The Boston University Center for Disease Control

New Employee Information Packet

*Name:*_____ *ID#:*_____

Dear Researcher,

Congratulations! You have been accepted into The Boston University Center for Disease Control's (B.U.C.D.C) Young Researchers Program. This year was a particularly strong year as the candidate pool was truly exceptional. However, your application clearly stood out above the rest. Therefore we would like to invite you to come visit our facilities the week of May 11th 2009 to begin your training.

The B.U.C.D.C. was founded in 1968. The mission of the center is two-fold; the first is to serve as a centralized storage facility for numerous pathogenic agents. In order to accomplish this first goal, specially designed to labs were constructed. Each is outfitted with a state of the art computer monitoring system which can detect the release of any pathogenic agent and take appropriate actions to limit public exposure to the agent. The second goal is to serve as the primary research facility for these agents with the goal of developing methods to identify, understand and than eliminate future outbreaks of these agents.

Please report to room 311 on the third floor at 9:00 AM sharp. Your training will begin with both a lab orientation and safety courses, both of which are mandatory, as you will receive your lab coat, goggles and ID number at this time. Afterwards we will adjourn to a pizza lunch reception to celebrate your first day with us.

Once again, congratulations on joining us at BUCDC. We look forward to working with you in the future.

Yours truly,

Pam Eskine
Managing Director
Boston University Center for Disease Control

Lab Safety Guide

STUDENT RESPONSIBILITIES:

The Center for Disease Control
@ Boston University

1. Determine the purpose and procedure of the experiment by reading completely the experiment before beginning.
2. Wear protective equipment (e.g., lab coat, gloves, and goggles).
3. Be aware of the dangers of long hair, long sleeves, and loose clothing.
4. Remain in your lab group throughout the experiment. Lab activities are team efforts.
5. Do the experiments as assigned and in the manner prescribed. Unauthorized experimentation is not permitted.
6. Keep your lab and other working areas neat and clean during lab sessions.
7. Running, horseplay and practical jokes are NOT allowed. Stay on-task and maintain quiet behavior during lab sessions. Loud and boisterous behavior is not acceptable.
8. Dispose of materials in the proper waste containers as instructed.
9. To avoid poisoning and/or contamination, no eating or drinking is allowed in the science classroom or laboratory.
10. Know the location and proper use of the emergency safety materials such as the fire blanket, eyewash, and fire extinguisher.
11. The instructor MUST be notified IMMEDIATELY of any accident, even if minor.
12. All laboratory equipment should be properly put away at the end of the lab session.

CHEMICAL SAFETY:

1. Replace caps on chemical bottles immediately after use.
2. NEVER return unused solutions or solids to stock containers as they can contaminate the stock chemical. Report suspected contamination to the teacher.
4. Never taste chemicals or drink from laboratory glassware.
5. Consider ALL chemicals to be dangerous. Most of them can be dangerous if used incorrectly.
6. REPORT IMMEDIATELY ANY CHEMICAL SPILLS.
7. Laboratory counters and tabletops should be cleaned and dried after each activity.
8. Follow directions for the proper disposal of wastes.
9. EYE SAFETY: If an accident occurs which involves splashing any chemicals into the eye, rinsing or washing of the eye must start immediately and continue for a minimum of 15 minutes. Students will be REQUIRED to wear protective goggles during laboratory periods that can involve any danger to the eyes.
10. TEACHER INSTRUCTIONS: Any additional safety instruction associated with any experiment as determined by the teacher MUST be followed.

ALL EMPLOYEES TAKING PART IN ANY SCIENCE LABORATORY ACTIVITY MUST HAVE A SAFETY CONTRACT ON FILE WITH THE B.U.C.D.C.

Lab Safety Contract

While I am an employee in the BUCDC facility, I agree to:

- Follow oral and written instructions.
- Protect my eyes, face, hands and body with appropriate safety gear when involved in science experiments
- Keep my work area clean and neat to avoid accidents and contamination.

• Contact the instructor immediately when help is needed fast.
• Know the locations of first aid equipment, eyewash, fire blanket and fire extinguisher.
• Act in a responsible way at all times so as to ensure the safety of others, as well as well as my own.

I, [*print name*]_____, have been instructed in the lab safety and emergency techniques needed for the science facility. I understand and agree to follow the lab safety regulations set forth above and in the Lab Safety Guide as stated by the instructors, lab manuals, and specific experiment instructions. I am aware that my safety and the safety of my colleagues depend on my behavior in the laboratory. With this in mind, I will closely follow the oral and written instructions provided by the instructors and/or the administration.

_____
Student Signature/Date

_____
Student Name (please print)

_____
Student Email Address

ACTIVITY 1: B.U.C.D.C ICE BREAKER

We will create a synthetic epidemic to show the ease with which microorganisms can be spread across a population, an ever present problem in the clinical area. One employee will unknowingly act as the epidemic initiator. His/her glove is contaminated with an infectious pathogen. The process of shaking hands will simulate the contact that is necessary for an epidemic to spread. By the end of this activity, you should be able to track down the original source of the "infection" using deductive reasoning, an approach similar to that used by public health officials and epidemiologists.

