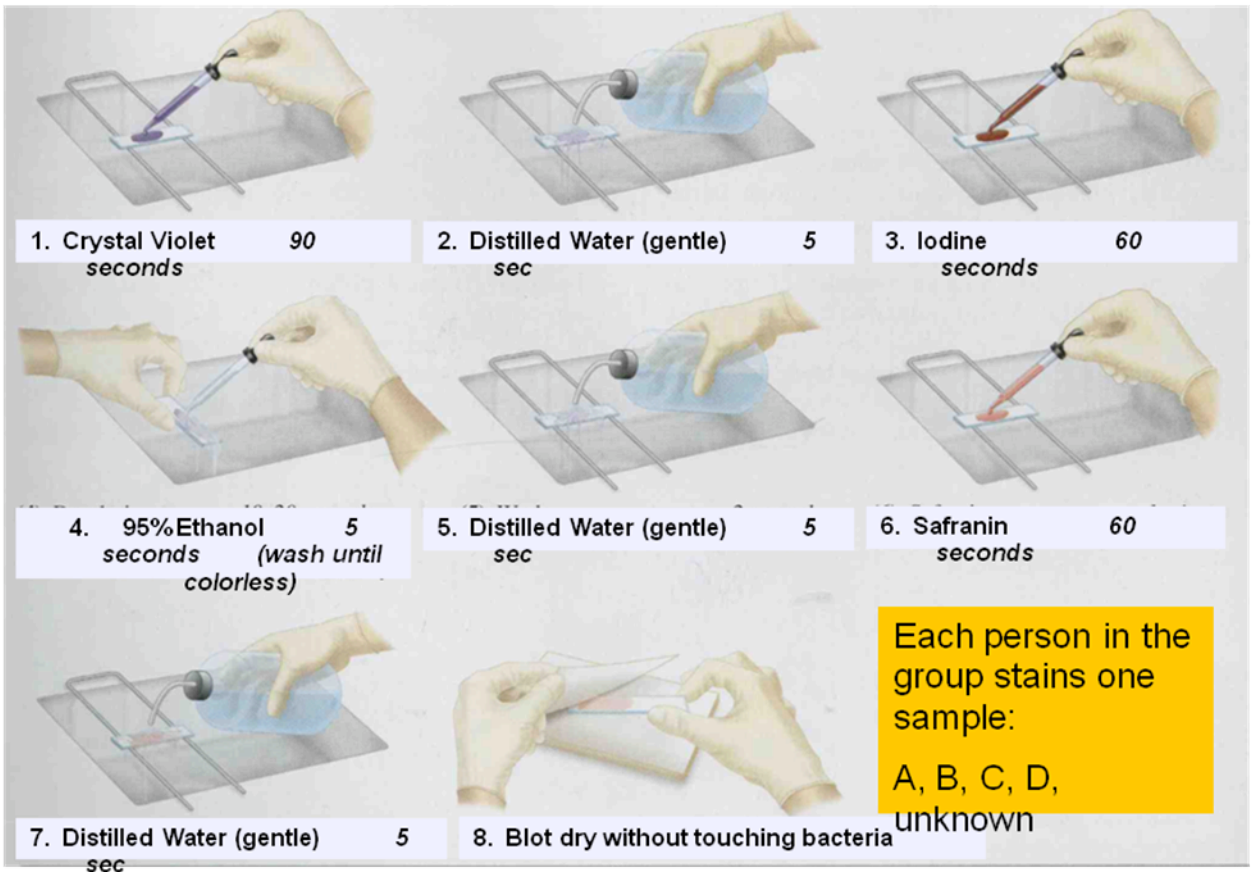
1. Bacteria stock (liquid broths) need to be prepped
2. Sample slides for each condition need to be made. See protocol below

Protocol

1. Smear preparation
    a. Use a sterile swab to place bacteria from broth onto the slide.
    b. Generously spread the bacteria onto the slide
    c. Grasp the end of the slide with a clothespin, and flame the slide from underneath the droplet GENTLY! Three or four quick passes over the flame should dry out the droplet. This fixes the bacteria to the slide. Heating the slide for too long will cause the slide to break. You should still be able to touch the slide without burning yourself. You should not smell burning bacteria.
2. Repeat steps for each organism
    a. Set of 5 slides/bench

Sample	Organism
A	*B. megatarium*
B	*S. epidermis*
C	*E. coli*
D	*S. epidermis*
U	*S. epidermis*

3. Gram Stain
    a. Place a drop of crystal violet on each dried bacterial smear and allow the smear to rest for one minute. Gently rinse the stain off with water. Crystal violet stains all cells purple, regardless of the cell structure.
    b. Stain the smears with Gram's iodine solution for one minute. Gently rinse the stain off with water. Gram's iodine causes large crystals to form in the peptidoglycan layer. Because the peptidoglycan layer is thicker in Gram+ cells, more iodine is retained in these cells.
    c. Apply a couple of drops of 95% ethanol to the smears for approximately ten seconds, then gently rinse off the slide with water. Ethanol removes the lipid layer of Gram- cells, removing the dye from the peptidoglycan layer. Gram- cells will now be colorless, while Gram+ cells will still be purple.
    d. Stain the smears with safranin for thirty seconds then gently rinse the slide with water and blot dry. Safranin stains all cells pink, but because Gram+ cells are already purple, only the Gram- cells display the pink color.
    e. Examine the cells under the microscope. Directions on how to use a microscope are below.



