Edvo-Kit # 161

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How Clean is Clean? Testing the Effectiveness of Antibacterial Cleaners

Experiment Objective:

In this experiment, students will explore the properties of bacteria by determining susceptibility to antibiotics and testing the effectiveness of household cleaners.

See page 3 for storage instructions.

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EDVOTEK®

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Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

Components	Storage	Check (✓)
• Ampicillin	4°C	❑
• *E. coli* BactoBeads™	4°C	❑

Store all components below at room temperature.

This experiment is designed for 10 lab groups.

IMPORTANT READ ME!

This experiment contains antibiotics. Students who have allergies to antibiotics such as penicillin, ampicillin, kanamycin or tetracycline should not participate in this experiment.

REAGENTS & SUPPLIES

- Bottle of ReadyPour™ Agar, sterile ❑
- Bottle of Recovery Broth ❑
- Sterlie water ❑
- Petri plates, large ❑
- 15 mL Conical tubes ❑
- Plastic microtipped transfer pipets ❑
- Wrapped 10 mL pipet (sterile) ❑
- Swabs (sterile) ❑
- Disks for antibiotics ❑

Requirements

- Microwave oven
- Adjustable Volume Micropipette (5-50 µL and 50-200 µL recommended) and tips
- Incubation Oven (37°C)
- Pipet pumps or bulbs
- Marking pens
- Gloves and safety goggles
- A Variety of household cleaners

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Background Information

Microorganisms, or "microbes", are living organisms that are too small to be seen with the naked eye. Examples of microbes include archaea, fungi, protists, and bacteria (Figure 1). When these microbes are responsible for diseases and infections they are called pathogens. Humans can spread these pathogens to each other and cause illness within the population.

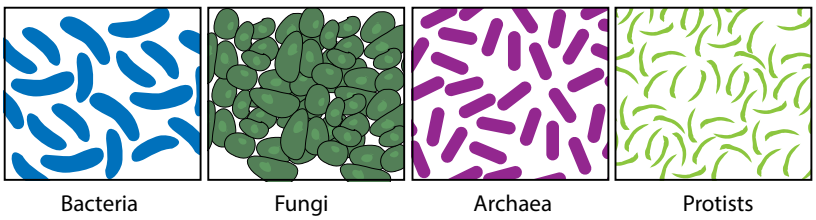


Figure 1: Examples of microbes.

Microbes aren't all harmful, though! Take bacteria for example. Bacteria are single-celled organisms that are microscopically small. They have no organelles but are surrounded by a cell membrane. Bacteria are a large and diverse group of organisms. While some like pathogenic *E. coli* can cause sickness, many others are beneficial to our health. Microbes help keep us healthy, so when we try to get rid of pathogenic microbes, we often upset the normal makeup of our microbiome. This is why when someone is prescribed antibiotics for an infection, their doctor will likely recommend they supplement their diet with probiotics and yogurt to help replace the good bacteria that's killed during the treatment. We have microbes all over our bodies, on our skin, in our nose, and in our digestive system. Our digestive system is lined with microorganisms that help us to digest food, fend off pathogenic microbes, secrete nutrients, and keep us healthy. Because microorganisms and bacteria and are so small, they make up only 1-3% of the body's *mass*. However, they outnumber human *cells* in our body 10:1!

Interestingly, microbes can be both helpful and harmful to other microbes as well. There are approximately $5x10^{30}$ bacteria on earth, and around 40 million bacterial cells in just 1 gram of soil. Scientists began noticing strange interactions between bacteria very long ago. The ancient Greeks, for example, used mold to treat infections. Fast forward hundreds of years, and in 1928 Sir Alexander Flemming noticed that a common fungus, *Penicillium notatum*, was able to inhibit the growth of *Staphylococcus*, a class of bacteria that causes many infections. With help, he was able to identify the substance that *Penicillium notatum* was secreting and named it penicillin. This new substance was able to inhibit the growth of bacteria, and the first official antibiotic was discovered.