MATERIALS SUPPLIED

- Employee ID sticker label

- 1 pair of disposable gloves
- Safety goggles
- Tray of powder
- 4 paper towels
- Table of hand-shaking order

PROCEDURE

1. Before you begin, stick the numbered employee ID on your lab coat.
2. Put on safety goggles and disposable gloves.
3. Wait for a signal from the instructor before starting each round.
4. Round 0: Rub your hands in the powder until it is spread out well over your gloves.
5. Stamp your right hand on the paper towel labeled 'Round 0'.
6. Round 1: Each employee will shake right hands with another employee for 5 seconds. Follow the table of hand-shaking order for Round 1. (E.g., #1 shakes hands with #20)
7. Stamp your right hand on the paper towel labeled 'Round 1'.
8. Round 2: Each employee will shake right hands with another student for 5 seconds. Follow the table of hand-shaking order for Round 2. (E.g., #1 shakes hands with #10)
9. Stamp your right hand on the paper towel labeled 'Round 2'.
10. Round 3: Each employee will shake right hands with another student for 5 seconds. Follow the table of hand-shaking order for Round 3. (E.g., #1 shakes hands with #7)
11. Stamp your right hand on the paper towel labeled 'Round 3'.
12. Carefully remove gloves and dispose in the biohazard container.
13. Follow the instructions from the instructor. Use the hand-held black light to identify "infected" individuals and mark them on the provided data chart.

****CLEAN UP:** Before moving on to the next activity, wipe down the bench with 70% ethanol, making sure that it is clean and free of powder.

RESULTS

Circle the employees that were infected for each round:

Employee ID	Infected Individuals			
	Round 3	***Round 2***	***Round 1***	***Round 0***
1	1	1	1	1
2	2	2	2	2
3	3	3	3	3
4	4	4	4	4
5	5	5	5	5
6	6	6	6	6
7	7	7	7	7

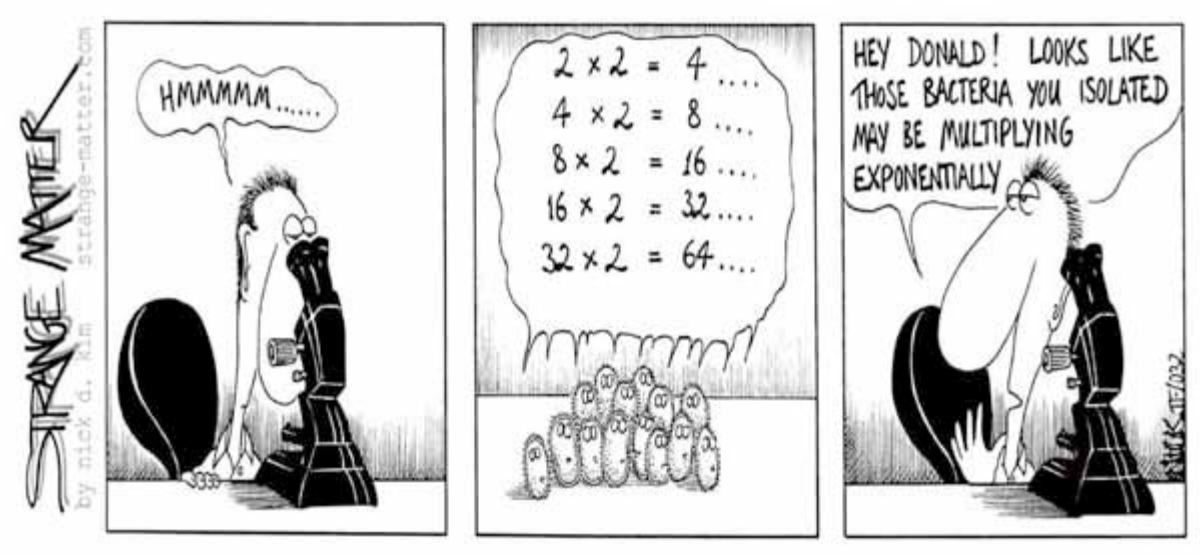
8	8	8	8	8
9	9	9	9	9
10	10	10	10	10
11	11	11	11	11
12	12	12	12	12
13	13	13	13	13
14	14	14	14	14
15	15	15	15	15
16	16	16	16	16
17	17	17	17	17
18	18	18	18	18
19	19	19	19	19
20	20	20	20	20
21	21	21	21	21
22	22	22	22	22
23	23	23	23	23
24	24	24	24	24
25	25	25	25	25
26	26	26	26	26
Total # Infected				

DISCUSSION QUESTIONS

1. Who is Patient Zero?

2. What is the most likely route of transmission for this pathogen? Explain.

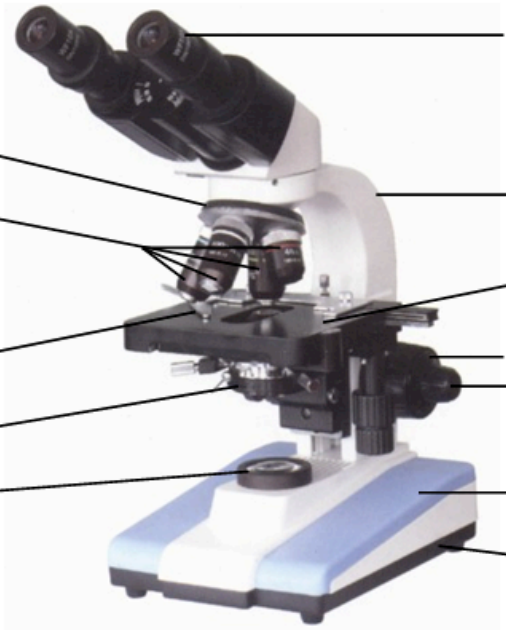
3. How would the transmission of the disease change if it were airborne? Waterborne?



