

ANTIBIOTIC SENSITIVITY TESTING

INTRODUCTION

Antibiotics are chemical compounds that selectively interfere with the growth of microorganisms while causing little or no damage to the animal or human host. Because of occasional adverse side effects and the increasing emergence of antibiotic resistant organisms, chemotherapeutic agents must be used properly and judiciously to safeguard their clinical usefulness.

ANTIMICROBIAL AGENTS

Classification two type : Bacteriostatic and Bactericidal Agents

Bacteriostatic agents, such as **tetracycline**, inhibit the growth and multiplication of bacteria. Upon exposure to a bacteriostatic agent, cells in a susceptible population stop dividing. However if the agent is removed, the cells once again multiply.

Bactericidal agents, such as **fluoroquinolones**, not only inhibit the growth of cells but also trigger pathways within the cell that lead to cell death. The actions of bactericidal drugs are irreversible so once susceptible cells are exposed to a bactericidal agent .

Overview of Commonly Used Susceptibility Testing Methods

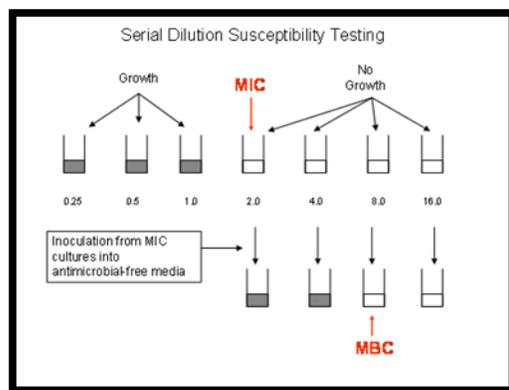
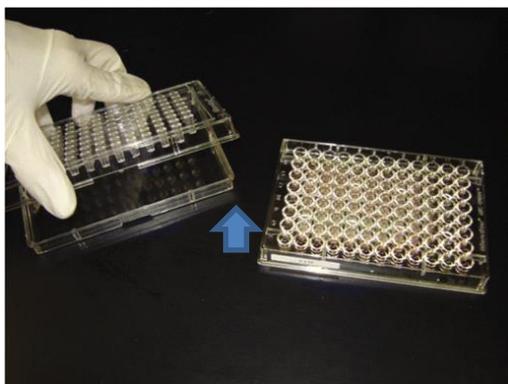
1- DILUTION METHODS

The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. **Microdilution testing** uses about 0.05 to 0.1 ml total broth volume and can be conveniently performed in a microtiter format. **Macrodilution testing** uses broth volumes at about 1.0 ml in standard

test tubes. For both of these broth dilution methods, the lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the minimal inhibitory concentration or MIC.

The minimum inhibitory concentration (MIC) is the concentration required to inhibit growth of a specific isolate in vitro under standardized conditions. It is determined by finding the lowest dilution without visible growth during serial dilution testing. This will vary for individual isolates.

The minimum bactericidal concentration (MBC) is the lowest dilution where the culture has been completely sterilized. It is not routinely determined. Treatment decisions are made related to MICs, and more specifically, the breakpoint MICs.



A broth microdilution susceptibility panel containing 98 reagent wells and a disposable tray inoculator

2- Kirby-Bauer Method (DISK DIFFUSION METHOD)

It is the most widely test used for determining the antibiotic(s) to be used to treat an infection. The suspected pathogen is isolated in *pure culture* from a clinical specimen and is tested for its sensitivity to clinically attainable levels of several antibiotics. The selection of drugs to be used for the test is based primarily on the knowledge of the infection, and *a carefully prepared Gram stain*.

After incubation, the diameter of the zone of growth inhibition is measured and scored according to the size of the zone and the particular antibiotic, as *sensitive, intermediate, or resistant*.

For example, this E.coli isolate on the right has a zone of inhibition of 10.1mm around ampicillin (AM); since the zone diameter interpretation chart is as follows:

Resistant: 13mm or less **Intermediate:** 14-16 mm **Susceptible:** 17 mm or more

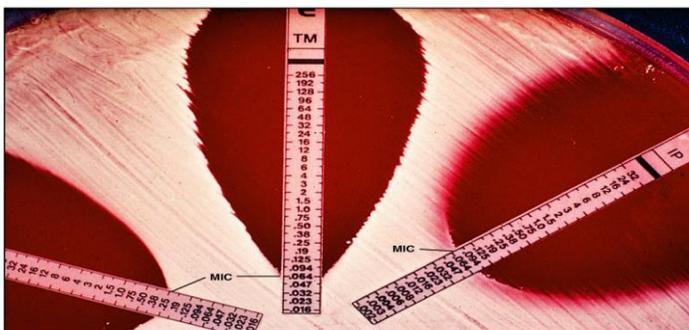
PERFORMING THE TEST

Once isolated colonies are available from an organism that has been identified as a potential pathogen, it is necessary to proceed as follows to perform the susceptibility test.

1. Select colonies
2. Prepare inoculum suspension
3. Standardize inoculum suspension
4. Inoculate plate
5. Add antimicrobial disks
6. Incubate plate
7. Measure inhibition zones
8. Interpret results

3. E-TEST

E-test is a commercially available test that utilizes a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic. The strip also displays a numerical scale that corresponds to the antibiotic concentration contained there in. This method provides for a convenient quantitative test of antibiotic resistance of a clinical isolate. However, a separate strip is needed for each antibiotic, and therefore the cost of this method can be high.



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