The discovery and distribution of penicillin radically changed the world. During World War II, penicillin was able to save 12-15% of the Allied forces' lives. Its ability to cure typhoid fever, tuberculosis, and pneumonia, among other diseases, led to its nickname "the miracle drug". However, it was soon apparent that this drug would not be the end to all bacterial diseases. Some people were allergic to penicillin and had severe allergic reactions. Penicillin could also have other side-effects in the body such as skin rashes and hives. Eventually, bacterial strains were becoming resistant to penicillin.

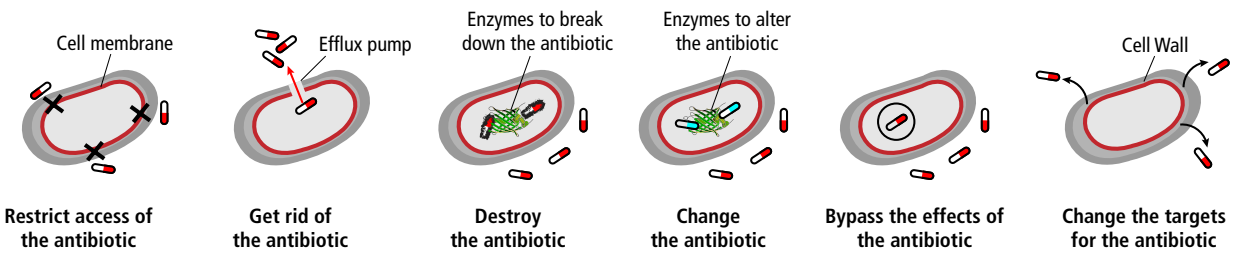


Figure 2: Resistance Mechanisms (Defense Strategies).

Antibiotic resistance happens when bacteria stop responding to antibiotics. There are many possible "resistance mechanisms" that bacteria have developed so as not to be susceptible to antibiotics (Figure 2). One major mechanism is by incorporating genes for antibiotic resistance into their DNA. These genes can be found on small pieces of DNA called plasmids. Plasmids can transfer genetic information from one bacteria to another, so other germs may also become resistant. As antibiotic use becomes more common, bacteria are able to utilize these resistance genes so that only the resistant bacteria multiply. Then, they pass that resistance onto the next generation. Eventually antibiotics will not be able to eliminate the infection.

Scientists and researchers have been coming up with new and better antibiotics to treat bacterial diseases. Other common antibiotics include ampicillin and doxycycline, both of which are commonly used to treat ear infections. However, as antibiotics become more prevalent in medicine, antibiotic resistance becomes more common.

When a doctor identifies an infection, they need to make a decision about the course of treatment. Today, doctors have a set treatment course for most infections. These recommended treatment courses came from scientific experiments showing which antibiotics each bacteria were most susceptible to. One common way for scientists to analyze the effectiveness of an antibiotic is on a particular infection through the Kirby-Bauer disk diffusion assay.

The Kirby-Bauer test (KB test) uses disks containing antibiotics to test the susceptibility of a particular microbe to antibiotics. Bacteria from an infected patient is cultured and plated onto a Luria broth (LB) agar plate. LB is a nutrient dense substance that is mixed with agar to form plates that hold a gel for bacteria to grow on. The bacteria cannot digest the agar, but they can access the nutrients in the LB. Bacteria grow exceptionally well on LB plates if they don't have any interference.

The disks in the KB test are made from thick filter paper cut into precise pieces and applied with antibiotics. There are many different possible experiments that can be done this way, but the two most common are using varying concentrations of one antibiotic and testing multiple antibiotics. When a bacteria is susceptible to the antibiotic on the disk, a zone of inhibition forms. A zone of inhibition is an area surrounding the disk without bacterial growth. The larger the zone of inhibition, the more susceptible the bacteria is (Figure 3).

Figure 3: Agar plate featuring zones of inhibition.

