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Antibiotic Effects Kit
Teacher's Guide

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 biochemical have adopted the name "antibiotics".

Historically, 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 named this new discovery "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 prevent 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 the bottom part of an R-Card®, will exhibit zones of inhibition when 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 by two different antibiotics, however, are not directly comparable because of differing abilities to diffuse through the gel. 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 such information, a doctor can prescribe an antibiotic regimen for treating a disease. Of course, other factors such as antibiotic 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 method. Because the kit uses R-CARD® 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. The R-CARD® has a different set of diffusion factors which makes such a direct 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 for understanding. 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 R-CARDs, each containing three different antibiotic disks, with one mL of broth culture. The broth culture should be deposited in the center of the R-CARD® base surrounded by the three antibiotic disks. The top should then be lowered on the water-containing RCARD® bottom and gelling will be complete within 2-3 minutes, at which time the R-CARD® may be handled and moved to be incubated. After 24-48 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 allow ample time to set up prior to the end of class.

Materials contained in one kit:

1. Total Count R-CARD® (each contains three antibiotic disks)
   Penicillin disks (blue)
   Tetracycline disks (red)
   Chloramphenicol disks (black)
2. 3 bacterial broth cultures
   a. Enterococcus faecalis (Gram-positive short chains)

b. Bacillus cereus (Gram-positive rods)
c. E.coli (Gram-negative rods)

3. Sterile 1 mL droppers
4. Teacher's Guide (You may copy the background portion for the student's use if desired)
5. Student Worksheet (Make 1 copy for each student)
6. Not included: forceps (1 for each group would be useful)

Procedure for Teachers

Day 1 of the experiment:

Prior to the class period, the teacher should: (Students may want to do these steps if capable)

1. Prepare all as follows:(Use a wax pencil or permanent marker (Fine tip "Sharpie")

2. R-CARDS to be used by each group will be labelled on the # line as follows:
   Label ⅓ of the R-CARD® with an "A" (Enterococcus)
   Label ⅓ of the R-CARD® with a "B" (Bacillus")
   Label ⅓ of the R-CARD® with a "C" (E.coli)

3. Label all the R-CARD® on the Date line with the current date and time of day. Therefore, there will be three sets of R-CARD®. ---Set #1 will be inoculated with "A" (Enterococcus), Set #2 will be inoculated with "B" (Bacillus), and Set #3 will be inoculated with "C" (E.coli).

NOTE: *IF YOU HAVE NOT USED ALL OF THE R-CARDS WITH THIS FIRST EXPERIMENT, YOU MAY WANT TO SAVE THEM FOR ANOTHER CLASS, OR YOU MAY WANT TO EXPERIMENT FURTHER BY TESTING OTHER MATERIALS THAN THE THREE ANTIBIOTICS BY MODIFYING THE CARDS BY REMOVING THE ANTIBIOTIC DISKS AND REPLACING THEM WITH OTHER DISKS (THAT YOU MAKE OF ABSORBANT PAPER) IMPREGNATED WITH OTHER MATERIALS SUCH AS MOUTHWASH, GARLIC JUICE, ETC.*

Preparation of the inoculum:

1. Provide each student group with 3 R-CARD® (one each from each set numbered 1, 2 or 3).

2. Each group will also receive (a) one sterile 1mL dropper. (b) three (3) pure broth cultures of the test bacteria--- one bottle labeled "A" (Enterococcus faecalis), one bottle labeled "B" (Bacillus cereus), and one bottle labeled "C" (E.coli).