Q: Why did the bacteria fail the math test?
A: He thought multiplication was the same as division.

ACTIVITY 2: INTRODUCTION TO THE LIGHT MICROSCOPE

PARTS OF THE MICROSCOPE



Always carry a microscope with one hand holding the **arm** and one hand under the **base**.

POWER OF MAGNIFICATION

To calculate the **power of magnification**, multiply the power of the ocular lens by the power of the objective.

For example, if you use a **10x** objective with a 10x ocular lens:

Total magnification = 10 x 10 = 100x

What happens as the power of magnification increases?

Power = 10 x 4 = 40 Power = 10 x 10 = 100 Power = 10 x 40 = 400

PROCEDURE

1. Turn on the microscope and then rotate the nosepiece to click the lowest power objective into place.
2. Place slide on the stage and secure it using the stage clips.

3. Use the coarse adjustment knob (large knob) to get it the image into view and then use the fine adjustment knob (small knob) to make it clearer.
4. Once you have the image in view, rotate to the next higher objective.
   ****Ensure that the objective does not crash into the glass slide!**
5. Draw what you see at two different magnifications in the space provided below
6. When done, turn off the microscope and return the sample slides.

DISCUSSION QUESTIONS

1. Calculate the power of magnification for each objective on your microscope.

Objective Power	Eyepiece Lens Power	Total Power of Magnification
4x	10x	
10x	10x	
40x	10x	
100x	10x	

2. What happens to the view of an image as the power of magnification is increased?

ACTIVITY 3: GRAM STAINING

Gram staining is a method commonly used to determine the chemical structure of the cell wall of bacteria, and is a very helpful technique for bacterial identification. The cell wall can stain either positive or negative, depending on its chemistry. Gram-positive cell walls consist of several layers of peptidoglycan. Gram-negative cell walls have one layer of peptidoglycan surrounded by a lipid-based outer membrane. If the bacteria stains positive it will retain a purple/blue color. If the bacteria stains negative, the bacteria will

not retain the purple/blue color, but rather have a pinkish/red color. Each bench will perform Gram staining on four known bacteria AND the unknown pathogen, and use a light microscope to compare the morphology and staining characteristics of the samples.

MATERIALS SUPPLIED

- 5 glass slides with heat-fixed bacteria smear (**A-D**, **Unknown**)
- Gram's crystal violet dye solution
- Iodine solution
- 95% ethanol solution
- Safranin stain solution
- distilled water
- Glass droppers
- Sharpie markers and color pencils
- Light microscope
- Disposable gloves

PROCEDURE

1. Put on a new pair of gloves.

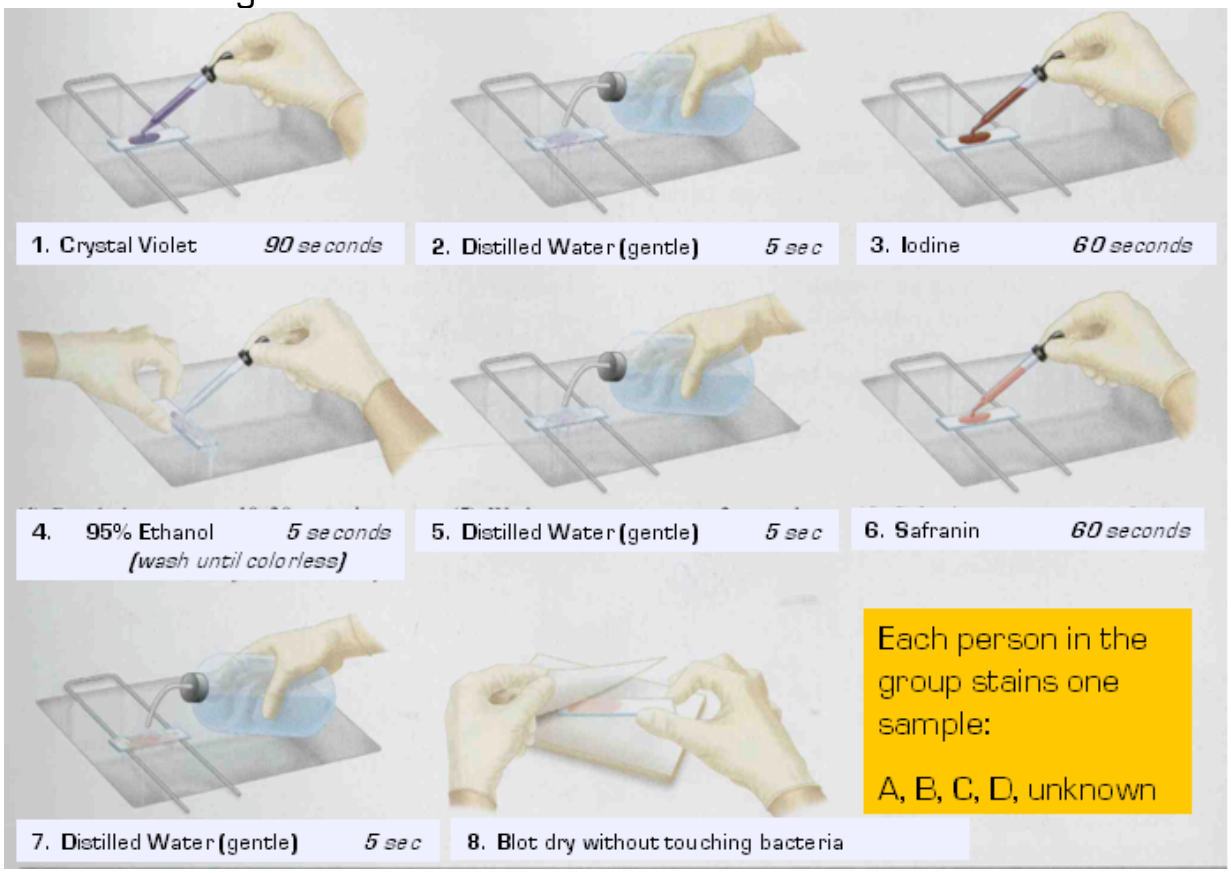
**For the following steps, WORK OVER THE SINK.