Basic Light Microscopy

1. Switch on your microscope's light source. This is a switch at the base of microscope.
2. Adjust the light using the wheel/slider bar on the base to 7-8.
3. **Adjust the diaphragm** to the largest hole diameter, allowing the greatest amount of light through. If you have an iris diaphragm, slide the lever till the most light comes through.
4. **Rotate the nosepiece** to the lowest-power objective (usually 4x for 40x magnification). It is easiest to scan a slide at a low setting, since you have a wider field of view at low power.
5. **Place a microscope slide on the stage**, either under the stage clips or clipped onto the mechanical stage if your microscope has one. Move the slide until the specimen is under the objective lens.
6. **Adjust the large coarse focus knob** until the specimen is in focus. Slowly move the slide to center the specimen under the lens, if necessary. Do this by nudging it gently with your fingers or by turning the slide control knobs if you have a mechanical stage.

7. **Adjust the small fine focus knob** until the specimen is clearly in focus. Then **adjust the diaphragm** to get the best lighting. Start with the most light and gradually lessen it until the specimen image has clear, sharp contrast.
8. **Scan the slide** (right to left and top to bottom) at low power to get an overview of the specimen. Then center the part of the specimen you want to view at higher power.
9. Rotate the nosepiece to the 10x objective for 100x magnification. **Refocus** and view your specimen carefully. **Adjust the lighting again** until the image is most clear (you will need more light for higher power). Repeat with the 40x objective for 400x magnification, which will enable you to see all of the specimen detail that's necessary for high school biology lab work.

Experiment Number: 3
Title: Catalase Test
Purpose: To explore the physiological (functional) characteristics of the bacterial samples.

Materials:

Catalase		
Item	Session	Total
Cultures *S. epidermis* on TSA plate (3 plates/ bench Labeled "C" " D" "U") *M. smegmatis* (2 plates/ bench Labeled "A" "B") BLANK organism on plate	12 plates "C", "D", "U" 8 plates "A", "B"	
5 slides/group of 2 (15 slides/table)	60	120
Wooden transfer sticks	60	1 Box
1 bottle 3% hydrogen peroxide in dropper bottler/group of 2	12	12

Pre-lab setup

1. Prepare cultures on TSA agar.

Sample	Organism
A	*M. smegmatis*
B	*M. smegmatis*
C	*S. epidermis*
D	*S. epidermis*

U	*S. epidermis*

Protocol

1. Using a wooden stick, smear a small amount of bacteria from the Petri dish onto a clean microscope slide
2. Label the slide using the Sharpie marker
3. Add 3 drops of H_2O_2 solution onto the smear.
4. Record your observations in the worksheet (Pg. 19).
5. Repeat steps 1-4 for all FIVE bacteria samples, using new materials each time.
6. When done, dispose of materials in the correct bins

Experiment Number: 4
Title: Antibiotic treatment
Purpose: The strain of bacteria has been identified. In this exercise students will be introduced to antibiotics and how effective they can be in treating bacterial infections. They will observe the effectiveness of three antibiotics on the organism that they have just identified.

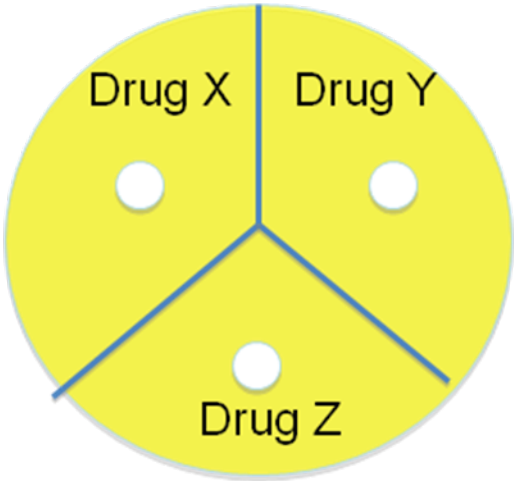
Materials

Antibiotic Resistance		
Item	Session	Total
15 Muller Hinton Agar plates	15	15
1 Broth culture of *S. epidermis*	1	1
Antimicrobic discs Penicillin Ampicillin Streptomycin	8 discs of each	1 vial of each antimicrobic
Ruler (Carolina Rulers in mm in Rm 440)	15	15

Pre-lab setup

1. Prepare Muller hinton agar plates
2. Plate out *S. epidermis* using a sterile swab
3. Place three antimicrobic discs onto the plate
    a. Spread them far apart from one another.
4. Let sit for 10 min before inverting and incubating at 38°C
5. After 24 hours store place at 4°C

Animicrobic	Animicriobic Name
X	Ampicillin
Y	Streptomycin
Z	Penicllin



Protocol

1. Have the students compare diameters of the zones of inhibition
    1. Measure in mm using rulers
2. Classify bacteria as
    1. R: resistant
        - Small/no zone of inhibition
    2. I: intermediate
        - Small to medium zone of inhibition
    3. S: susceptible
        - Large zone of inhibition

Key

Gram Stain

Sample	Organism	Result
A	*B. megatarium*	Gram + Rod
B	*S. epidermis*	Gram + Cocci
C	*E. coli*	Gram - Rod
D	*S. epidermis*	Gram + Cocci
U	*S. epidermis*	Gram + Cocci

Catalase Test

Sample	Organism	Result
A	*M. smegmatis*	Negative
B	*M. smegmatis*	Negative
C	*S. epidermis*	Positive
D	*S. epidermis*	Positive
U	*S. epidermis*	Positive

Antibiotics
Sample "D" *S. epidermis*

Animicrobic	Animicriobic Name	Result
X	Ampicillin	Sensitive (S)
Y	Streptomycin	Intermediate (I)
Z	Penicllin	Resistant (R)