Unit - 3

Staining :-

Staining is the technique used for the demonstration of microorganism

Aim of Staining :-

- To increase the visibility
- Development of contrast
- Study of morphology and composition
- To detected the extracellular and intracellular of microbes
- It is helpful on primary level for identification of microbes for the purpose of diagnosis and to determine cause of disease and also helpful on the treatment

Stain

- Stain is defined as an organic compound containing both chromophore and auxochrome linked to benzene ring or aromatic ring
- Stain may be natural and artificial compound Stain provide contrast to microorganism

Types of Stain

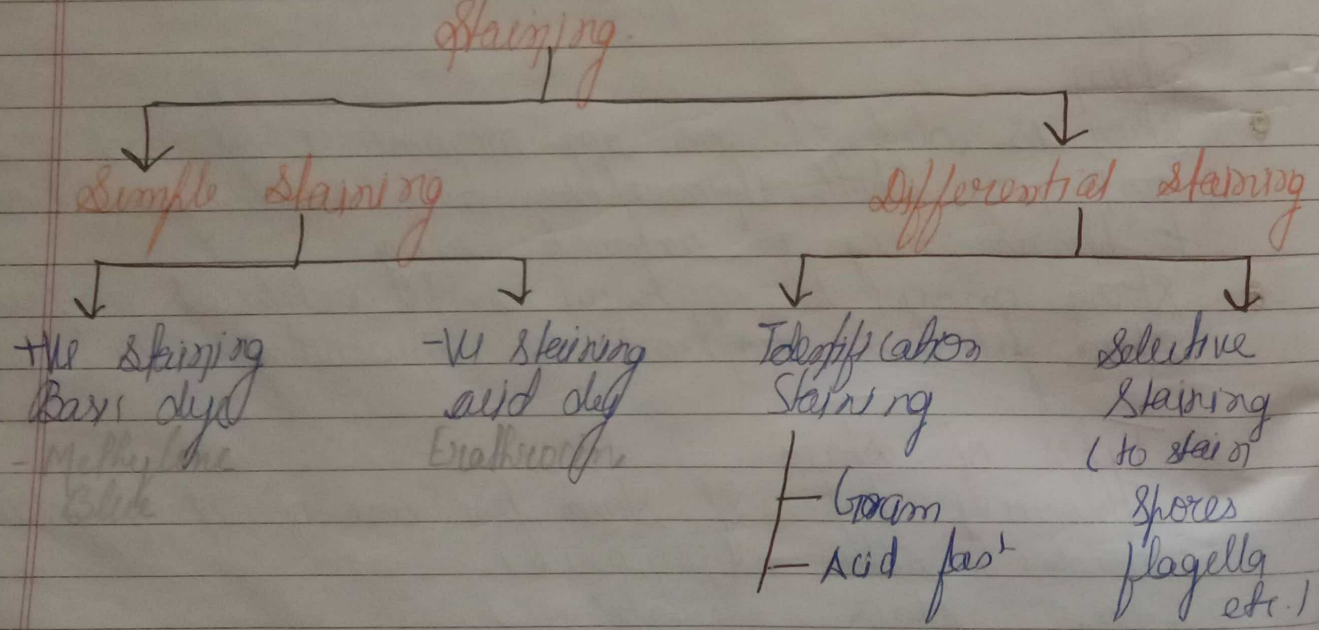
Following types of stain for use of staining technique.

1. Acidic stain (anion)
2. Basic stain (cation)

Stain :-
Stain are dyes are reagent used for differential colouring of microorganism to observe there morphology which much clarity under microscope

Purpose of staining :-
- To view the organism with much clarity
- To determine particular structure like cell wall shape, flagella etc.
- To differentiate one organism and other

Types of staining



Simple Staining - Simple staining is carried out to visualize bacteria and to compare morphological shape and arrangement of bacterial cell

In this technique bacteria smear is stain with single basic dye like crystal violet and methylene blue.
Bacteria cells usually carry negative charge on surface show they are mostly colour by basic stain.

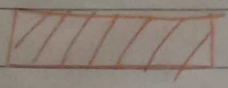
Procedure of Simple staining:

Slide → Wash → Dry.
Transfer using inoculation loop (sterile).
(Prep of smear)

Heat fix
Stain.
Leave for 1-2 min
wash → to remove excess dye.
air d

Gram staining :-

Technique is developed by Hans Christen gram in 1884 to differentiate g+ve gram +ve and gram -ve bacteria.



add - Crystal violet - 1° Stain
Iodine sol^{n} ⟶ Mordant

↕

Alcohol wash

↓ ↓

Color attach ↓ — Purple colour

Colour remove ↓ — (2° Stain) Safranin (counter stain) ↕ — red or pink colour

Acid fast staining technique :- This technique is used for staining by mycobacterium tuberculosis or mycobacterium leprae as these microbes have waxy material their cells.

