

Antibiotic Effects Kit

Teacher's Guide

Background:

The use of antibiotics has revolutionized clinical medicine. Antibiotics allow modern doctors to treat a far wider range of diseases more effectively and economically. The net result of the development of antibiotics has been a historically unprecedented rise in the health and quality of life of a large portion of the world's population.

Technically, an antibiotic is a biochemical produced by a microorganism that inhibits the growth of, or kills, another microorganism. Biochemists, however, are now able to synthesize many antibiotics and derivatives of antibiotics. These substances are technically referred to as semi-synthetic antimicrobial agents. In practice, however, the whole spectrum of antimicrobial biochemicals has adopted the name "antibiotics".

Many antibiotics trace their roots to the *Penicillium* mold which Alexander Fleming serendipitously noticed inhibiting the growth of the bacterium *Staphylococcus aureus* on an agar dish. Fleming was able to isolate a chemical from the mold which produced the same type of inhibition. He dubbed this chemical "penicillin."

Penicillin is a member of a class of antibiotics known as **cell wall inhibitors**. The penicillin molecule contains a beta-lactam ring which disrupts cell wall synthesis in growing bacteria by mimicking a component of the peptidoglycan layer of the cell wall. The result is a weaker and more permeable peptidoglycan layer. Autolysins ("self-lysing" enzymes produced by the cell) attack the peptidoglycan layer, and osmotic pressure from the liquid outside the cell causes the cell to burst (lyse), killing the bacterium. Note that penicillin does not affect mature bacteria because their cell walls have already been formed with a normal peptidoglycan layer.

Penicillin and related antibiotics are primarily specific against Gram-positive bacteria because of the higher percentage of peptidoglycan in the cell walls of these organisms. The presence of a thinner layer of peptidoglycan which is shielded by a thick layer of proteins, phospholipids and lipopolysaccharides accounts for the resistance of the cell walls of most Gram-negative bacteria to penicillin. Because penicillin and most related antibiotics are only effective against certain Gram-positive organisms, they are referred to as **narrow spectrum antibiotics**.

Other antibiotics, such as chloramphenicol and tetracycline, are termed **inhibitors of protein synthesis**. Chloramphenicol and tetracycline function by bonding to a cell's ribosomes, preventing peptide bonds and thus proteins from forming. Since all bacteria depend on protein synthesis to some degree, chloramphenicol and tetracycline are effective against a greater variety of microorganisms. For this reason, they are called **broad spectrum antibiotics**.

One way to test the effectiveness of an antibiotic against a specific microorganism is the Bauer-Kirby test which measures the degree of inhibition produced by antibiotic disks (disks which contain a known amount of antibiotic) when placed on an agar dish inoculated with the desired microorganism. The antibiotic disks produce zones of inhibition (clear areas of no growth) which are measured and compared to a standardized table in order to determine the susceptibility of the microorganism to the different antibiotics used in the test. For a specific antibiotic, the larger the zone of inhibition, the more effective the antibiotic is at killing or inhibiting the growth of the microorganism. The zones of inhibition produced by two different antibiotics, however, are not directly comparable because of differing abilities to diffuse through agar. Based on the results of the Bauer-Kirby test a given microorganism is declared either sensitive, intermediately sensitive or resistant to a given antibiotic.

Based on this information, a doctor can prescribe an antibiotic regimen for treating a disease. Of course, other factors such as side-effects and the ability of the antibiotic to remain in an active form inside the human body must be taken into account.

Experiment Design:

This kit is designed to help acquaint students on a fundamental level with antibiotics and the Bauer-Kirby test. Because the kit uses **Easygel** instead of agar dishes, comparison of the data collected in this experiment with the data used to evaluate standard Bauer-Kirby tests is not possible. **Easygel** has a different set of diffusion factors which makes such a comparison inaccurate. The results, therefore, can only be used for internal comparison.

The kit assumes a certain degree of background knowledge, most of which can be provided in one lecture that covers the development, types and uses of antibiotics. The preceding section of this guide, entitled "Background", is designed to refresh your memory and summarize the knowledge which your students will need to understand. This kit can also be adapted for use at a less advanced level as a lesson in health and sanitation.

