

# Antibiotic Sensitivity Tests - A description

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## Introduction

**P**athogenic bacteria often exhibit very great strain variations in their susceptibility to antibiotics and chemotherapeutic agents. Selection of a suitable antimicrobial agent is essential for effective treatment and prevention of bacterial resistance. Hence an in-vitro antibiotic sensitivity test is an essential prerequisite for the rational use of antimicrobial agents.

Antibiotic sensitivity tests are of two types, diffusion tests and dilution tests. Of these the former is the most simple and rapid method which is explained here.

## Principle

In diffusion tests, the drug is allowed to diffuse through a solid medium so that a gradient is established, the concentration being the highest near the site of application of the drug and decreasing with distance. The test bacterium is seeded on to the medium and its sensitivity to the drug is determined from the inhibition of its growth. Filter paper discs of 6.0 mm in diameter impregnated with antibiotics have been used for the application of the drug.

## Reagents

1. Muller Hinton Agar/Nutrient Agar/Trypticase Soy Agar
2. Trypticase soy Broth
3. Barium Sulphate standard.
4. Sterile normal Saline.

## Procedure

### 1. Preparation of the Petri Plates:-

Suspend the agar (quantity depends on the type of agar used) in 100 ml distilled water. Boil to dissolve the medium completely. After autoclaving pour it into sterile petriplates. The depth of the medium should be approx. 4mm. After the medium has solidified, dry the plates for 30 mts in an incubator (35-37°C) to remove the excess moisture from the surface.

### 2. Preparation of inoculum

a) It is ideal to use pure culture for sensitivity testing. Perform gram staining

before preparing an inoculum. When ever the results are required in a hurry, the swab or other clinical material is directly applied on the surface of petriplates. But in such cases a subsequent testing of isolate is also necessary.

b) Select 4 to 5 ml similar colonies and transfer them into a tube containing 5 ml to Trypticase soy Broth/Nutrient Broth with the help of wire loop.

c) Incubate the broth culture at 35-37°C for 2 to 5 hrs to obtain moderate turbidity.

d) Dilute the broth culture with sterile normal saline to obtain a turbidity equivalent to that of Barium Sulphate Standard. Barium sulphate standard is prepared by adding 0.5 ml of 1.175% Barium Chloride Solution to 99.5 ml of 0.36N sulphuric acid (This standard is equivalent to half the density of MC Farland standard.1)

## 3. Inoculation

a) Dip a sterile cotton swab into the diluted culture and rotate it while pressing against the upper inside wall of the test tube, above the fluid level to remove excess inoculum

b) Streak the agar surface of the plate in three directions, turning the plate by 60° between each streaking.

c) Replace the lid of the petri dish and keep it at room temperature for 5 to 10 mts. to dry the inoculum.

## 4. Application of discs.

Carefully take out the antibiotic discs from their respective vials with flamed forceps and place them in petri plates at least 15mm away from the edge. Discs should be placed in such a way that they are at equal distance and sufficiently separated from each other to avoid overlapping of the zone of inhibition. Finally press the disc lightly to the agar surface with the forceps to get a complete contact with the surface of the medium. Keep the plates at room temp for 30 mts.

## 5. Incubation

Incubate the petriplates for 16 to 18 hrs at 35-37°C

## 6. Reading the results:-

Measure the diameter of the zone of

### Disc Concentration and interpretation of disc diffusion test

Drug	Disc	Inhibition zone diameter in mm.		
		Resistant mm or less	Intermediate mm	Sensitive mm or more
1. Tetracycline	30 mcg	14	15-19	20
2. Sulphonamides	300 mcg	14	15-19	20
3. Streptomycin	10 mcg	12	13-16	17
4. Penicillin	10 units	20	21-29	30
5. Kanamycin	30 mcg	13	14-17	18
6. Gentamycin	10 mcg	12	13-14	15
7. Chloramphenicol	30mcg	12	13-17	18
8. Ciprofloxacin	5 mcg	15	16-20	21
9. Ampicillin	10 mcg	20	21-29	30
10. Pefloxacin	5 mcg	12	13-16	17
11. Amoxycillin	10mcg	13	14-17	18
12. Cotrimoxazole	25mcg	10	11-15	16

inhibition in millimeter at the end of incubation (6 to 8 hrs after in urgent situations). Results can be interpreted on the basis of zone size interpretative chart given at the end of this literature. The results are usually reported as 'sensitive', 'intermediate' or 'resistant' to different drugs.

Factors that can influence the results are the density of the inoculum, depth and composition of the medium, diffusibility of the drug, pH and time of incubation etc.



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