2. **Crystal Violet Stain:**
   - Cover the bacteria smear with 10-15 drops of crystal violet dye, making sure it is evenly covered. Leave for 90 seconds.
   - Rinse slide with a gentle stream of distilled water for 5 sec. DO NOT RUB.
3. **Iodine Stain:**
   - Cover the bacteria smear with 10-15 drops of Iodine, making sure it is evenly covered. Leave for 60 seconds.
   - Rinse slide with a gentle stream of distilled water for 5 sec. DO NOT RUB.
4. **95% Ethanol:**
   - Add 10-15 drops of 95% ethanol for only 5 seconds.
   - Immediately rinse slide with a gentle stream of distilled water for several seconds. DO NOT RUB.
5. **Safranin Stain:**
   - Cover the bacteria smear with 10-15 drops of Safranin stain, making sure it is evenly covered. Leave for 60 seconds.
   - Rinse slide with a gentle stream of distilled water for 5 sec. DO NOT RUB.
6. Carefully dry the slide by blotting with a paper towel. DO NOT RUB.
7. Place a cover slip over the smear and visualize using the light microscope.
8. **Gram-positive** bacteria will stain dark purple/blue.
9. **Gram-negative** bacteria will stain pink.
10. Focus the smear using the **4x** objective lens. Follow the microscope tips given earlier on page X.
11. Switch to higher objectives (**10x** and **100x**). Use OIL IMMERSION at 100x.

12. Draw and label your observations. The color and shape of bacteria should be accurate in your drawings
13. Move to the next microscope station at your bench and record your observations. Draw all five bacteria samples: **A**, **B**, **C**, **D** and **Unknown**.

* ***CLEAN UP:** Before moving on to the next activity, dispose of your slides in the biohazard glass disposal container. Wipe down the bench and microscope (if necessary) with 70% ethanol. Dispose gloves in the biohazard container.

Gram Staining Procedure:

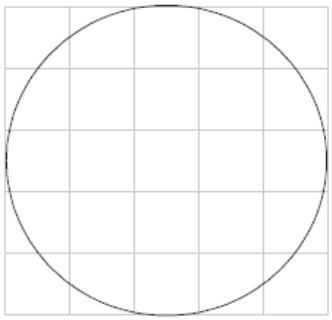
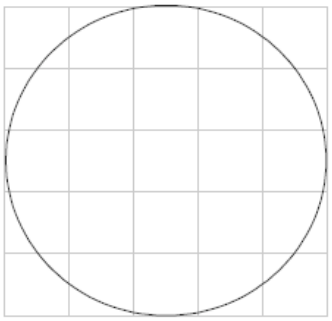


RESULTS

Draw and label your observations of all FIVE bacteria at **100x** and **1000x** magnifications. Refer to the picture below for different shapes of bacteria. *The color and shape of the bacteria should be exact*.

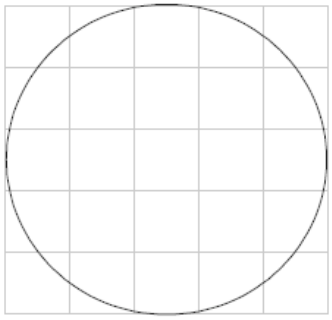
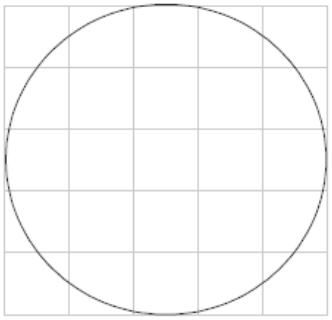
Magnification: **100x** Magnification: **1000x**

Bacteria Sample:_____
Gram stain color: _____
Gram stain (+/-): _____
Shape: _____

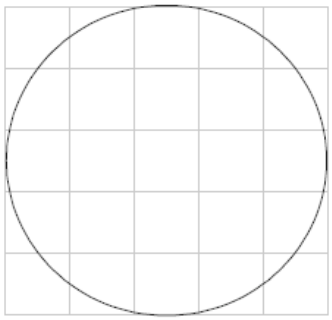
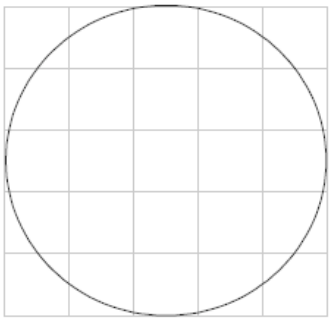
Magnification: **100x** Magnification: **1000x**