German bacteriology this technique was developed by German bacteriologist Franz Ziehl and German pathologist are Adolf Neelsen.

Procedure :-

Heat fix smear

↓

Add Carbofuschin (Reagent)

Warm (15-20 min), room temp.

↓

Dry

↓

Add Alcohol (decolourising)

↓

Color removes

↓

Colorless

↓

Methylene blue (counter stain)

↓

blue

↓

Acid dissolve to the waxy substance

↓

red color

↓

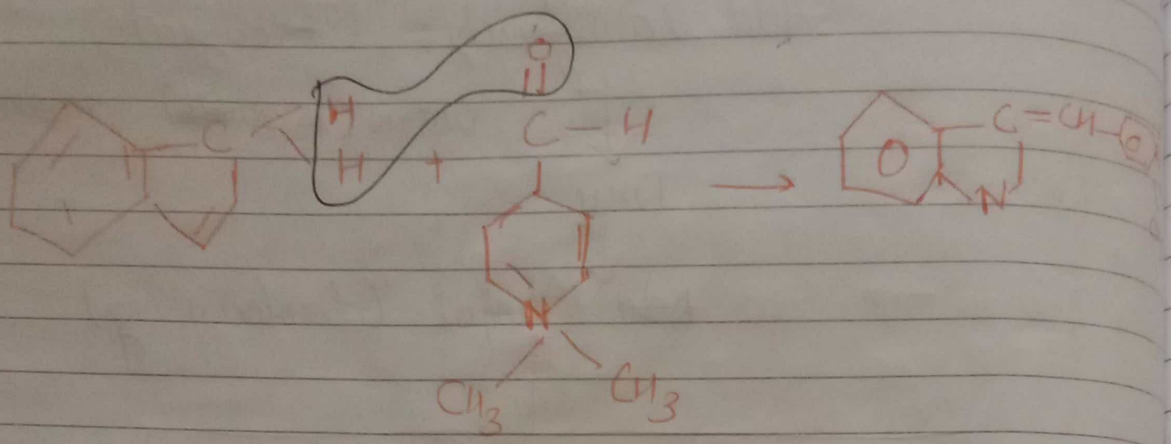
acid fast bacteria

IMViC → Indole test
Methyl red
Voges Proskauer
Citrate utilisation test

This is to detect the presence of enterobacteria or enteric bacteria like E Coli, Pseudomonas, salmonella typhi

Indole test :-

Medium — Tryptophan broth
Reagent → Kovac's (P-dimethyl amines benzal
+ HCl + alcohol)



Indole test is used for detection of indole as some bacteria produce indole during tryptophan hydrolysis.
Indole presence can be detected by Kovac reagent.

Procedure

Prepare tryptophan broth and dispose 5ml of it in a test tube
Inoculate broath tube.

MR-VP test :-

This test is used to differentiate b/w E. Coli & Enterobacter aerogens

Some bacteria produces acid during fermentation of glucose and some time produce Acetyl Methyl Carbinol)

If acid is produced by bacteria then red colour is produced after adding methyl red, if the medium turns to yellow then the MR test is negative.

If glucose ⟶ Formic acid
Lactic acid

add Methyl red

Yellow colour
MR -ve

Red colour
MR +ve

Now, Yellow medium is further treated with VP reagent (VP reagent 1 - 5% α naphthol & VP reagent 2 - 40% KOH solution).

Procedure :-

Prepare glucose phosphate broth and dispose 5 ml of it in sterile test tube
Inoculate with organism and incubate at

35°C for 48 hours
Add 5 drop of methylene red and observe colour change.
If test is negative add VP reagent 1 and VP reagent - 2 shake for 30 sec. and remove the plug.
Allow to stand for 15-30 minute
If pink colour is produced then ~~test~~ V.P. test is positive.

Citrate Utilisation Test

Some bacteria metabolises citrate or citric acid leading to breakdown of ammonium salt which results in formation of ammonia.

M → Simon's citrate agar medium
R → Bromothymol blue

pH will turn to basic then add bromothymol blue as a indicator, if green colour converts to blue after fixed seconds then the test is positive.

Citrate → ammonium salt → ammonia
↓
alkaline (green)
↓
Bromothymol blue
↓
green → blue.

