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Identification of Bacteria

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Unit II

10 Hours

Identification of bacteria using staining techniques (simple, Gram's &Acid fast staining) and biochemical tests (IMViC). Definition of D value & Z value and its significance.

Study of principle, procedure, merits, demerits and applications of physical, chemical gaseous,radiation and mechanical method of sterilization.

Evaluation of the efficiency of sterilization methods.

Equipments employed in large scale sterilization.

Sterility indicators

BACTERIAL STAINING TECHNIQUES

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Simple staining

Gram's staining

Acid fast staining)

Biochemical tests (IMViC).

Identifiction means

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Taxonomy of bacteria: 3 interrelated area

Classification: arranging organisms into related groups.

Nomenclature refers to the assignment of names to these groups, guided by a set of rules.

Identification is the process of determining to which established taxon a new isolate or unknown strain belongs.

Need

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Microbiologists must identify bacterial isolates for several practical reasons:

- **Medical diagnostics** — identifying a pathogen isolated from a patient.
- **Food industry** — identifying a microbial contaminant responsible for food spoilage.
- **Research setting** — identifying a new isolate which carries out an impor tant process.

INTRODUCTION

- As bacteria consist of clear protoplasmic matter, differing but slightly in refractive index from the medium in which they are growing, it is difficult with the ordinary microscope, except when special methods of illumination are used, to set them in the unstained condition.
- Staining, therefore, is of primary importance on the recognition of bacteria.
- Staining may be simple staining and differential staining.

DYES

Why we need to stain bacteria?

- Bacteria are transparent and colorless, so they would be invisible to naked eye if observed under a microscope thus bacteria should be stained with certain dyes in order to visualize bacterial cell or their internal structures using the light microscope.

DYE (stain) :

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Colored organic compound in the form of salt, composed of positive and negative ion, one of these ions is responsible for color called chromogen.

TYPES OF DYES:

1. BASIC DYES
2. ACIDIC DYES

DYES(CONTD.)

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- **BASIC DYES:**
  - In which chromogen is the positive ion (cation).
  - Basic dye has the form: dye+Cr-
  - E.g.; crystal violet, methylene blue and safranin.
- **ACIDIC DYES:**
  - In which the chromogen is negative ion(anion).
  - Acidic dye has the form :Na+dye-
  - E.g.; nigrosin and India ink.

Staining of Bacteria

- **Types of staining technique:-**

Simple staining
(use of
a single basic stain)

For visualization of morphological shape & arrangement.

Differential staining
(use of two contrasting stains separated by a decolorizing agent)

Identification
- Gram stain
- **Acid fast stain**

Visualization of structure
- **Spore stain**
- Capsule stain

SIMPLE STAINING

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- These show not only the presence of organism but also the cellular contents of exudates.
- A single stain is used.
- Examples are methylene blue, polychrome methylene blue, dilute Carbol fuschin.
- The principle staining may involve ion exchange reaction between the stain and active site or within the cell structure as flagella spore.

SIMPLE STAINING

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- The surface of a bacterial cell has an overall acidic characteristic because of large amount of carboxyl groups located on the cell surface due to acidic amino acids. Therefore, when ionization of carboxyl groups takes place it imparts negative charge to the cell surface as per the following equation.

$$COOH \rightarrow COO^- + H^+$$

SIMPLE STAINING

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- H+ is removed and the surface of the bacteria becomes negatively charged and a positively charged dye like (methylene blue) attaches to the negatively surface and gives it a coloured appearance.

Methyleneblue chloride → Methylene Blue++ Cl-

POSITIVE SIMPLE STAINING

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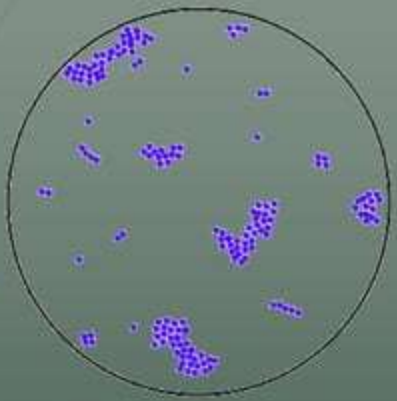
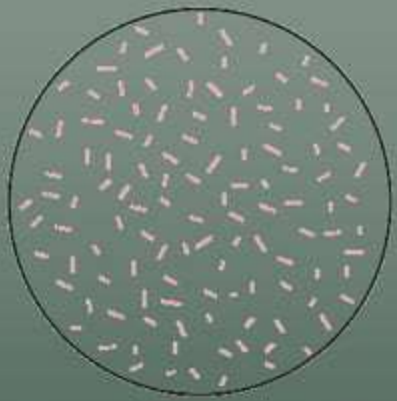
- Prepare a separate bacterial smears of the each micro-organism and fix it by heat.
- Place slide on the staining tray and flood the smear with one of the indicated stains using the appropriate exposure time.
- Pour off the staining solution and wash the slide in running tap water.
- Dry the slide between blotting papers and examine the stained smear under microscope using oil-immersion objective.