Each group of students will inoculate each of their three **Easygel** dishes with a different culture and then place three different antibiotic disks on each dish. After 24 hours, the students will measure the diameter of the zones of inhibition which have resulted. From this information they should be able to determine which antibiotics are most efficacious against which organisms and offer reasons as to why this should be.

During the first class period, we suggest that the teacher start the experiment even prior to taking attendance if possible. This will allow the liquid ample time to set up prior to the end of class (it takes approximately 40 minutes for the dish to completely gel). Steps 1-3 should take 5-10 minutes.

Materials:

1. 30 units Total Count **Easygel** (must be used with Easygel petri dishes)
2. 30 **Easygel** pretreated petri dishes
3. 3 bacterial broth cultures
 - a. *E. aerogenes* (Gram-negative rods)
 - b. *B. cereus* (Gram-positive rods)
 - c. *S. lutea* (Gram-positive cocci)
4. 3 tubes of antibiotic disks (**disks must be kept frozen until use**)
 - a. 30 penicillin
 - b. 30 tetracycline
 - c. 30 chloramphenicol
5. 6 sterile 1 mL droppers
6. Teacher's Guide (You may copy the background portion for the student's use if desired)
7. Student Worksheet (Make 1 copy for each student)

Not included: 10 pairs of clean forceps (1 for each group for gripping antibiotic disks).

Procedure for Teachers:

Day 1 of the experiment:

(In the interest of time we are recommending that the teacher do the actual inoculation of the culture into the bottle of **Easygel** prior to the class period on the day of the experiment.)

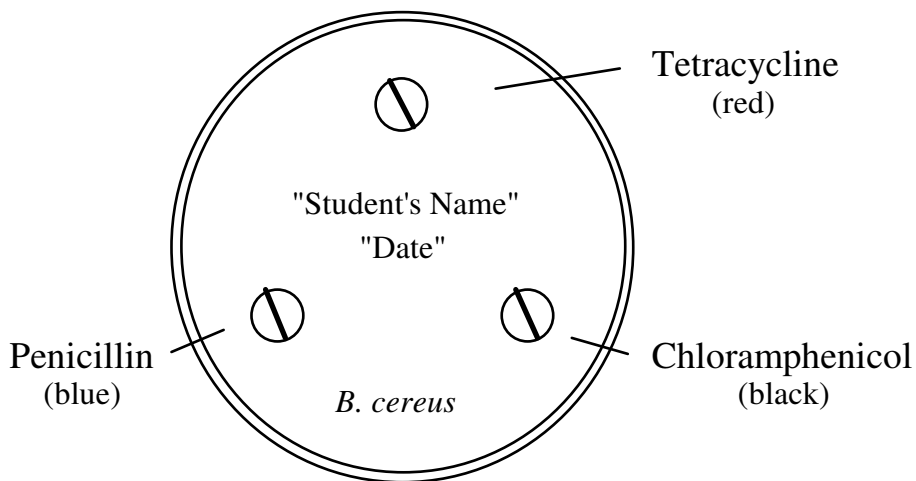
Prior to the class period, the teacher should:

- 1) Prepare all 30 petri dishes as follows: (Use a wax pencil or permanent marker)
 - a. Label the top of 10 of the dishes with a 1
 - b. Label the top of 10 of the dishes with a 2
 - c. Label the top of 10 of the dishes with a 3
- 2) Prepare all 30 bottles of **Easygel** as follows:
 - a. Label the caps of 10 of the bottles with a 1
 - b. Label the caps of 10 of the bottles with a 2
 - c. Label the caps of 10 of the bottles with a 3
 - b. Inoculate the bottles of set 1 with *Enterobacter aerogenes* as follows:

Loosen the caps of all 10 bottles so you will be able to lift the cap off with one hand. Shake the *Enterobacter aerogenes* bottle to re-suspend the culture. Remove the cap of the culture bottle. Open the bulb end of a package containing a sterile dropper and remove, touching only the bulb. Place the tip of the dropper in the culture and remove approximately 1 mL. Remove the cap of the first **Easygel** bottle and place 2 drops in the bottle. Replace cap and inoculate the other 9 bottles of set #1 the same way. Tighten the **Easygel** bottles, and recap and tighten the culture bottle.
 - c. Repeat the inoculation procedure for set #2 with *Bacillus cereus* and set #3 with *Sarcina lutea*.