Bacteria Sample: _____
Gram stain color: _____
Gram stain (+/-): _____
Shape: _____

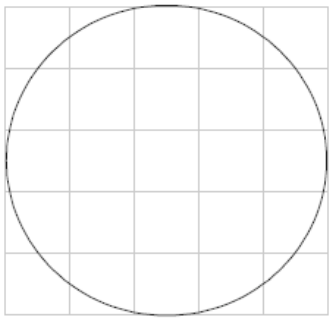
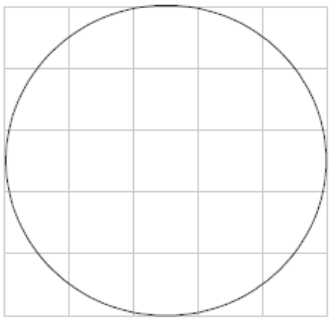
Magnification: **100x** Magnification: **1000x**

Bacteria Sample:_____
Gram stain color: _____
Gram stain (+/-): _____
Shape: _____

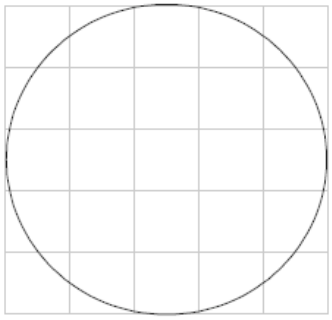
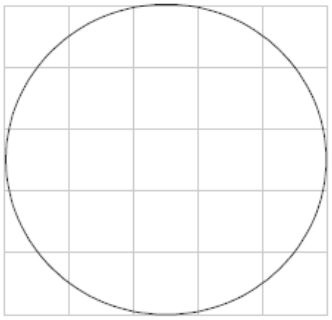
Magnification: **100x** Magnification: **1000x**

Bacteria Sample:_____
Gram stain color: _____
Gram stain (+/-): _____
Shape: _____

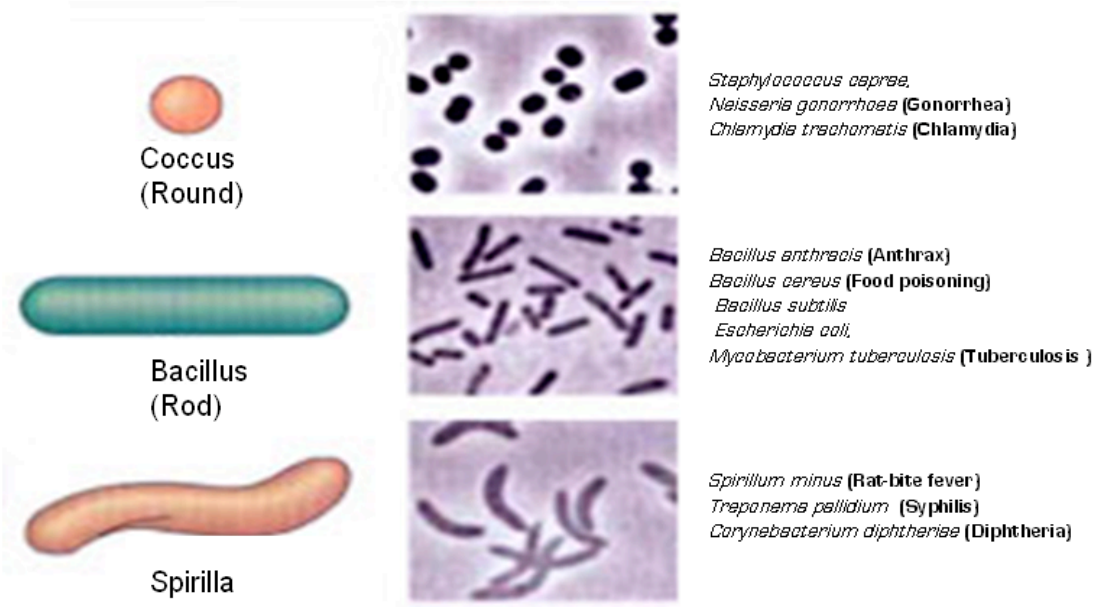



Magnification: **100x** Magnification: **1000x**

Bacteria Sample:_____
Gram stain color: _____
Gram stain (+/-): _____
Shape: _____

Reference for Bacteria Morphology (Shape):



DISCUSSION QUESTIONS

1. What characteristics can be determined in a Gram stain?

2. What can happen to make Gram-positive cells appear Gram-negative?

ACTIVITY 4: CATALASE TEST

Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O (water) and O_2 (oxygen).

$$2\ H_2O_2 + \text{catalase} \rightarrow 2\ H_2O + O_2$$

Hydrogen peroxide is often used as a topical disinfectant in wounds, and the bubbling that is seen is due to the evolution of O_2 gas. H_2O_2 is harmful to a cell; because of this,

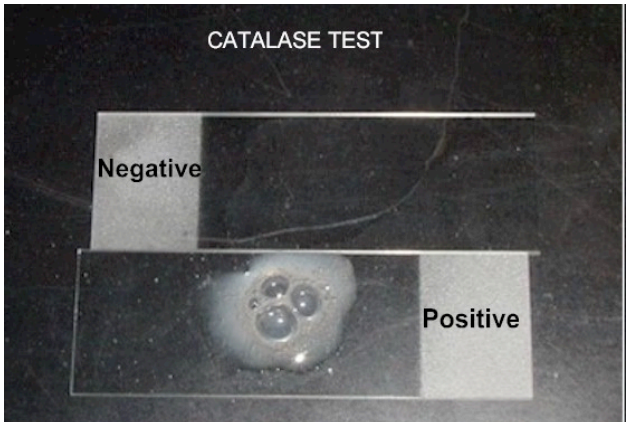
any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the peroxide. One of those ways is to make catalase. We will now test for the presence of catalase in the bacteria as a means to distinguish them.