Record your observation from the microscope.



Simple Positive Staining



Simple NegativeStaining

RESULT OF SIMPLE STAINING PROCEDURE

- Simple positive staining: all bacteria are colored.
- Simple negative staining: background is dark, bacteria are without any color .

DIFFERENTIAL STAINING

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- This type of staining is to differentiate two organisms.
- Mainly used differential staining methods are

1. GRAM'S STAINING.
2. ACID-FAST STAINING.

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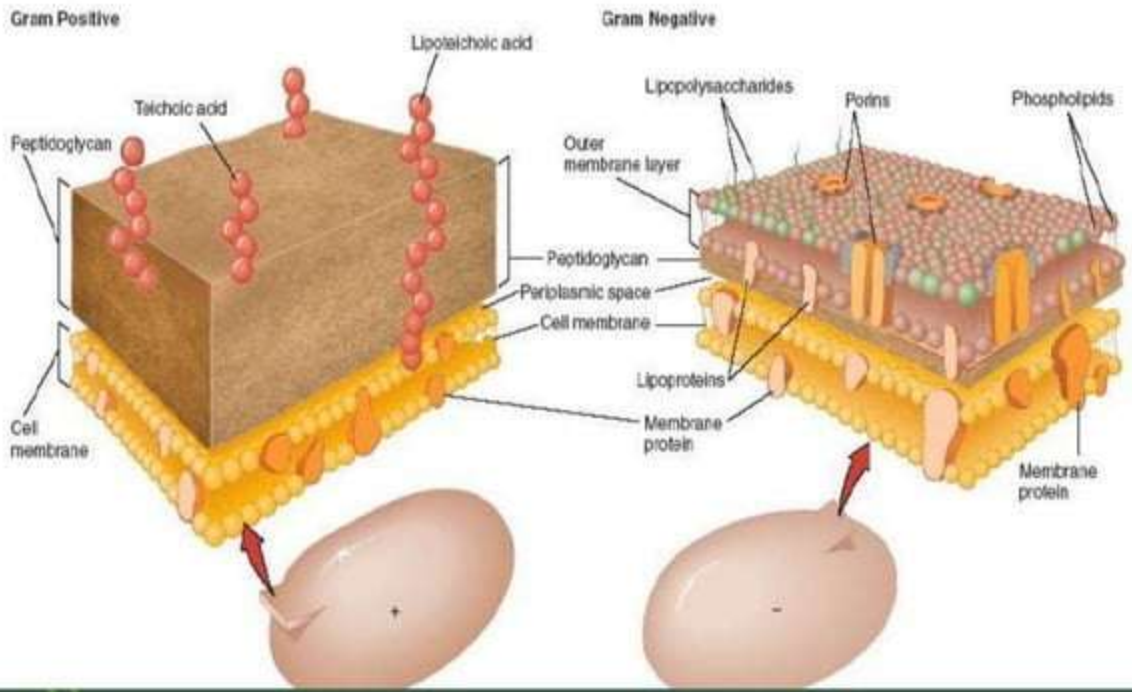
GRAM'S STAINING

- Gram staining is developed in 1884 by the Danish physician Christian Gram , is the most widely used method in bacteriology.
- It is first and usually the only method employed for the diagnostic identification of bacteria in clinical specimen.

HANS CHRISTIAN GRAM

S.D.Mankar

CELL WALL OF GRAM POS & NEG



PRINCIPLE

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This test differentiates the bacteria into Gram-Positive and Gram-Negative Bacteria, which helps in the classification and differentiation of microorganisms. The Gram stain separates bacteria into two groups: (1) Gram-positive microorganisms that retain the primary dye (Crystal violet) and (2) Gram-negative microorganisms that take the color of the counterstain (usually Safranin O).

Procedure:

- Prepare a smear from provided bacterial suspension on clean grease free slide.
- Allow smear to air dry and then heat fix in the usual manner.
- Cover the smear with crystal violet (primary stain) and keep for 1 min.
- Wash the smear with water and cover it with Gram's iodine for 1 min.

Procedure:

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- Wash the smear with water & decolorized with 95 % ethanol very carefully till the washing does not contain violet color.
- For normal smear 10-15 seconds are enough. Rinse the smear with water and counterstain with safranin or dil. Carbol fuchsin for 1 min. wash the smear with water and allow it to air dry.
- Put a drop of oil on the smear and examine under oil immersion objective.