Class Period

1. Divide students into ten groups. Provide each group with 3 pretreated petri dishes and 3 **Easygel** bottles (one each from sets 1, 2 and 3).
2. Instruct the students to swirl their **Easygel** bottles, open them and pour them into their appropriate petri dishes (bottle #1 into dish #1, etc.) They should then replace the lid and swirl and rock the petri dishes gently until the liquid completely covers the bottom of the dish.
3. Instruct the students that after the dishes have been swirled, and the bottoms covered with the liquid, the dishes should not be picked up until the end of class. If they need to be moved, they can gently slide them across the bench.
4. Have the students put their name or initial, and date on the top of the dish. Also have them write the name of the culture on each dish (#1 = *E. aerogenes*, #2 = *B. cereus*, #3 = *S. lutea*).
5. Demonstrate how the bottles were inoculated with the three bacterial cultures. Note that each bottle was inoculated with over 100,000 bacteria. Have the students look at the liquid in their petri dishes (without moving the dishes) and notice the clarity of the liquid. After growth occurs, the dish will appear cloudy except where there is antibiotic inhibition.
6. Provide each student group with a forceps (tweezers). Pour the tetracycline antibiotic disk set onto a piece of paper.
7. Instruct students to use their forceps to pick up three tetracycline disks and place them on their own clean piece of paper to carry back to their petri dishes. They should then drop one of each of the disks on each of the dishes. Discs that fall upside-down will work. Do not touch the discs after they have touched the liquid in the petri dish. **At no time should the disks be touched by hand.**
8. Repeat using the penicillin and chloramphenicol disks. The dishes should look similar to this:



9. Forty to forty-five minutes after the petri dishes have been poured, they can be picked up and moved. Instruct the students to seal the dishes using two small pieces of tape so that it cannot fall open (allow room for air flow so that the dish can "breathe"). Collect dishes and incubate upright at 25-35° Celsius for 24 hours. For more information on incubation see Appendix II. Students should end the day by washing their hands thoroughly.

Day 2 of the experiment:

10. Remove dishes from the incubator and return to student groups. Students should now be instructed to measure the diameter of the zones of inhibition in millimeters by placing a ruler against the bottom of the petri dishes. Data gathered should be recorded in the chart on the student worksheet. **Petri dishes should not be opened under any circumstances.** (For further information on interpreting results see Appendix III.)
11. Dispose properly of used petri dishes and culture bottles. (For further information see Appendix III.)

Study Questions: (for answers see Appendix V)

1. Which antibiotic seems to be most effective in inhibiting *E. aerogenes*? Which antibiotic seems to be least effective? Justify your choices.
2. Which organism was penicillin most effective against? least effective against? How can you explain this difference?
3. If the zones of inhibition of two antibiotic disks (A and B) on a *Sarcina lutea* dish measure 17 and 18 mm respectively, which antibiotic is more effective against *Sarcina lutea*. Why?
4. Do the antibiotics kill the bacteria or only inhibit the growth? Design a procedure to determine whether the antibiotics are bacteriostatic (inhibit) or bactericidal (kill).
5. If the antibiotic concentration is doubled, will the growth zone be twice as large? Explain.
6. If a doctor were prescribing medicine for a person with a systemic *E. coli* infection (a systemic infection is one that is spread throughout the body by the circulatory system), which antibiotic might the doctor choose? Hint: *E. coli* belongs to the same general class of bacteria as *Enterobacter aerogenes*.

Appendix I: Incubation.