MATERIALS SUPPLIED (work individually)

- 5 labeled petri dishes containing bacteria samples (A-D, Unknown)
- Glass slides
- 5 Wooden sticks
- 3% hydrogen peroxide solution
- Glass dropper
- Sharpie marker

PROCEDURE

1. Place a small amount of bacteria growth from your culture broth onto a clean microscope slide using a wooden stick.
2. Add a few drops of H_2O_2 solution onto the smear. If needed, mix with a wooden stick.
3. A **positive** result is the rapid evolution of O_2 gas as evidenced by bubbling.
4. A **negative** result is no bubbles or only a few scattered bubbles.
5. Dispose of your slide in the biohazard glass disposal container. Dispose of the wooden sticks in the biohazard sharps container.
6. Repeat experiment for all bacteria samples, using new slides and sticks.



RESULTS

Record your data in the following table:

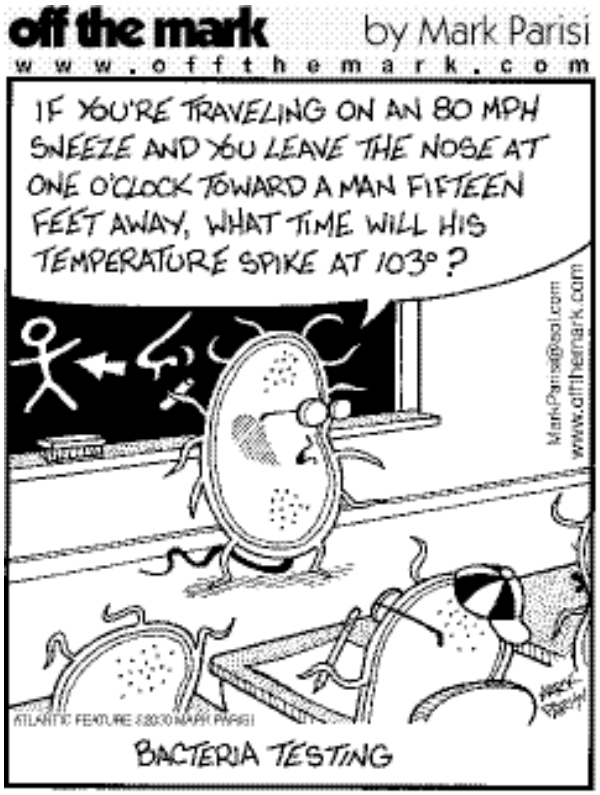
Bacteria Sample	Observations	Catalase test result (+/-)

DISCUSSION QUESTIONS

1. Which bacteria tested positive for catalase? Which tested negative?

2. What conclusions can you make about the unknown pathogen?

3. What is the purpose of using new slides and sticks for each bacteria species?



ACTIVITY 5: IDENTIFYING THE UNKNOWN PATHOGEN

Now we can finally put together all the information that we have obtained from the earlier activities to positively identify the unknown pathogen that is infecting our group. Summarize your results in a table and use your deductive and reasoning skills to identify the unknown sample.

Fill in the following table with the information from the various tests that you have performed:

Bacteria Sample	Scientific Name	Shape	Gram Staining (+/-)	Catalase Test (+/-)