By using disks with varying levels of antibiotic, you can determine a certain antibiotic's minimum inhibitory concentration (MIC) for a specific bacteria. The MIC is the lowest concentration of antibiotic that is able to create a zone of inhibition. The MIC can be determined by plotting the diameter of the zone of inhibition in relation to the concentration of antibiotic. Doctors use the MIC score in a number of ways to treat patients with a bacterial infection. First, it can allow them to choose the correct dose. Due to the side effects of most antibiotics, clinicians want to find the antibiotic that takes the lowest concentration to be effective. Second, if a patient isn't responding to a dose of antibiotics, they can test to see if the concentration they are giving the patient is correct.

The second major role that the KB test is used for in medicine is to determine which antibiotic to use. There are many different antibiotics available today, and some bacteria are already resistant to certain antibiotics. Using the KB test, doctors can find out which antibiotics the infection is most susceptible to and will have the greatest chance of eliminating the pathogenic bacteria.

In this lab, you will be doing two experiments to explore the properties of bacterial growth. First, you will determine the MIC of a bacterial strain to Ampicillin in order to determine the correct dosage to kill an infection. Then, you will test different household cleaners to determine which is most effective in preventing bacterial growth.

Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will explore the properties of bacteria by determining susceptibility to antibiotics and testing the effectiveness of household cleaners.

LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:
- Record your observations in your lab notebook.

After the Experiment:
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Laboratory Safety

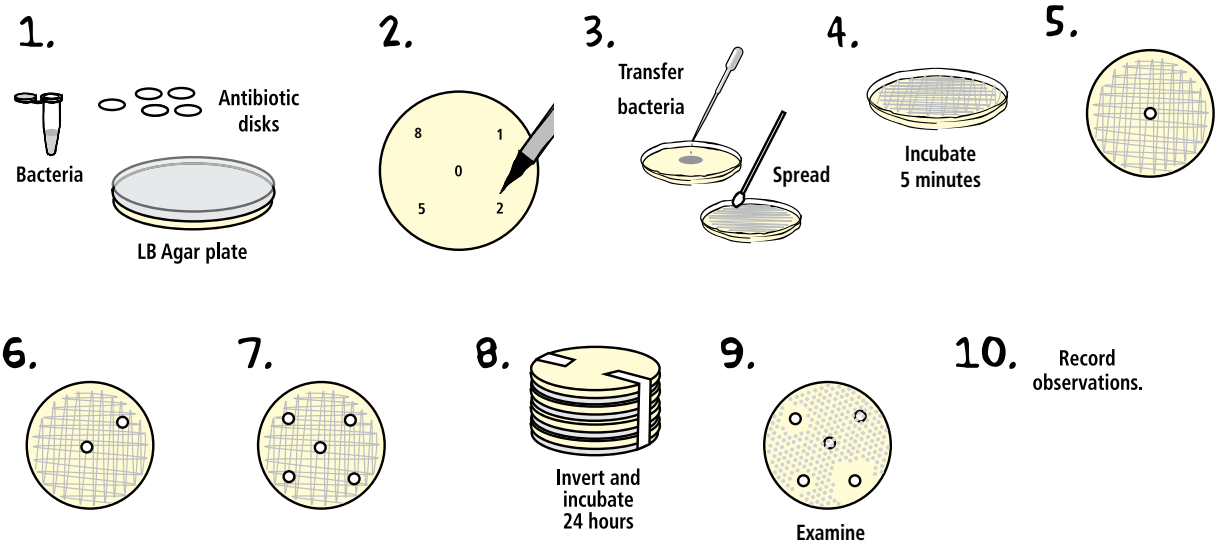
IMPORTANT READ ME!

This experiment contains antibiotics. Students who have allergies to antibiotics such as penicillin, ampicillin, kanamycin or tetracycline should not participate in this experiment.

