

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.

McFarland standard

McFarland standards are suspensions of either barium sulfate or latex particles that allow visual comparison of bacterial density . A 0.5 McFarland standard is equivalent to a bacterial suspension containing 1.5×10^8 CFU/ml . A 0.5 McFarland standard may be prepared by adding 0.5 ml (% 1.175) barium chloride solution to 99.5 ml (%1) sulfuric acid resulting in a barium sulfate precipitation .



McFarland Standards provide a reference for standardization of bacterial suspensions used for susceptibility testing and other procedures that require a standardized inoculum .

There are many different procedures that microbiologists use to study the effects of various antimicrobial agents in treating an infection caused by different microorganisms. **Mueller Hinton Agar** is considered as best for the routine susceptibility testing since it has batch-to-batch reproducibility , low concentration of inhibitors of sulphonamide, trimethoprim and tetracyclines and produce satisfactory results for most of the non-fastidious pathogens.

Fastidious organisms which require specific growth supplements need different media to grow for studying the susceptibility patterns.

The Kirby Bauer test is a qualitative assay whereby disks of filter paper are impregnated with a single concentration of different antibiotics or any chemicals that will diffuse from the disk into the agar. The selected antibiotic disks are placed on the surface of an agar plate which has already been inoculated with test bacteria. During the incubation period, the antibiotics/chemicals diffuse outward from the disks into the agar. This will create a concentration gradient in the agar which depends on the solubility of the chemical and its molecular size. The absence of growth of the organism around the antibiotic disks indicates that, the respected organism is susceptible to that antibiotic and the presence of growth around the antibiotic disk indicates the organism is resistant to that particular antibiotic. This area of no growth around the disk is known as a zone of inhibition, which is uniformly circular with a confluent lawn of growth in the media (Figure 1).

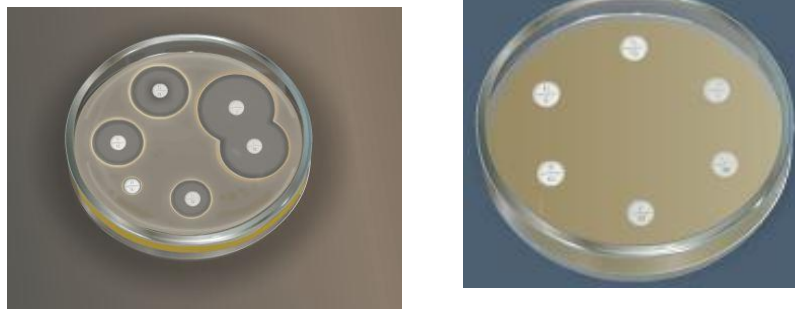


Figure 1: Antibiotic Susceptibility Testing

The diameters of the zone of inhibition are measured (including disk) using a metric scale or a sliding caliper. The measured zone diameter can be compared with a standard chart for obtaining the susceptible and resistant values. There are zone of intermediate resistance which means that the antibiotic may not be sufficient enough to eradicate the organism from the body.

THE PROCEDURES:

1. Prepare bacterial suspension and standardization with 0.5 McFarland standard .
2. Swab a Mueller-Hinton plate with the bacteria . Dip a sterile swab into the broth and express any excess moisture by pressing the swab against the side of the tube or add 0.1μ of bacteria suspension .
3. Spreading the surface of the agar completely with L- shape spreader or swap it . (you do not want to leave any unswabbed agar areas at all).
4. After completely swabbing the plate, turn it 90 degrees and repeat the swabbing process. (It is not necessary to re-moisten the swab.) Run the swab around the circumference of the plate before discarding it in the discard bag.
5. Allow the surface to dry for about 5 minutes before placing antibiotic disks on the agar.
6. Place the appropriate antibiotic discs on the surface of the agar using sterile forceps .
7. Gently press each disc on to the agar to provide uniform contact . DO NOT move the disc once it has contacted the agar because some of the antibiotics diffuse almost immediately . Disc must be placed in such a way that thy are at least 20 mm from one another .
8. Incubate the plates in 35 -37° C for 16-18 hr.

INTERPRETATION:

1. Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to the other edge. HOLDING THE PLATE UP TO THE LIGHT MIGHT HELP.
2. Use millimeter measurements. The disc diameter will actually be part of that number.
3. If there is NO zone at all, report it as 0---even though the disc itself is around 7 mm.

4. Zone diameter is reported in millimeters, looked up on the chart, and result reported as sensitive, resistant, or intermediate.
5. Record the results for your table, as well as the table below ;

Details of the antibiotics that were used in the study to test for antibiotic resistance

| Group | Antibiotic | Abbreviation | Generally accepted antibiotic disc concentrations (μg) | Inhibition zone (mm) | | |
|-----------------|-------------------|--------------|---|----------------------|------------------------|-------------|
| | | | | Resistant | Intermediate resistant | Susceptible |
| Aminoglycosides | Streptomycin | S | 10 | ≤ 11 | 12 – 14 | ≥ 15 |
| Macrolides | Erythromycin | E | 15 | ≤ 13 | 14 – 22 | ≥ 23 |
| Tetracyclines | Oxytetracycline | OT | 30 | ≤ 14 | 15 – 18 | ≥ 19 |
| Beta-lactams | Ampicillin | AP | 10 | ≤ 11 | 12 – 14 | ≥ 15 |
| | Penicillin G | PG | 10 | ≤ 20 | 21 – 28 | ≥ 29 |
| | Methicillin | MT | 5 | ≤ 9 | 10 – 13 | ≥ 14 |
| Glycopeptides | Vancomycin | V | 30 | ≤ 9 | 10 – 11 | ≥ 12 |
| | Nitrofurantoin | NI | 300 | ≤ 14 | 15 – 18 | ≥ 19 |
| Sulphonamides | Sulphamethoxazole | Smx | 300 | ≤ 10 | 11 – 15 | ≥ 16 |

Source: The concentration used as well as the inhibition zone measurements were according to the National Committee on Clinical Laboratory Standards²³

Note: The abbreviations are as they appeared on the antibiotic discs.