3. To make your inoculum, first take the Broth cultures that matches your R-CARD® (if your card is a #1, you will get broth culture "A")

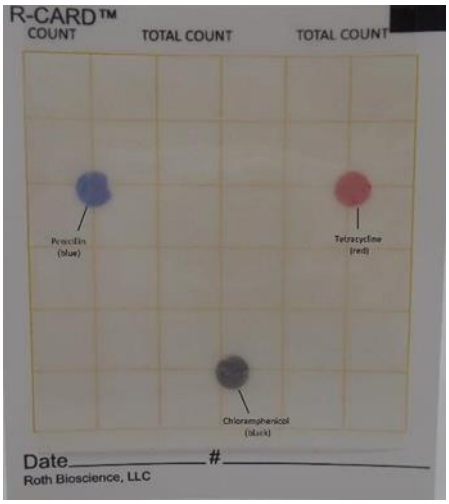
Inoculation Procedure:

1. Open the broth culture (shake gently first) and then remove the dropper from its sheath. Insert the dropper end into the bottle of broth culture and suck up 1 mL into the dropper.

2. Lift the R-CARD® top and carefully deposit the 1 mL broth culture onto the center of the R-Card® and drop the top onto the pool of broth culture so that it spreads over the card base in a circle covering the three antibiotic disks.

3. Allow the card to stand for 2-3 minutes to gel and it can be picked up and moved into your incubator.

4. Repeat using the penicillin and chloramphenicol disks. The R-CARD® should look similar to this:

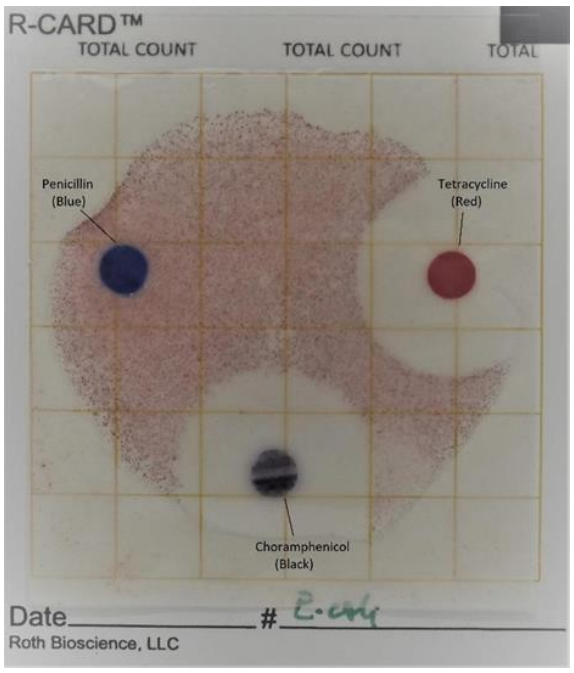


5. Incubate at 25-35º Celsius for 24 hours. The gelled R-CARD® may be stacked on top of others. For more information on incubation see Appendix II. Students should end the day by washing their hands with soap and warm water thoroughly.

Day 2 of the experiment:

1. Remove R-CARD® 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 top of the R-CARD®. Data gathered should be recorded in the chart on the student worksheet. ***Cards should not be opened by lifting the top film under any circumstances without detailed instructions.*** (For further information on interpreting results see Appendix III.)

2. Dispose properly of used R-CARD® 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.coli? 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 on a dish measure 17 and 18 mm respectively, which antibiotic is more effective against the bacterium? 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). (you may call Roth Bioscience LLC for advice or help)

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?

Appendix I: Incubation.

If possible, R-CARDs should be incubated in an incubator set at 32-35º Celsius. If an incubator is not available, cards may be kept at room temperature. However, bacterial growth will be slower. Slower bacterial growth may necessitate reading cards after 48 hours instead of 24 and may also result in larger zones of inhibition. If possible, put the R-CARDs in a sealed container and place in a warm, dark place. Be careful, however, that temperatures do not exceed 35º.

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 disposal. 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 R-CARDs and open culture bottles in a pressure cooker and cook at 15 lbs. for 15 minutes. (This is the same as autoclaving.)

2. Place R-CARDs and open culture bottles in an oven-proof bag, seal it, and heat in an oven at 300º Fahrenheit for 45 minutes.

3. Place R-CARDs and open 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.coli, 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.coli. E.coli 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. B. cereus and E.coli 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.coli. E.coli.' 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 cannot 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 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 B.cereus, 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.