Wear gloves and safety goggles

1. Wear gloves and goggles while working in the laboratory.
2. Exercise extreme caution when working in the laboratory - you will be heating and working with high voltages, which could be dangerous if performed incorrectly.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. The bacteria used in this experiment is not considered pathogenic. Regardless, it is important to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.
   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.
   B. All materials, including petri plates, pipettes, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:
   - Autoclave at 121°C for 20 minutes.
     Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.
   - Soak in 10% bleach solution.
     Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.
5. Always wash hands thoroughly with soap and water after working in the laboratory.
6. If you are unsure of something, ASK YOUR INSTRUCTOR!

Module I-A: Finding the MIC



1. **COLLECT** bacteria, LB agar plate, and antibiotic disks from instructor.
2. Using the image above as a reference, **FLIP** your plate upside down and **LABEL** areas 0, 1, 2, 5, and 8. The labels should be at least 1.5 cm from the edge of the dish. **FLIP** your plate back upright.
3. Using a transfer pipette, **TRANSFER** the bacteria onto the agar. **SPREAD** around the entire plate with a sterile cotton swab.
4. **INCUBATE** at room temperature for 5 minutes to allow the bacteria to soak into the agar.
5. Using forceps, **PLACE** the 0 antibiotic disk on top of the "0" spot on the agar. This disk does not have antibacterial agents on it and should not inhibit bacterial growth.
6. Using forceps, **PLACE** the "1" antibiotic disk on top of the "1" spot on the agar. This disk has 1 µg of antibiotic on it.
7. **REPEAT** step 6 for the remaining 3 antibiotic disks.
8. **PLACE** plates upside-down in incubator and **INCUBATE** for at least 24 hours.
9. **EXAMINE** plates for bacterial growth.
10. **RECORD** results in your lab notebook.

TABLE A

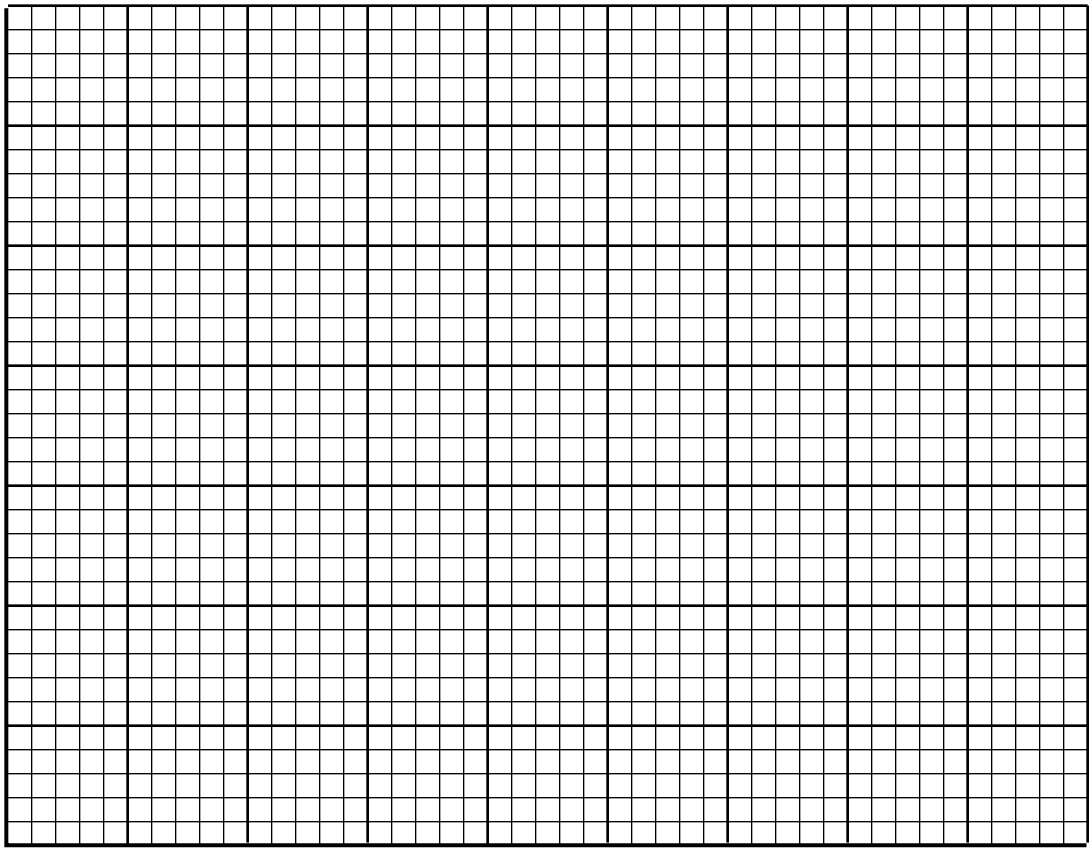
Amt. of Antibiotic	Zone of inhibition (cm)
8 µg	
5 µg	
2 µg	
1 µg	
0 µg	