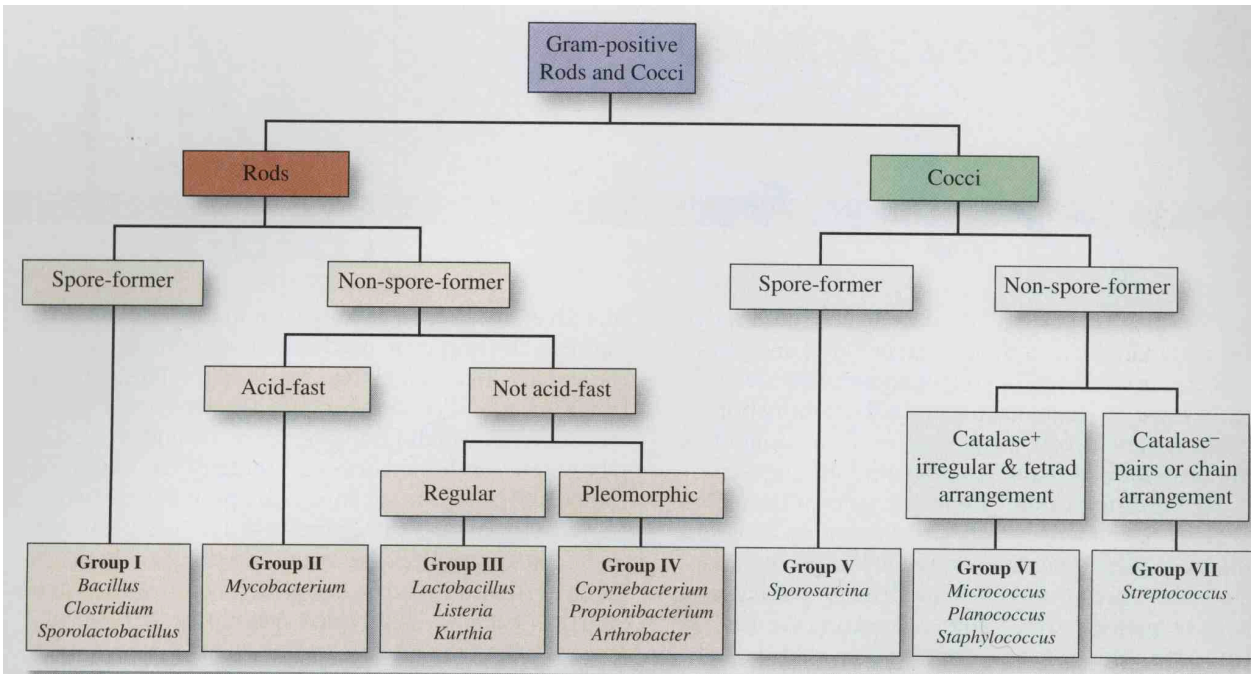
A				
B				
C				
D				
Unknown				

CONCLUSIONS

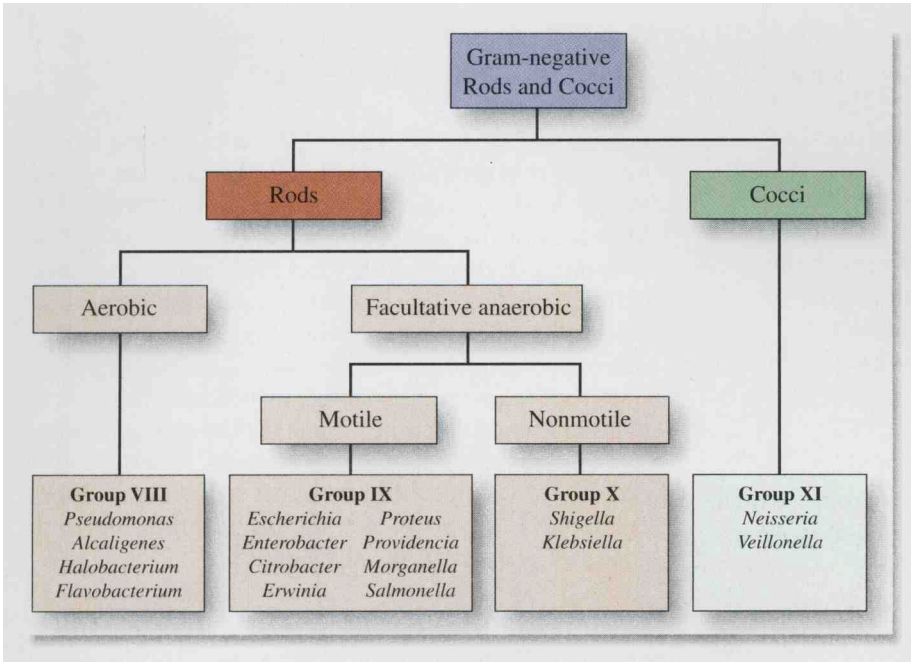
Summarize the characteristics of the unknown bacteria in 1 paragraph. Be descriptive.

By comparing its characteristics to known bacteria samples, we have identified the unknown infectious pathogen as _____.

Bergey's Classification of Gram-Positive Bacteria:



Bergey's Classification of Gram-Negative Bacteria:



ACTIVITY 6: ANTIBIOTIC TESTING

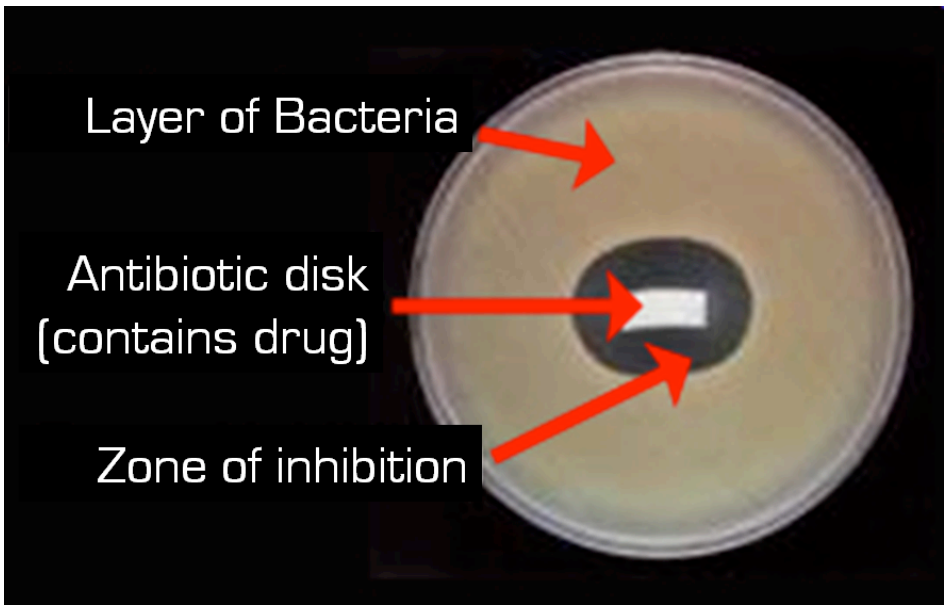
Antibiotics are a class of antimicrobials produced by living organisms which, even in tiny amounts, can inhibit the growth of or kill bacteria. In order to find an effective cure

for the pathogen infecting our facility, we have to determine which antibiotic compound is the most effective against the infectious unknown pathogen by measuring zones on inhibition on bacterial culture plates. Following inoculation of an agar medium, discs containing antibiotics are placed onto the surface of the agar so that the antibiotic will diffuse into the agar. Inhibition of the organism is evident following incubation as a clear region around the disc, called a "zone of inhibition", in which no growth has occurred. The diameter of the zone of inhibition for an antibiotic indicates how effective it is for use in treating that particular infectious organism. We will now test the unknown pathogen against three different antibiotics and recommend the most effective one by measuring their zones of inhibition.

