

Note: this lab is completed online. Visit the following address and click on "Lab 3"

<http://labs.7bscience.com/unit-9.html>

**Purpose:** The purpose of this lab is to see how antibiotics affect bacteria. In this activity you will observe the effects of antibiotics on bacteria that were cultures in a petri dish.

### Part One - Background

Fill in the missing blanks using the information on the web site.

For decades medical doctors have been giving patients \_\_\_\_\_ to help

fight infections. An antibiotic is a substance that \_\_\_\_\_

\_\_\_\_\_. There are a variety of antibiotics. Not all

antibiotics work on every bacteria. Therefore it is important to know which antibiotics

are most effective against bacteria. How do you determine that?

This lab shows you one method of measuring the effectiveness of an antimicrobial agent against bacteria grown in culture. This is called the \_\_\_\_\_

\_\_\_\_\_ method, and here is how it works. The bacteria of

interest is swabbed uniformly across a culture plate. Then a filter-paper disk, covered

with the \_\_\_\_\_ to be tested, is placed on the surface of the agar. The

compound diffuses out from the filter paper into the agar. The concentration of the

compound will be \_\_\_\_\_ next to the disk, and will decrease gradually as

distance from the disk increases. If the compound is effective against bacteria at a

certain concentration, no \_\_\_\_\_ will grow wherever the concentration in the

agar is greater than or equal to that effective concentration. This region where no

colonies of bacteria grow is called the "\_\_\_\_\_."

Thus, the size of the zone of inhibition is a measure of the compound's effectiveness:

Name \_\_\_\_\_

Period \_\_\_\_\_

Date \_\_\_\_\_

the larger the clear area around the filter disk, the more effective the compound.

(Second paragraph taken from [Sciencebuddies.org](http://Sciencebuddies.org))

### Part Two - Observations and Data

In class we prepared petri dishes following the Kirby-Bauer disk-diffusion method. On each dish we added the bacteria *Bacillus subtilus* and three antibiotic discs: penicillin, streptomycin, and tetracycline. We also added a blank disc to act as the control. The purpose of the control is two-fold. First, it shows us what normal growth of bacteria looks like. Second, it shows us that the antibiotics, and not the paper, are affecting the bacteria. We left the petri dishes in an incubator over night so they could grow. The next day we observed the affects of the antibiotics on the bacteria.

Below you fill find a sample image of what a similar lab looks like. The person who conducted this lab used the same antibiotics except streptomycin. In the place of streptomycin they used amoxicillin, another common antibiotic.

### Procedure

1. Measure the zone of inhibition around each antibiotic disk. To do this, measure the diameter of the zone of inhibition. *Picture on next page of worksheet.*
2. Record this data in the appropriate space on your data table.

Antibiotic	Diameter of the Zone of Inhibition (mm)
A. Erythromycin	
B. Chloramphenicol	
C. Cephalothin	
D. Tetracycline	
E. Vancomycin	
F. Novobiocin	
G. Streptomycin	
H. Penicillin	



Picture source: Microbe Library, <http://archive.microbelibrary.org/ASMOnly/Details.asp?ID=3023>

### Part Three - Analysis

Answer the following questions. Some of the answers may be found in your background information and from your data.

1. What is an antibiotic? \_\_\_\_\_  
\_\_\_\_\_
2. What is the zone of inhibition? \_\_\_\_\_  
\_\_\_\_\_

**Name**

**Period**

**Date**

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3. Using the Kirby-Bauer disk-diffusion method, how can you tell which antibiotic is most effective? \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

4. Which antibiotic was the least effective against the bacteria in the picture? How do you know? \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

5. Which antibiotic was the most effective against the bacteria in the picture? How do you know? \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

6. What is the purpose of putting a control disk, or blank disk, into the petri dish?

\_\_\_\_\_  
\_\_\_\_\_