Module I-B: Determining the MIC

1. Using a ruler, **MEASURE** the diameter of the zone of inhibition for each plate. **RECORD** in Table A. **GRAPH** your results below.

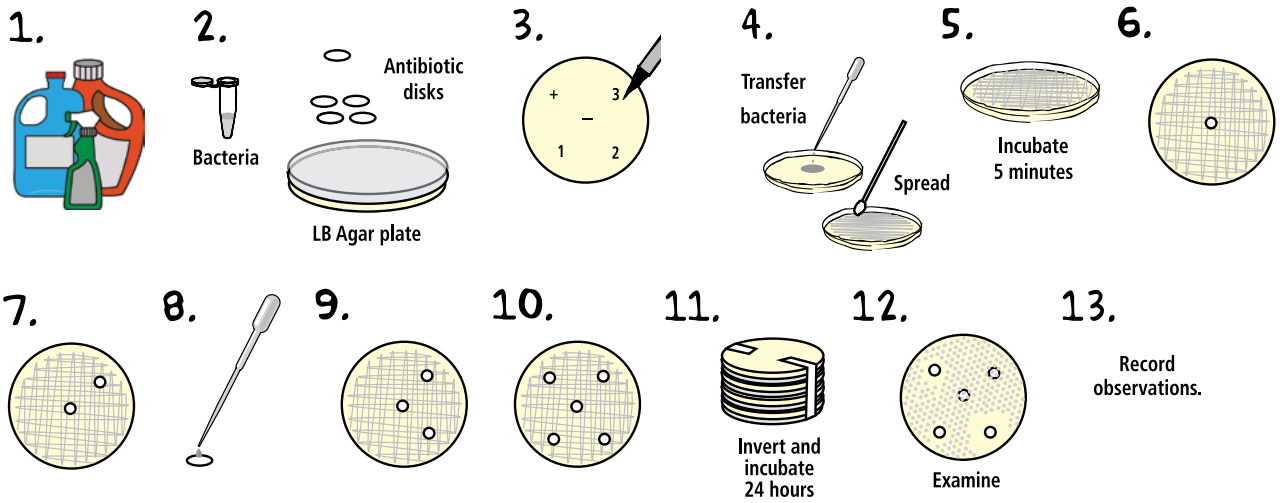
2. **DETERMINE** the MIC.

The Clinical and Laboratory Standards Institute (CLSI) has classified *E.coli* as being susceptible to an antibiotic if its zone of inhibition is >15 mm (1.5 cm). Using your graph below, or a graphing software, determine at which concentration the bacteria are susceptible to the antibiotic.



MIC: _____

Module II: Which Cleaner Works Best?



