

## **Antibiotic Sensitivity Test**

Antimicrobial susceptibility tests are very essential step for the proper treatment of infectious diseases. Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth.

There are two methods for this test:-

- 1- Dilution method.
- 2- Disk method.

### **1-Dilution method**

The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. Each antimicrobial agent is tested using a range of concentrations commonly expressed as ( $\mu\text{g}$ ) of active drug/ ml of broth (i.e.,  $\mu\text{g}/\text{ml}$ ). Typically, the range of the concentrations tested for each antibiotic are series of doubling dilution (e.g., 16, 8, 4, 2, 1, 0.5  $\mu\text{g}/\text{ml}$ ).

For 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 MIC is thus the minimum concentration of the antibiotic that will inhibit this particular isolate.

### **2- Disk method**

Culture medium is evenly inoculated with the organism to be tested and plotting paper discs containing the antibiotics are put on the surface. During incubation, antibiotic diffuses radially from the disc in to the medium. If the organism is sensitive to the drug in the concentrations achieved, its growth is prevented in a circular zone around the disc.

***Factors affecting the results at disc diffusion tests:***

- 1- Rate diffusion of drug.
- 2- The culture medium.
  - a) The effects of medium constituents.
  - b) The pH of the medium.
  - c) Minerals and salts.
  - d) Carbohydrates.
  - e) Blood.
- 3- Depth of medium.
- 4- Density of inoculums.

***The Kirby – Bauer method:***

**1- Preparation of plates:**

Only Mueller – Hinton medium can be used. The medium is prepared and sterilized as directed by the makers. defibrinated blood may be necessary for tests on fastidious organisms in which case the medium should be allowed to cool to 50°C before 5 per cent of blood is added. The medium should be poured into Petri dishes on a flat horizontal surface to a depth of 4 mm. (25ml in an 8.5 cm circular dish). Poured plates are stored at 4 °C and used for one week of preparation.

PH should be checked at the time of preparation and should be (7.2 - 7.4)

**2- Preparation of inoculums:**

At least four morphologically similar colonies from an agar medium are touched with a wire loop and the growth is transferred to a test tube containing sterile saline. The density of the suspension is standardized by dilution with sterile saline or broth to a density visually equivalent to a barium – sulphate standard. Comparison should be made against a white background with a contrasting black line. The standard should be kept in screw capped tubes. Before use the standard should be shaken vigorously.

### **3- Inoculation:**

Plates are inoculated with in 15 min of preparation of the suspension so that the density of the inoculum dose not change. A sterile cotton – wool swab is dipped in to the suspension and surplus removed by rotation of the swab against the side of the tube above the fluid level. The medium is inoculated by even streaking of swab over the entire surface of the plate in three direction.

### **4- Antibiotic disc:**

After the inoculum has dried, single discs are applied with forceps, or a sharp needle and pressed gently to ensure even contact with the medium. Not more than six discs can be accommodated on an 8.5cm circular plate.

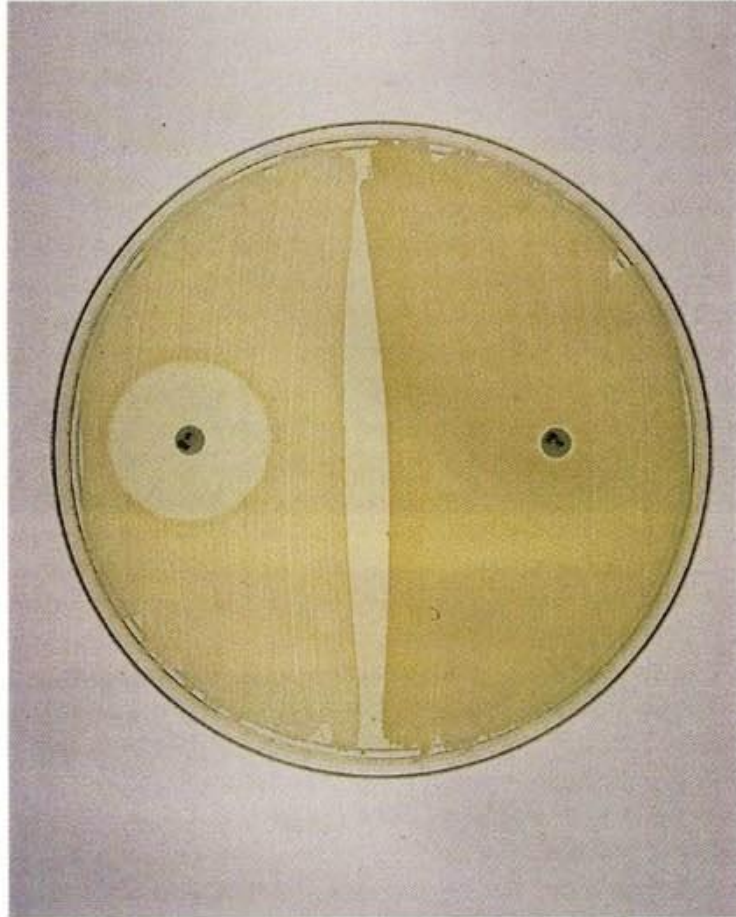
Discs should be stored at 4 °C in sealed containers, and should be allowed to come up to room temperature before the containers are opened. Discs should be used before the expiry date on the label.

