

The Kirby-Bauer Antimicrobial Susceptibility Test

Introduction

Today you will perform a frequently used procedure called the Kirby-Bauer antimicrobial susceptibility test. Be sure to keep a record of which culture you have selected. The instructor will demonstrate how to inoculate an agar plate with a cotton swab in order to evenly distribute the culture over the surface of the agar. You will inoculate your own plate of Mueller-Hinton agar. To the inoculated plate, you will then aseptically add sterile filter paper discs which contain known concentrations of antibiotics. As soon as the antibiotic discs touch the agar, the antibiotics will begin to diffuse into the surrounding agar. Meanwhile, the bacteria you inoculated onto the agar will begin to grow. As the antibiotics diffuse and the bacteria grow, organisms which are inhibited by the antibiotic will fail to grow where the antibiotic is highest in concentration, leaving a clear area called the zone of inhibition. When performed in a clinical microbiology laboratory, the Kirby-Bauer test is a very standardized procedure. For instance, the petri plates are filled with a standard volume of Mueller-Hinton agar. The bacterial inoculum is also standardized by adjusting it to the optimal concentration of 1×10^8 to 2×10^8 colony forming units per ml. Several methods could be used to produce an inoculum of the proper concentration. One method uses a spectrophotometer. The inoculum will be at the right concentration at a final absorbance between 0.08 and 0.10 measured at 625 nm. A second commonly used method compares the inoculum density to a turbidity standard known as a 0.5 McFarland standard. Either method will result in a bacterial concentration between 1×10^8 and 2×10^8 colony forming units per ml.

During the next lab period, you will observe your plates, measure the diameter of each zone of inhibition, and use a standard interpretation table to interpret your results. Organisms may be susceptible, intermediate in resistance, or resistant, to the antibiotic. After you obtain results from your own plate, you will join the rest of your class to pool data and discuss the results.

First Lab Period - Materials and Procedure

1. Select a culture to test. This broth culture has been diluted to the appropriate concentration, equivalent to the 0.5 McFarland standard. Record the number of the culture you have selected here:

2. You will need the following materials at your workbench:
 - Container of sterile cotton swabs
 - Discard container for contaminated swabs
 - Petri plate containing Mueller-Hinton agar (one per student)

- Sterile filter paper discs impregnated with antibiotics
 - Forceps
 - Beaker containing 70% alcohol (The alcohol will be used to disinfect the forceps. **Your instructor will discuss how to flame your forceps safely.**)
 - Marking pen
 - Masking tape (optional)
3. Before inoculating your petri plate, examine the surface of the agar and the sides of the plate carefully. Be sure there are no visible colonies or drops of water on the agar. If there are colonies, the plate is contaminated and should be discarded. If there is water on the plate, use some sterile cotton swabs to gently remove the water.
 4. Resuspend the bacteria in your culture tube as you gently dip a sterile dry cotton swab into the culture. Remove the excess liquid from the swab by rolling the swab tip around the test tube above the level of the broth as you take the swab out of the tube.
 5. Inoculate your Mueller-Hinton agar plate in three directions to ensure even spreading of bacteria over the surface. The pictures used to illustrate this process only show you the direction of the swab's zig zag. The zig zags you will make on your plate should be much closer than illustrated and should cover the plate completely to create an even lawn over the plate.

- a) Pick up your Mueller-Hinton agar plate and zig zag your bacteria-damp cotton swab over the surface of the agar, covering it thoroughly from one end to the other.



- b) Without dipping the swab into your culture again, rotate the plate approximately 30 degrees (1/3 around) and repeat the zig-zag inoculation in the new direction.



- c) Finally, rotate the plate 30 degrees once again, and inoculate it for a third time. This three-way inoculation results in an even confluent growth of bacteria called a lawn.



- d) Discard your contaminated cotton swab into the proper container at your workbench.
6. Allow the plate to absorb the liquid for 2 to 3 minutes, then begin placing the filter paper antibiotic discs onto the surface of the agar. Five different antibiotic discs will fit in a 100-mm petri plate. Place one in the middle and space the other four discs evenly midway between the edge of the plate and the center disc. Use the illustration on the final page of this handout as a guide for disc placement.
 7. Regardless of the method you use to place the discs onto the agar surface (a disc dispenser, a cartridge, or forceps), you will tap the discs onto the agar surface using the tip of a disinfected forceps.
Disinfecting and flaming forceps is different than flaming a transfer loop and needle. When preparing the forceps, alcohol is the disinfectant, and while you do pass the forceps through the flame, the purpose is to merely burn the alcohol off, rather than to sterilize through heat. Place the forceps into the alcohol for several minutes, then pass them quickly through the flame only long enough to ignite the alcohol. **Be careful to keep the forceps away from the alcohol in the beaker and also be careful not to tip the forceps up so that a drop of flaming alcohol runs onto your fingers.**
 8. Label the agar side of the plate as instructed. Generally, your name or initials and the organism are required. If there are multiple lab sections, include your section identification. The plate will be incubated at 37°C for 24 hours and then refrigerated until your next lab period, when you will observe the results.

Second Lab Period - Materials and Procedure

1. Obtain your petri plate and observe the bacterial growth on the plate.
2. Clear areas where bacteria did not grow around the antibiotic discs are called zones of inhibition. Turn the plate so that you can place a ruler against the back of it and measure the diameter of each zone of inhibition in millimeters. Record your data in Table 1 below.
3. Using the zone diameter interpretative chart, determine how susceptible your organism is to each antibiotic.

TABLE 1. Individual culture antibiotic susceptibility

Antibiotic	Disc code	Diameter of zone of inhibition (mm)	Interpretation: susceptible, intermediate, or resistant

4. Bring your data to the blackboard and enter them into the tables drawn there. Record the class data in the tables on the following pages. Discuss these results with your classmates.

Analysis

1. For each species, identify resistance or susceptibility to the antibiotics used in the exercise.
2. Analyze the data you have for each species.
Are the data consistent for each species?
If the data are not consistent, what hypothesis can you derive?
If the data are not consistent, what conclusions can you make?
3. Describe how patient treatment would be affected by results such as these.

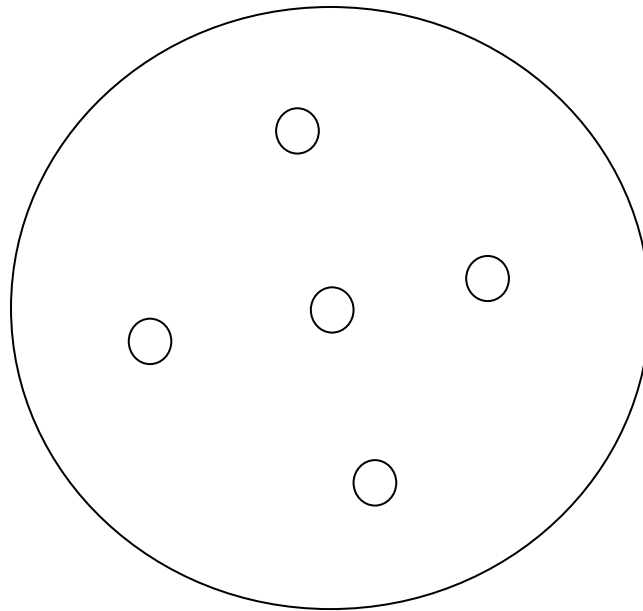


FIG 1. Guide to antibiotic disc placement in the petri plate.