If possible, dishes should be incubated in an incubator set at 32-35° Celsius. If an incubator is not available, dishes may be kept at room temperature. However, bacterial growth will be slower. Slower bacterial growth may necessitate reading dishes after 48 hours instead of 24 and may also result in larger zones of inhibition. If possible, wrap the petri dishes in a towel and place in a warm spot. Be careful, however, that temperatures do not exceed 35°.

Appendix II: Interpreting Results.

In the case that the zone of inhibition is not a uniform circle, the diameter can be determined in one of two ways:

- 1) in the case that the zone of inhibition has no degree of uniformity, measure several representative "diameters" and average them in order to determine an approximation of the true diameter
- 2) in the case that the zone of inhibition is basically uniform with the exception of one area of aberration, simply ignore the area of aberration and measure the diameter across an area of the zone of inhibition which was not affected by the aberration.

Appendix III: Disposing of Materials.

Because the materials used in this kit contain living cultures, they should be sterilized before they are disposed. If an autoclave is available, heat at 15 lbs. pressure for 15 minutes. If an autoclave is not available, several other methods will suffice:

1. Place dishes and culture bottles in a pressure cooker and cook at 15 lbs. for 15 minutes. (This is the same as autoclaving.)
2. Place dishes and culture bottles in an oven-proof bag, seal it, and heat in an oven at 300° Fahrenheit for 45 minutes.
3. Place dishes and culture bottles in a large pan, cover with water and boil for 45 minutes.

Appendix IV: Answers to Study Questions.

1. Both chloramphenicol and tetracycline are effective against *E. aerogenes*, whereas penicillin produces little to no inhibition. Chloramphenicol generally has a slightly larger zone of inhibition than tetracycline; however, this difference can be explained in terms of either diffusion factors or the relative potencies of the antibiotics against *E. aerogenes*. *E. aerogenes* is a Gram-negative organism. Since its cell walls contain a small percentage of peptidoglycan, it is able to readily resist the narrow spectrum penicillin.
2. Penicillin should exhibit the greatest degree of effectiveness, judged by the size of the zone of inhibition, against the Gram-positive coccus *Sarcina lutea*. This is due to penicillin's action as a cell-wall inhibitor and the high percentage of peptidoglycan present in the cell walls of *S. lutea*. *B. cereus* also has a high percentage of peptidoglycan in its cell walls, but other factors seem to provide it with a greater degree of resistance. Penicillin was probably least effective against the Gram-negative *E. aerogenes*. *E. aerogenes'* low percentage of peptidoglycan makes it resistant to the effects of penicillin.
3. Because both the potency of the antibiotic and the antibiotic's diffusion factor determine the size of the zone of inhibition, you can not conclusively state that B is a more potent antibiotic than A on the basis that its zone of inhibition is a millimeter larger.
4. Penicillin is considered bacteriostatic in that it does not alter mature cells, but instead works by preventing these bacteria from reproducing. With regard to developing bacteria, however, penicillin has to be considered bactericidal because it interferes with the development of their cell wall and allows the osmotic pressure of the environment to cause lysis (a rupturing of the cell wall). Chloramphenicol and tetracycline are both bactericidal because they function by interfering with a bacterium's ability to synthesize vital proteins, disabling and eventually killing the bacterium. An experiment may involve exposing bacteria to the antibiotic, and then finding out if it will grow after it has been removed from the antibiotic source and placed in/on a suitable growth medium.
5. The size of the zone of inhibition depends on both the concentration of the antibiotic and its ability to diffuse through the agar medium over time (the diffusion factor). For two different antibiotic concentrations, the diffusion factor is potentially, but not necessarily, the same. Therefore, doubling the concentration does not mean that either the diameter or volume of the zone of inhibition will be doubled. In other words, the relationship between the antibiotic concentration and the size of the zone of inhibition is potentially linear, but in reality is probably more complex.
6. Since *E. coli* is similar to *E. aerogenes*, the student should select either chloramphenicol or tetracycline for the purpose of treating the systemic infection. Of the two, tetracycline is probably the antibiotic of choice because of chloramphenicol's toxicity to human cells. Of course, when actually prescribing antibiotics, numerous other factors must be taken into consideration such as the antibiotic's ability to stay potent in the human body and attack the area of infection.