### **5-Incubation:**

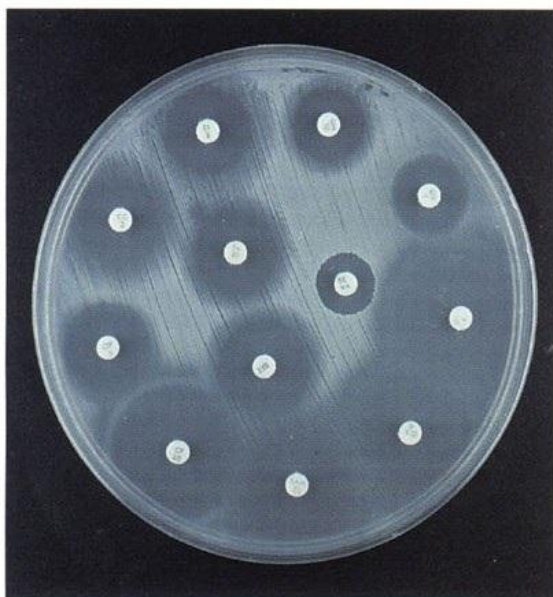
Plates are incubated inverted for (16-18) hr at (35 – 37) °C.

### **6- Reading of zones of inhibition:**

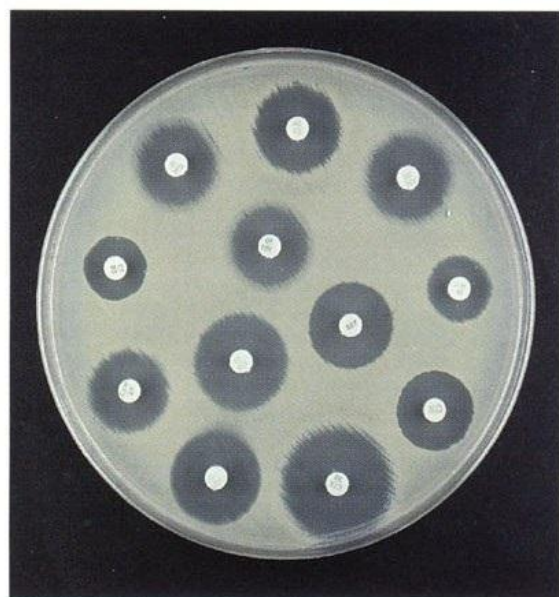
The diameters of the zones are measured to the nearest (mm) with calipers or millimeter rule.



**5-15 Novobiocin susceptibility test**, used to differentiate *S. saprophyticus* (resistant; microorganism on the right) from other species of coagulase-negative staphylococci (susceptible; microorganism on the left) encountered in urine specimens.



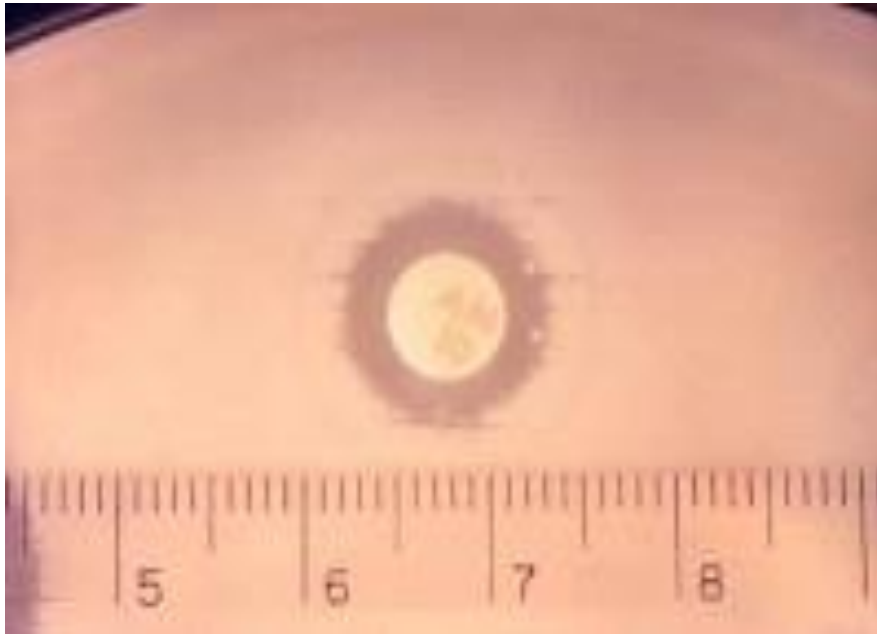
**13-5 Disk diffusion method—*Staphylococcus aureus*** American Type Culture Collection (ATCC) 25923.



**13-6 Disk diffusion method—*Escherichia coli*** ATCC 25922.

**Interpretation:**

- \* Sensitive: Infection treatable with normal dosage.
- \* Intermediate: Infection that may respond to therapy with high dosage .
- \* Resistant: Not treatable with this agent.



- 1- Koneman's Color Atlas and Text book of Diagnostic Microbiology.
- 2- Bauer, A. W.; Kirby, W. M.; Sherris, J. C. and Turck, M. (1966).Antibiotic Susceptibility Tests by A Standardized Single Disk Method.Am. J. Clin. Path. 45:493-496.