1. **COLLECT** 3 household cleaners to test. **LABEL** them "1", "2", and "3" and record in Table B.
2. **COLLECT** bacteria, LB agar plate, antibiotic disk (+ control), and 4 plain disks from instructor.
3. Using the image above as a reference, **FLIP** your plates upside down and **LABEL** areas -, +, 1, 2, and 3.
4. **FLIP** your plate back upright. Using a transfer pipet, **TRANSFER** the bacteria onto the agar. **SPREAD** bacteria around the entire plate with a sterile cotton swab.
5. **INCUBATE** at room temperature for 5 minutes to allow the bacteria to soak into the agar.
6. Using forceps, **PLACE** one plain disk on top of the "-" spot on the agar. This disk does not have antibacterial agents on it and should not inhibit bacterial growth.
7. Using forceps, **PLACE** the antibiotic disk on top of the "+" spot on the agar. This disk has antibiotics and will inhibit bacterial growth.
8. Using a small transfer pipet, pipet **ONE DROP** of the household cleaner you designated as sample 1 onto a plain disk. Let sit for 1 minute.
9. Using forceps, **PLACE** disk on top of the "1" spot.
10. **REPEAT** steps 8 and 9 for the remaining 2 household cleaners.
11. **PLACE** plates upside-down in incubator and **INCUBATE** for at least 24 hours.
12. **EXAMINE** plates for antibacterial properties in the 3 household cleaners.
13. **RECORD** your results in your lab notebook or in Table B.

TABLE B

Label	Cleaner	Zone of inhibition (cm)
+	---------	
-	---------	
1		
2		
3		

Most efficient cleaner:

_____

Study Questions

1. What are antibiotics?
2. What are some positives and negatives of using antibiotics to treat infections?
3. Did different cleaners give the same or different results? Why do you think that happened?
4. How can we fight antibiotic resistance?

Instructor's Guide

IMPORTANT READ ME!

This experiment contains antibiotics which are used to show antibiotic resistance. Students who have allergies to antibiotics such as penicillin, ampicillin, kanamycin, or tetracycline should not participate in this experiment.

Preparation For:	What to do:	When:	Time Required:
Modules I & II	Pour LB Agar Plates	2-7 days before use.	1 hour
	Prepare Antibiotic Disks	Up to 3 days before the lab.	1 hour
	Prepare and aliquot bacteria	Up to 1 week before the lab.	10 min.
Module II	Ask students to bring in, or acquire, household cleaners	Anytime before the lab.	None

Red = Prepare immediately before module. Yellow = Prepare shortly before module. Green = Flexible / prepare up to a week before the module.

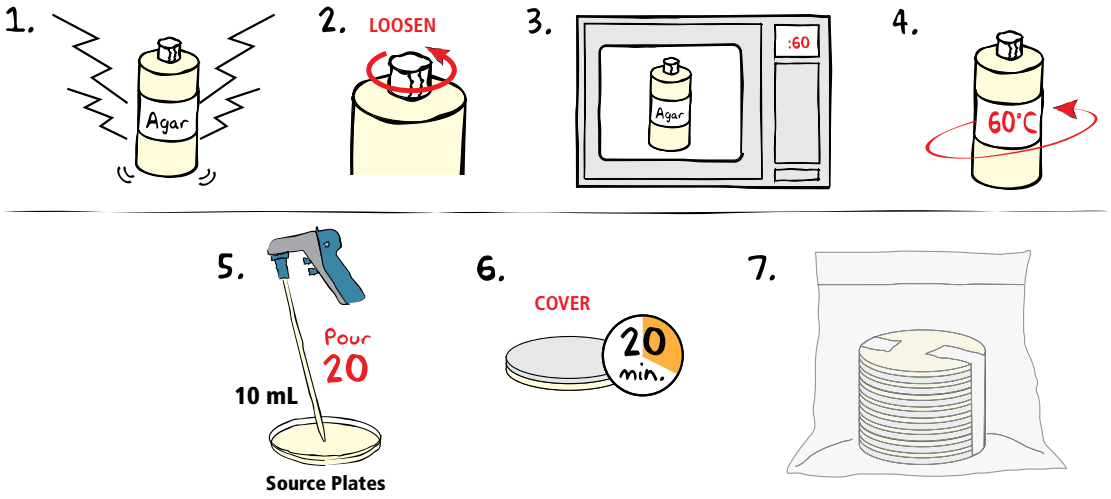
For Module I, each group will need:

- 1 LB agar dish
- 1 antibiotic disk with 25 µg of antibiotic
- 1 antibiotic disk with 12.5 µg of antibiotic
- 1 antibiotic disk with 5 µg of antibiotic
- 1 antibiotic disk with 2.5 µg of antibiotic
- 1 antibiotic disk with sterile water
- Forceps
- 200 µL of bacteria
- Sterile cotton swab

For Module II, each group will need:

- 1 LB agar dish
- 1 antibiotic disk with 5 µg of antibiotic (+)
- 1 antibiotic disk with sterile water (-)
- 3 plain antibiotic disks
- Forceps
- 200 µL of bacteria
- Sterile cotton swab
- 3 household cleaners to test

Pouring LB Agar Plates



1. **BREAK** solid ReadyPour™ Agar into small chunks by vigorously squeezing and shaking the plastic bottle.

2. **LOOSEN**, but DO NOT REMOVE, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. ***CAUTION: Failure to loosen the cap prior to heating may cause the bottle to break or explode.***

3. **MICROWAVE** the ReadyPour™ Agar on high for 60 seconds to melt the agar. Carefully remove the bottle from the microwave and mix by swirling the bottle. Continue to heat the solution in 30-second intervals until the agar is completely dissolved (the amber-colored solution should be clear and free of small particles).

4. **COOL** the ReadyPour™ Agar to 60°C with careful swirling to promote even dissipation of heat.

5. **POUR** 10 mL of the cooled ReadyPour™ Agar into each of the twenty large petri dishes using a 10 mL pipet and pipet pump.

6. **LEAVE** plates at room temperature overnight to solidify.

7. **STORE** plates at room temperature for no more than two days. Plates should be inverted and placed in a sealable plastic bag to ensure that they do not dry out. Inverted and bagged plates can also be stored in the refrigerator (4°C) for up to two weeks.

PreLab Preparations

PREPARE ANTIBIOTIC

1. Add 1 mL of sterile water to the vial of Ampicillin. Vortex or pipet to dissolve completely.
2. Label four 15 mL conical tubes: 8 µg, 5 µg, 2 µg, and 1 µg.
3. Add 4 mL of sterile water to the "8 µg" tube and transfer the 1 mL of dissolved ampicillin into it. Invert or vortex to mix.
4. Transfer the remaining 2 mL of "8 µg" solution into the 15 mL conical tube labeled "5 µg" and add 1.2 mL sterile water. Invert or vortex to mix
5. In the tube labeled "2 µg", mix 1.5 mL sterile water and 1 mL ampicillin from the "5 µg" solution tube. Invert or vortex to mix.
6. In the tube labeled "1 µg", mix 1 mL sterile water and 1 mL ampicillin from the "2 µg" solution tube. Invert or vortex to mix.
7. Dilutions can be stored for up to 1 week in the refrigerator (4°C).

PREPARE DISKS

1. Label 5 petri dishes: 8 µg, 5 µg, 2 µg, 1 µg, and 0 µg. Use forceps to transfer 10 disks into the 8 µg, 2 µg, and 1 µg dishes and 20 disks into the 5 µg and 0 µg dishes..
2. Spread disks out in each plate so they aren't touching one another.
3. Take the cover off of the dish labeled "8 µg". Pipette 5 µL (1 drop if using a transfer pipet) of the 8 µg stock solution onto each disk. Put the cover back onto the dish.
4. Take the cover off of the dish labeled "5 µg". Pipette 5 µL (1 drop if using a transfer pipet) of the 5 µg stock solution onto each disk. Put the cover back onto the dish.
5. Take the cover off of the dish labeled "2 µg". Pipette 5 µL (1 drop if using a transfer pipet) of the 2 µg stock solution onto each disk. Put the cover back onto the dish.
6. Take the cover off of the dish labeled "1 µg". Pipette 5 µL (1 drop if using a transfer pipet) of the 1 µg stock solution onto each disk. Put the cover back onto the dish.
7. Take the cover off of the dish labeled "0 µg". Pipette 5 µL (1 drop if using a transfer pipet) of sterile water onto each disk. Put the cover back onto the dish.
8. For module 1, each group should receive one disk of each concentration (5 disks per group). For module 2, each group will receive one disk with no antibiotics ("0 µg", students will label as "-"), one disk with antibiotic ("5 µg", students will label as "+"), and an additional 3 disks with nothing on them. Disks can be placed on paper towels labeled with their concentration to be handed out.