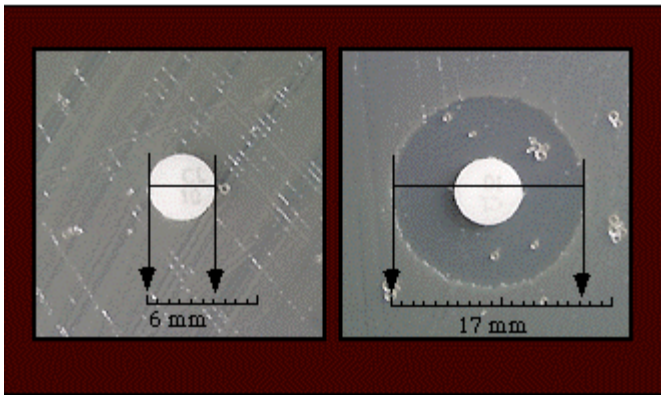
PROCEDURE

1. Put on a new pair of disposable gloves.
2. You have been given a Petri dish containing the unknown bacteria that has been divided into three equal areas. Each area contains a different antibiotic disc.
3. Examine each area for the presence of a zone of inhibition.
4. Keeping the lid on, measure the clear zones (in millimeters) using a ruler. Take two measurements for each antibiotic disc, then calculate the average diameter of the zone of inhibition for each antibiotic.

* ***CLEAN UP:** Put away the Petri dishes as instructed. Wipe down the bench (if necessary) with 70% ethanol. Dispose of gloves in the biohazard container.



Example zone of inhibition measurement:



RESULTS

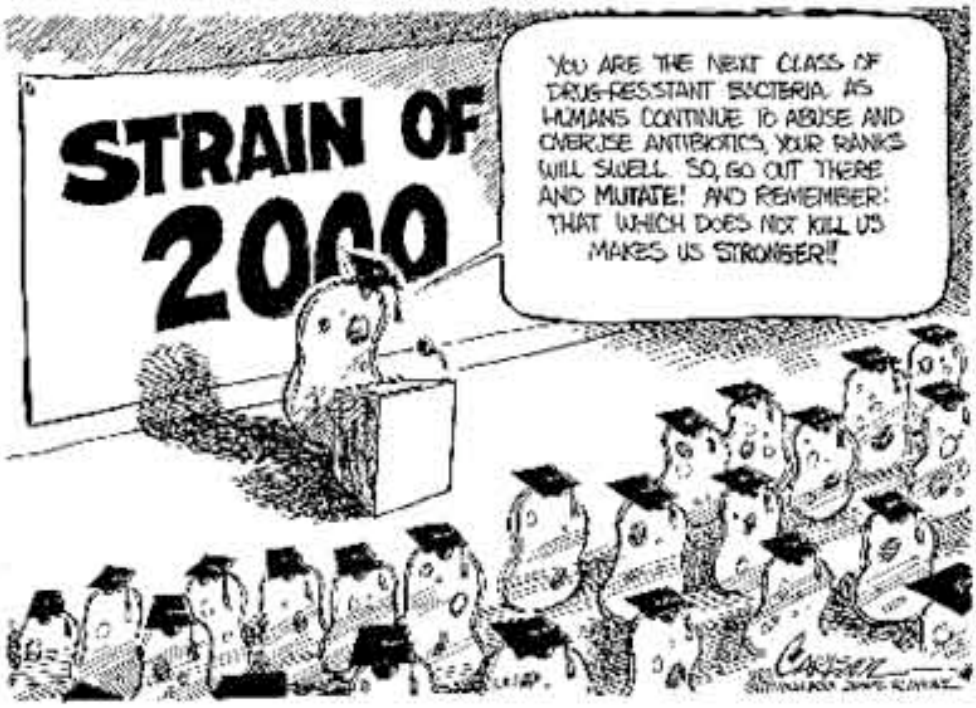
Record your data in the following table:

Type of Antibiotic	Zone of inhibition (mm)		Average Zone of Inhibition (mm)
	Trial 1	*Trial 2*	

DISCUSSION QUESTIONS

1. Determine if the bacteria is **resistant**, **intermediate** or **susceptible** to each of the three antibiotics.

2. Which antibiotic has the most potential for treating this infectious disease?


STRAIN OF 2000
YOU ARE THE NEXT CLASS OF DRUG-RESISTANT BACTERIA. AS HUMANS CONTINUE TO ABUSE AND OVERUSE ANTIBIOTICS, YOUR RANKS WILL SWELL. SO, GO OUT THERE AND MUTATE! AND REMEMBER: THAT WHICH DOES NOT KILL US MAKES US STRONGER!!