


Antibiotic sensitivity Test



A test done to check the effectiveness of a drug against a bacterium and to select the best drug that acts against the bacterium.

There are two methods for this test:-

1 - Dilution method.

2- Disk method.



According to this the m.o classified
into 3 categories

Sensitive

Intermediate

Resistant

1 - Dilution methods

- * Used to determine the minimal concentration of antibiotic to inhibit or kill the microorganism.
- * Achieved by dilution of antibiotic in either agar or broth media.

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.

difibrinated 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 inoculum

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 within 15 min of preparation of the suspension so that the density of the inoculum does not change. A sterile cotton-wool swab is dipped into 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 directions.

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.



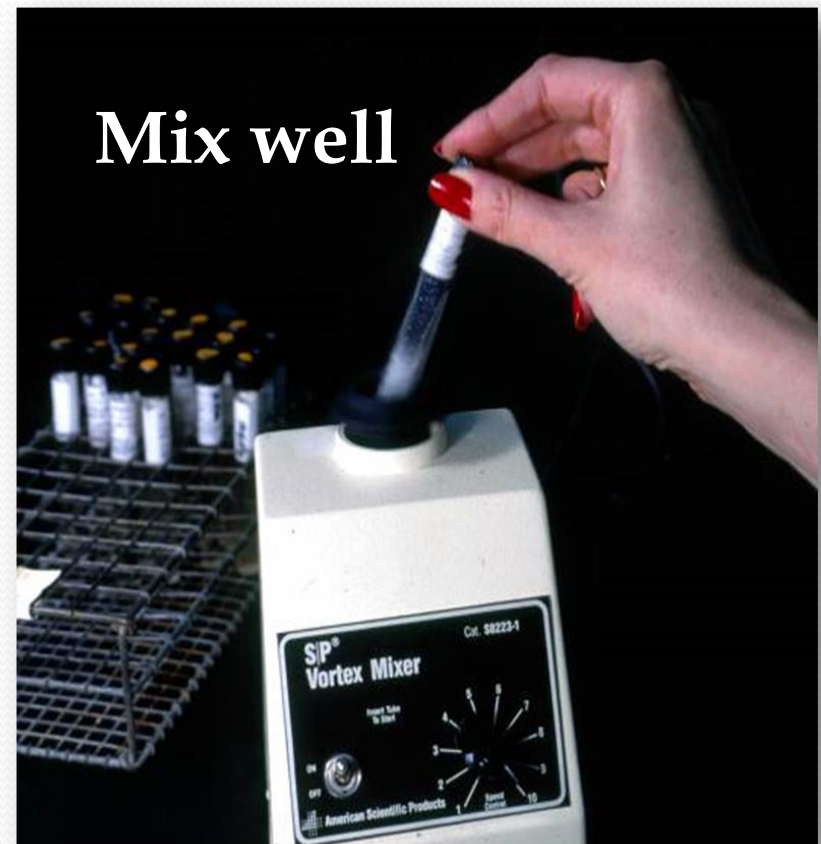
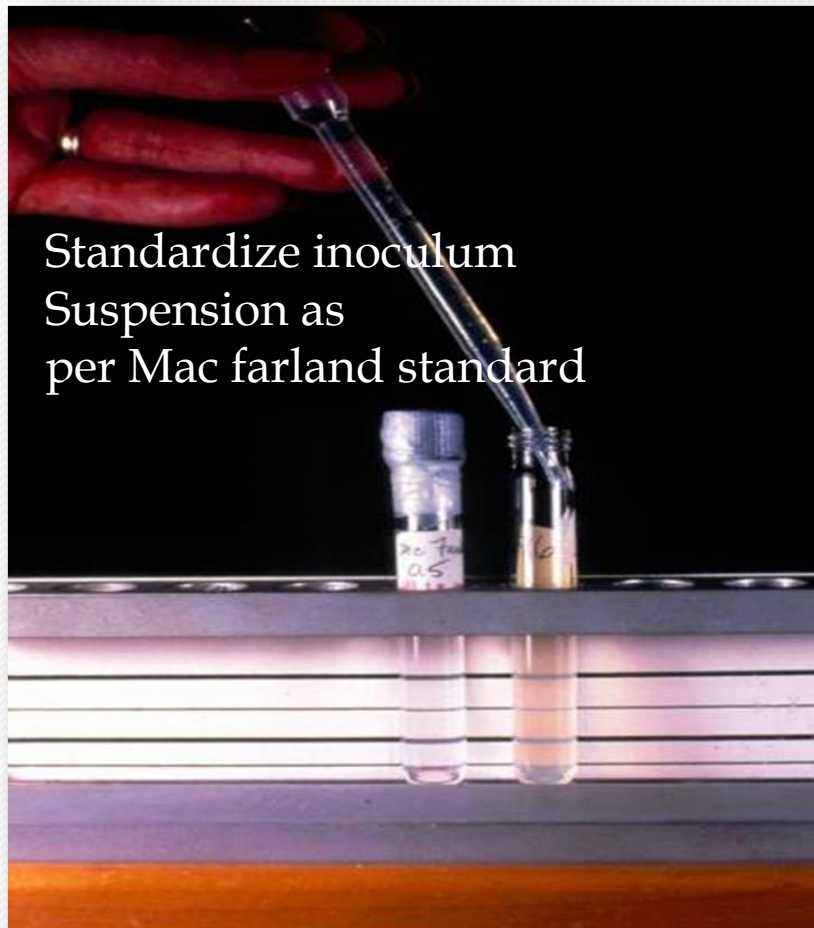
Interpretation:

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

Disk Diffusion Test



Prepare the Material for Inoculation



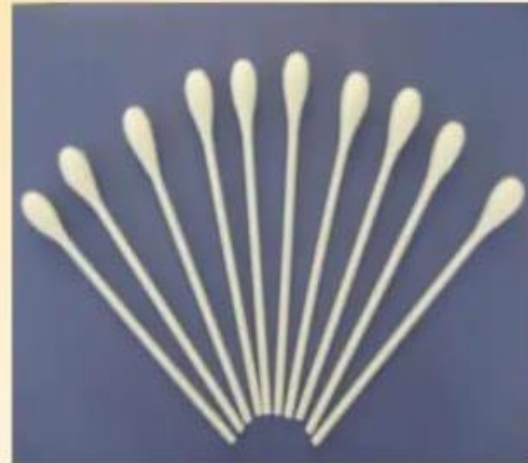
Swab the plate with optimal sample



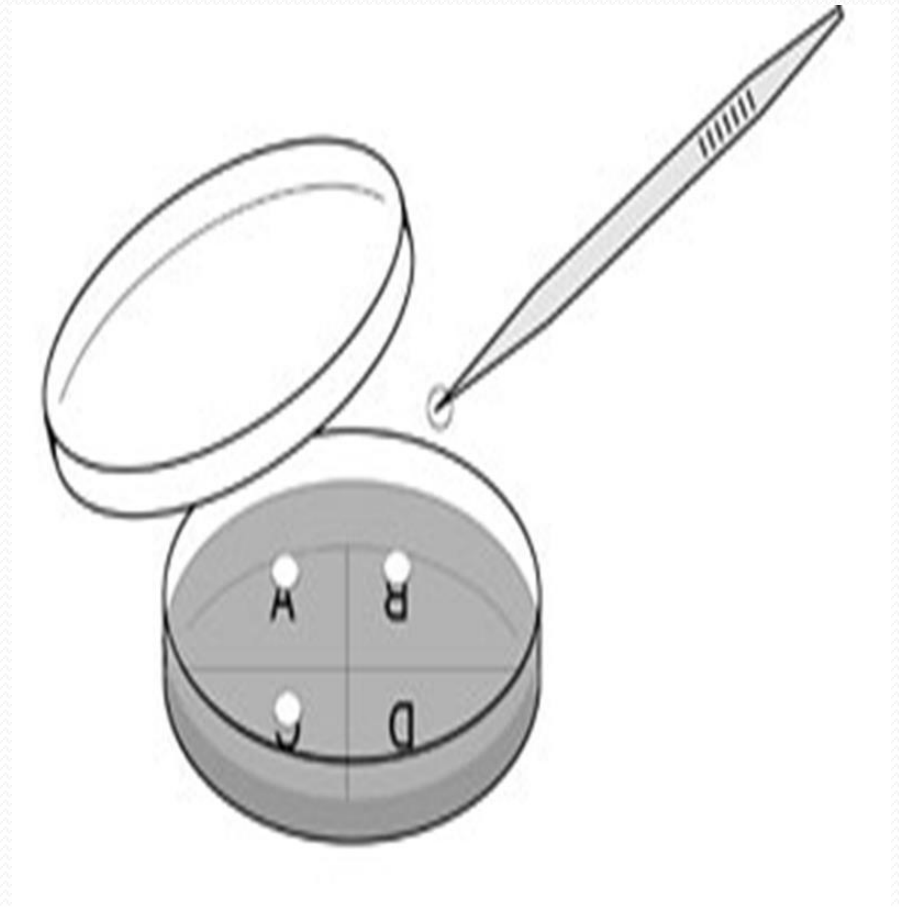
Methicillin resistance in *Staphylococcus aureus*



Cotton Swabs



Select the Disks and Apply



Incubate Overnight