160 – 2hrs

Sterilization

Sterilization is a process that effectively killed all microbes from surface equipments / glasswares, culture media etc

Physical method of sterilization

- Heat
- Radiation
- Filtration

Heat Sterilization

This is the most reliable, rapid and widely used method of sterilization

Principle

Protein denaturation and oxidative damage

Dry heat Sterilization (160-180°C)

This method is used for thermostable product.
Efficiency of heat to inactivate microorganism depend on degree of heat, exposure time, presence of water.
It employes higher temp. and require exposure time upto 2 hours.

Advantage :-

This method is suitable for sterilisation of substance destroyed by moisture
This method do not require water.
Equipment used is much smaller as compared to autoclave but is equally effective

Disadvantage
High temp and long heating time is required
This method uses dry heat so some organism like Prion may not be killed by this method.

Moist heat Sterilization
This technique involved use of killing by hot water or steam

I. Pasteurization $< 100^{\circ}C$
II. Tyndallization $- 100^{\circ}C \rightarrow 20$ min
↓
3 successive days
III. Autoclave $\rightarrow \geq 100^{\circ}C$

Radiation Sterilization :-
→ Ionizing
→ Non-ionizing

Radiation
→ Ionising
→ Non-Ionising

Prepacked item

Plastic
Syringes.
Fibres

Radiation.

Many ~~Sterilization~~ used for Sterilization like electromagnetic radiation (UV & gamma radiation) particulate radiation.

The major target for these radiation is microbial DNA.

Radiation Sterilization are generally used for the product ex dry stable like plastic Injection Surgical equipment ect.

Sterilization

Chemical Sterilization –

Several chemical reagent are used as Antiseptic or disinfectant

Properties of chemical reagent used as Antiseptic and disinfectant –

The chemical disinfectant need to have broad Spectrum of activity against all microorganism.
High penetration power is an important property of the chemical agent.
Chemical reagent needs to be chemically stable under both acidic and basic environment.

Alcohol.

Ethyl alcohol and Isopropyl alcohol are frequently used as chemical agent for disinfectant.
They facilitated protein denaturation
70% ethanol is used for disinfectant

Aldehyde :-
10% formalin solution is of standard chemical disinfectant.
Aldehyde produces bactericidal, sporicidal and virucidal action.

Glutaraldehyde
It has activity against mycobacterium tuberculosis, fungi and viruses.
Used as 2% buffer solution.
Can kill spores and is known for its less toxic nature.

Dyes :-
They have low bactericidal activity but are bacteriostatic

Two type of dye generally used

Acridine :- It is more effective against gram +ve bacteria.

Acridine :-

Chlorine
Chlorine is used to disinfectant water supply swimming pool, food dairy industries along with Hypochlorite they produces Bactericidal action.

Gaseous Sterilization

The chemical reactive gases ethylene oxide & formaldehyde posses broad spectrum biocidal activity.

They are used for sterilization of Reusable Surgical instruments.

This method involve exposing equipment to different gases enclosed in closed chambers.

Sterilization process using ethylene dioxide is most commonly used process of sterilization.

They work by causing DNA degradation.

Ethylene dioxide

This is the recognized sterilization method em B.P (British Pharmacopeia)
It is mixed with 10% CO_2 or 8.6% with hydroflourocarbon to hazard.
Ethyle oxide treatment usually conducted to 30-60° C for several hours

Formaldehyde :-

This is another important highly reactive gas used for sterilization.
It posses broad spetrum biocidal activity
This gas is obtained by heating formalin to a temperature of 37 - 80° C
It posses low penetration power ethylene oxide and used for sterilization of paper and cotton fabric.

Nitrogen dioxide :-

This is rapid and effective method of sterilization
This method is used for removal of fungi bacteria and even spores
Nitrogen dioxide have low B.P. of 20° C that allow high vapour pressure at standard temperature
No condensation of the gas occurs on the surface of the device b/c of low level of gas used and high vapour pressure

Ozone :-
This is highly reactive industrial gas which is commonly used to sterilized air and water and as disinfectant for surface
This gas is hazadarous. So used as a concentration of 5 ppm (parts per million). which is 160 time less than ethyle dioxide.

Mechanical Sterilization

Filtration :- Filtration does not destroy but removes the microorganism
It prevent passage of both viable and non-viable particles

Mechanism

Sieving
Adsorption
Trapping within the matrix of filter media

Sterilization grade filters are used in treatment of opthalmic solution, gases for supply to Asptic area, biological products etc.

e.g HEPA Filter
The filter are generally made up of cellulose acetate or cellulose nitrate