TABLE C

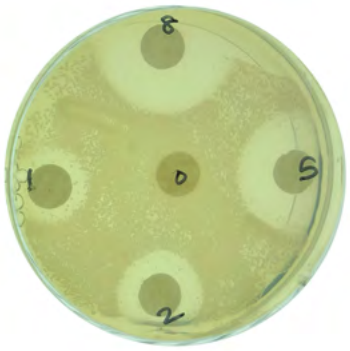
Label on Dish *(amt. of antibiotic on disk)*	Antibiotic Concentration
8 µg	1.6 mg/mL
5 µg	1 mg/mL
2 µg	0.4 mg/mL
1 µg	0.2 mg/mL

PREPARE BACTERIA

1. Pour all of the BactoBeads™ into the bottle of recovery broth. Invert to mix.
2. Label 20 microcentrifuge tubes as "Bacteria".
3. Aliquot 500 µL of the Recovery Broth/Bacteria mix to each tube.
4. Bacteria can be used immediately or aliquoted and stored in tubes for up to 1 week in the refrigerator (4°C).

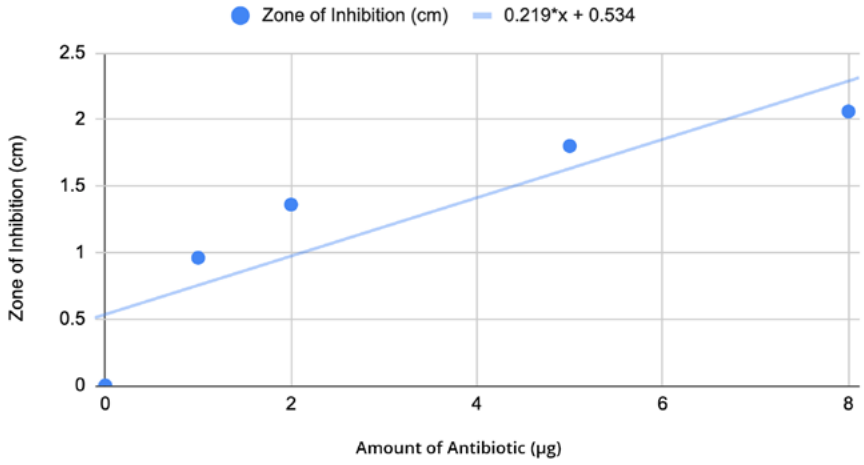
Experiment Results and Analysis

MODULE I-A



Label on Dish *(amt. of antibiotic on disk)*	Antibiotic Concentration
8 µg	1.6 mg/mL
5 µg	1 mg/mL
2 µg	0.4 mg/mL
1 µg	0.2 mg/mL

MODULE I-B



The MIC is 4.41 µg (or 0.885 mg/mL).

NOTE: The MIC for your class may vary slightly depending on when the antibiotic was made and how long the plates were incubated.

MODULE II

Label	Cleaner
+	---------
1	Generic grocery store "All Purpose Cleaner"
2	Seventh Generation Disinfecting Multi-surface Cleaner
3	Myer's Clean Day Multi-surface Everyday Cleaner
4	Lysol Kitchen Pro Antibacterial Cleaner

NOTE: In your student's plates they will only use 3 cleaners, one positive control, and one negative control. We suggest using a wide range of cleaners and letting the students explore whatever cleaners they choose.

Please refer to the kit insert for the Answers to Study Questions