Antibiotic Effects Kit

Student Worksheet

The use of antibiotics has revolutionized clinical medicine. Antibiotics allow modern doctors to treat a far wider range of diseases more effectively and economically. By definition, an antibiotic is a biochemical produced by a microorganism that inhibits the growth of, or kills, another microorganism. 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 swabbed with the desired microorganism. The antibiotic disks produce zones of inhibition (clear areas of no growth) which are measured in order to determine the susceptibility of the microorganism to the different antibiotics used in the test.

Day 1 of the experiment:

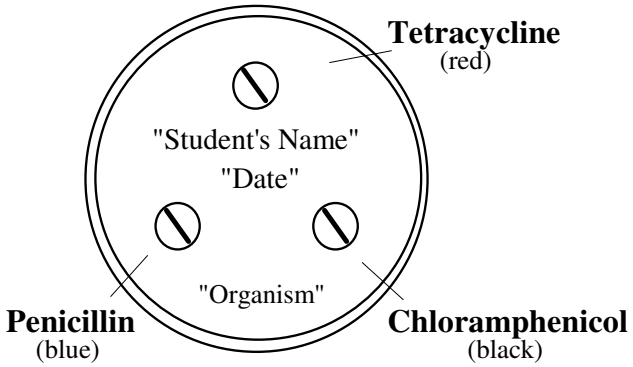
1. Your group will be provided with 3 petri dishes and 3 bottles of **Easygel**.
2. Swirl each bottle of **Easygel** and pour the liquid from bottle #1 into petri dish #1 as instructed by your teacher. Repeat with bottle and dish sets #2 and #3. Gently swirl and rock the dish while it sits on the counter top until the bottom is covered by the liquid.
3. Once the bottom of the petri dish is covered, do not pick the dish off the counter until instructed to do so by your teacher. If the dishes need to be moved, they can be gently slid to a new location.
4. Write your name or initials, and the date on the top of the petri dish. Then write the name of the culture with which each dish is inoculated (Dish #1 = E. aerogenes, dish #2 = B. cereus, dish #3 = S. lutea).
5. Notice how clear the liquid is in each dish. This will become cloudy by day 2 of the experiment.
6. Using your forceps, take 3 tetracycline disks from the teacher and place on a clean sheet of paper. Take them back to your counter, and place one of these disks on the gel in each dish. It is alright if they fall upside down on the gel.
7. Repeat with the penicillin and chloramphenicol disks. Try to place them on the dish as the illustration shows. However, once they are in the dish, do not try to pick them up or move them. **At no time should the disks or the gel be touched by hand.**
8. When the teacher instructs you to do so, seal the petri dishes with two one-inch pieces of tape. Incubate dishes upright at 25-35º Celsius for 24 hours. Wash hands using soap and warm water.

Day 2 of the experiment:

9. Measure the diameter of the zones of inhibition in millimeters by placing the ruler against the bottom of the petri dishes. Record results in Chart I. **Petri dishes should not be opened under any circumstances.**

Study Questions:

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 method 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. A doctor is prescribing medicine for a person with a systemic *E. coli* infection (a systemic infection is disseminated throughout the body by the circulatory system). Which antibiotic might a doctor choose? Hint: *E. coli* belongs to the same class of bacteria as *Enterobacter aerogenes*.



Dish Diagram

Organism	Chloramph.	Penicillin	Tetra.
E. aerogenes			
B. cereus			
S. lutea			

Chart 1: Zones of Inhibition (diameter in mm)