Antibiotic Sensitivity testing: The Kirby-Bauer Disk Diffusion Test

INTRODUCTION

Certain bacteria can display resistance to one or more antibiotics. Determining bacterial **antibiotic resistance** – whether a bacterium can survive in the presence of an antibiotic - is a critically important part of the management of infectious diseases in patients. The **Kirby-Bauer (K-B) disk diffusion test** is the most common method for antibiotic resistance/susceptibility testing. The results of such testing help physicians in choosing which antibiotics to use when treating a sick patient.

The Kirby-Bauer (K-B) test utilizes small filter disks impregnated with a known concentration of antibiotic. The disks are placed on a Mueller-Hinton agar plate that is inoculated with the test microorganism. Upon incubation, antibiotic diffuses from the disk into the surrounding agar. If susceptible to the antibiotic, the test organism will be unable to grow in the area immediately surrounding the disk, displaying a **zone of inhibition** (see figure below). The size of this zone is dependent on a number of factors, including the sensitivity of the microbe to the antibiotic, the rate of diffusion of the antibiotic through the agar, and the depth of the agar. Microorganisms that are resistant to an antibiotic will not show a zone of inhibition (growing right up to the disk itself) or display a relatively small zone.

In this module, you will be testing a number of bacteria against various antibiotics. Following inoculation and incubation you will assess the results by observing whether any zones of inhibition are formed, recording their sizes, and comparing your results with those obtained by other class members.

DAY ONE ACTIVITIES

Materials needed:

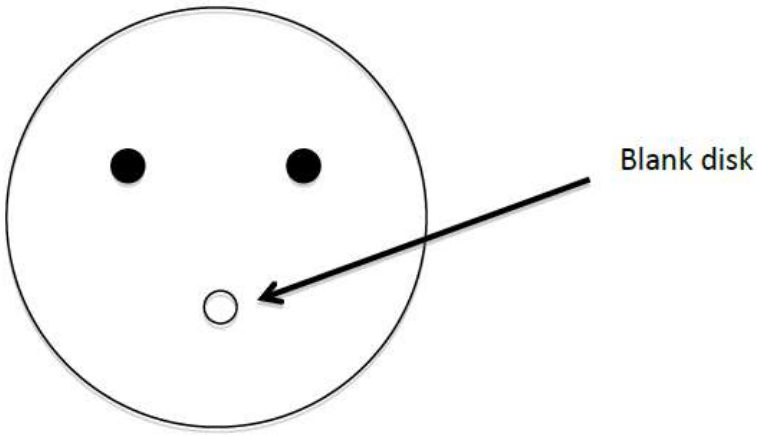
- Test tube rack
- Forceps
- Sterile swabs
- Two agar plates
- Cultures of 2 bacteria
- Antibiotic disks – each pair of students will be using:
  - (i) **TWO** different antibiotics
  - (ii) Antibiotic-free disks (BLANK)

Record antibiotics you are using

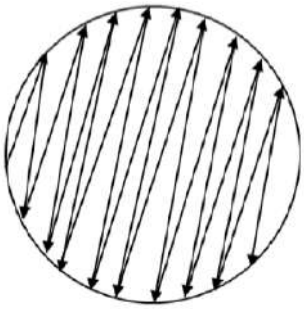
1. _____
2. _____

Procedure:

1. Label the agar plates with the usual 5 items. Also mark, using dots, where you will put the antibiotic disks AND the BLANK (antibiotic-free) disk.
   a. Disks should be a minimum of 20 mm apart.
   b. Disks should not be placed near the edge of the plate



2. Inoculate one plate with your first bacterium as follows:
    a. Heat the inoculating loop. Let it cool. Swab slant culture.
    b. Thoroughly swab the surface of the plate, making sure to cover the entire surface. See picture.
    c. Do NOT get more culture.
    d. Turn the plate approximately 60 degrees and repeat the previous step (2nd swabbing).
    e. Do NOT get more culture.
    f. Repeat the previous step (3rd swabbing).



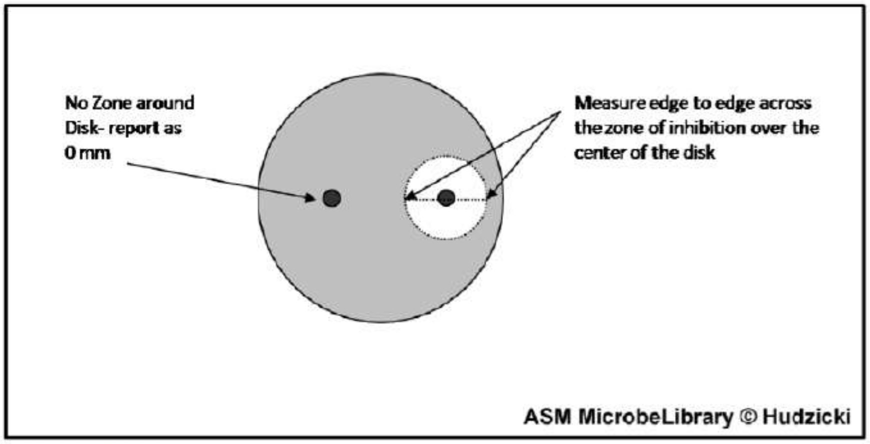
(Hudzicki, 2009)

3. Heat the loop to kill remaining bacterial.
4. Place one antibiotic disk onto the surface of the agar, using aseptic technique as follows:
    a. Heat the tips of the forceps by placing them in the flame of the Bunsen burner.
    b. Cool the forceps by waving them in the air for about 10 seconds.
    c. Carefully pick up your test disk with the forceps, and gently place it in the appropriate spot on the plate.
    d. To ensure that the disk is flat on the agar, gently push it down with the forceps.
    e. Reheat the tips of the forceps as above to kill any bacteria.
5. Repeat the procedure with the second antibiotic disk.
6. Repeat the procedure with the BLANK disk.
7. Repeat steps 1 – 6 on a new agar plate with your second bacterium.
8. Incubate both plates.

DAY TWO ACTIVITIES

Activity 1: Observation of K-B test plates.

1. Observe both agar plates.
2. If present, measure the **diameter** of the zone of inhibition **in mm** (see the two figures below), and record your results in the Table on the next page. If there is no zone present, record your result as 0 mm.
3. We will then compare the results for the entire class.



(Hudzicki, 2009)

Bacteria	Blank	Chloramphenicol	Streptpmyocin	Kanamycin	Tetracycline	Penicillin	Novobiocin	Meomyocin	Erythromycin

QUESTIONS

1. List the bacteria/antibiotic combinations that displayed zones of inhibition:

2. Did the Gram-positive and the Gram-negative bacteria produce different results? If yes, think about the possible reason(s).

3. Did the two bacteria used show different results for each antibiotic? If yes, what is one possible explanation for the observed differences?

4. What would happen if we didn't use aseptic technique to put the antibiotic disk on the plate? What would this look like?

5. What would happen if we increased or decreased the concentration of the antibiotic?

6. Antibiotics can be **bactericidal** (they kill the microbe) or **bacteriostatic** (they inhibit microbial growth, but do not kill). Did your K-B results allow you to determine whether the antibiotics used were bactericidal or bacteriostatic? Why?

7. When performing K-B testing, it is important that the test conditions are kept very standardized (e.g. same kind of media, agar depth, age of culture). Why?

8. Are there any other substances besides antibiotics we could evaluate using this method?

9. Does the presence of a zone of inhibition necessarily mean that the bacterium being tested is susceptible to the antibiotic?