Staining technique Pract.3

1- Simple stain

2- Differential stain → Gram`s stain

→ Acid fast stain

Dyes: The chemical materials used for staining the cell of bacteria and may be acidic or basic.

Cell staining is a technique that can be used to better visualize cells and cell components under a microscope. By using different stains, one can preferentially stain certain cell components, such as a nucleus or a cell wall, or the entire cell. Most stains can be used on fixed, or non-living cells, while only some can be used on

living cells; some stains can be used on either living or non-living cells.

Acidic stain: (-ve charge), like Indian ink stain, Eosin stain, Nigrosin stain also this stain called negative stain.

Basic stain: (+ve charge), like Crystal violat, Safranin, Methylene blue, Malachite green

Note:

** Natural stain like Giemsa stain used for higher cells like blood smear.

** We must used basic stain to stain bacteria cells if we need to study the morphology characters in general shape.

**Staining is not limited to biological materials, it can also be used to study the morphology of other materials for example the lamellar structures of semicrystalline polymers or the domain structures of block copolymers.

1- Simple stain: At its simplest, the actual staining process may involve immersing the sample (before or after fixation and mounting) in dye solution, followed by rinsing and observation. Many dyes, however, require the use of a mordant a

precipitate. When excess dye solution is washed away, the mordanted stain remains.

Staining technique steps:

1- Preparation of slide.
2- Fixation of smear.
3- Staining.
4- Examining under microscope.

1- Preparation of slide

- Passes the slide on flame .
- By wax pencil draw two lines and put the(X) sign behind the slide.
- Heat the loop on flame.
- Put a drop of water in the center of slide and mixing well with bacterial growth to make a thin smear.
- Let the smear to in air.

2- Fixation of smear

- Fixed the smear on flame by passestfTe slide three times on flame genitally and be careful do not burned the smear. Sometimes heat fixation is used to adhere and alter the specimen so it will accept stains also killing the bacteria by decreases the viability and activity of cells by pulling the moisture from them ; removing the motility of cells.

• Wet preparation :

- The wet mount preparation of bacterial culture can be very informative because tell us much about morphology characters like (shape and arrangement)of both cell and motility.

- This way useful in clinical laboratories to give an idea on sample taken from patients like urine, faces, blood, pus, etc...

Antibiotic Sensitivity Test (Disk method)

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 (µg) of active drug/ ml of broth (i.e., µg/ 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 µg/ 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 of 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 to50°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 form 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:

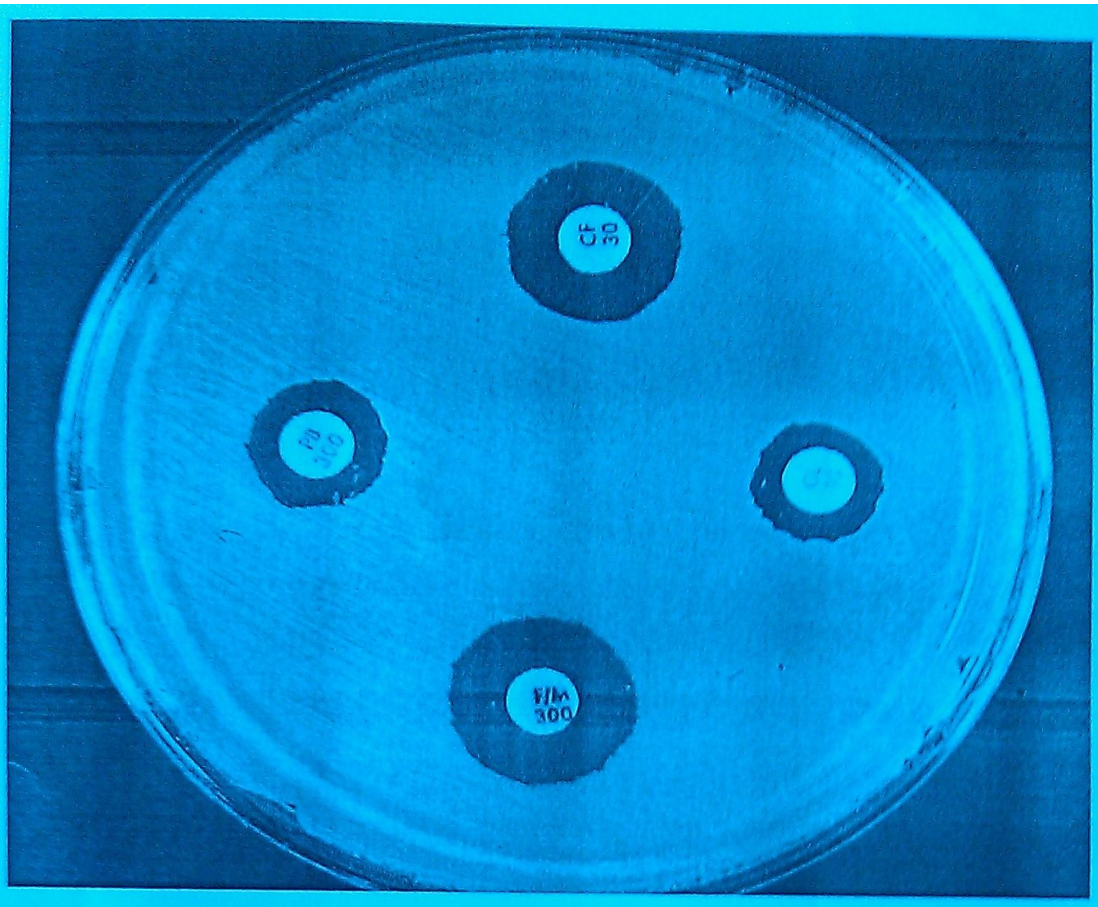
Plates are incubated inverted for (16-18) hr at (35 – 37) °C.

6- Reading of zones of inhibition:

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

Interpretation:

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



DISC DIFFUSION METHOD

- Place the appropriate drug-impregnated disc on the surface of the inoculated agar plate
- Invert the plates and incubate them at 35 °C, o/n (18-24 h)
- Measure the diameters of inhibition zone in mm