- Gram-positive cells have a thick peptidoglycan cell wall that is able to retain the crystal violet-iodine complex that occurs during staining, while Gram-negative cells have only a thin layer of peptidoglycan.

Thus Gram-positive cells do not decolorize with ethanol, and Gram-negative cells do decolorize. This allows the Gram-negative cells to accept the counter stain safranin.

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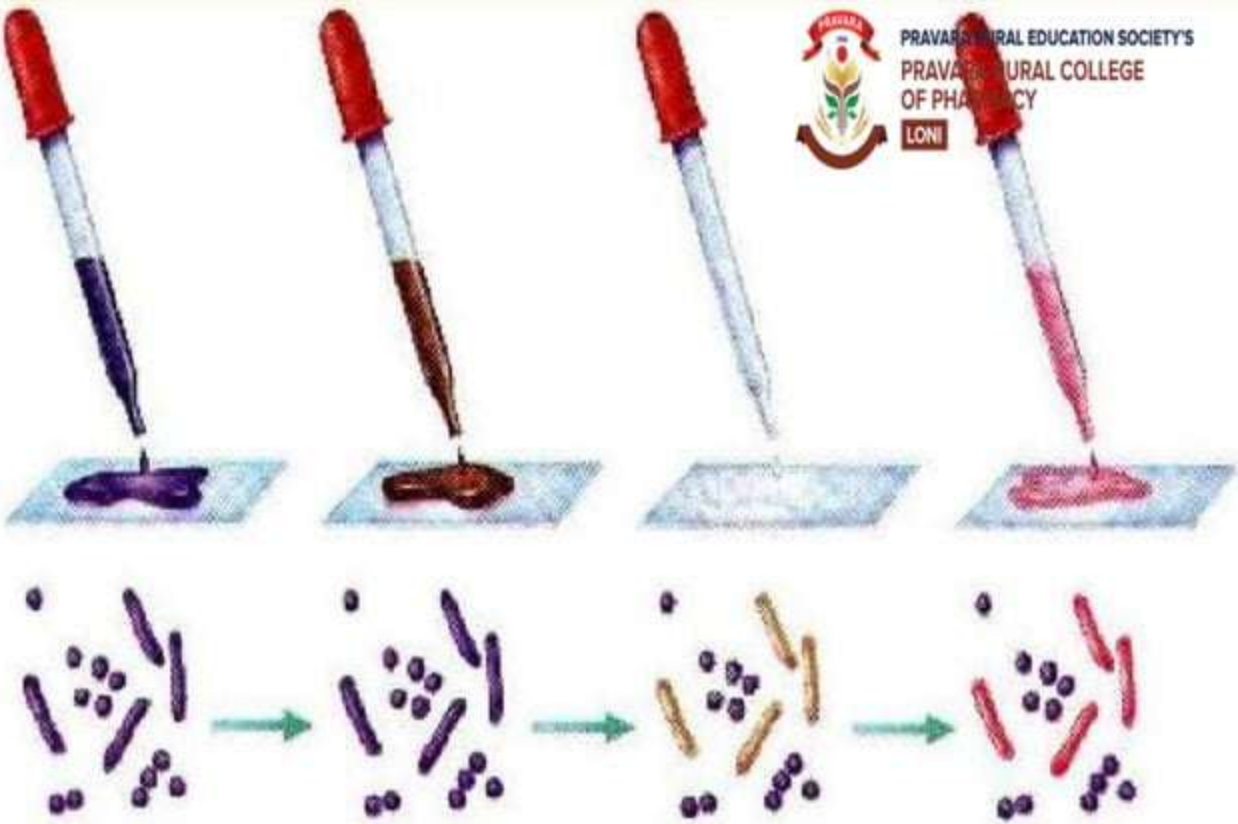
PROCEDURE

- Gram positive cells remain purple. Gram negative cells appear red.



(a) Application of crystal violet

(b) Application of iodine

(c) Alcohol wash

(d) Application of safranin

OBSERVATION

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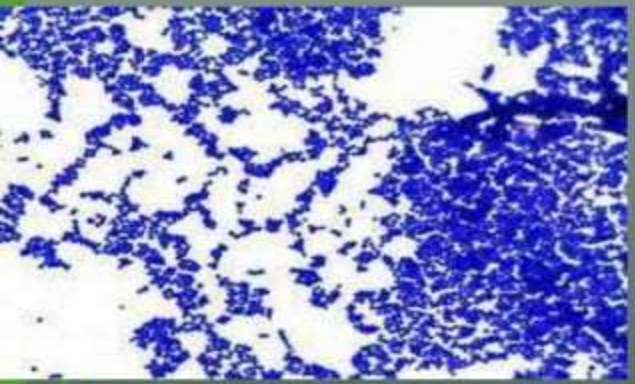
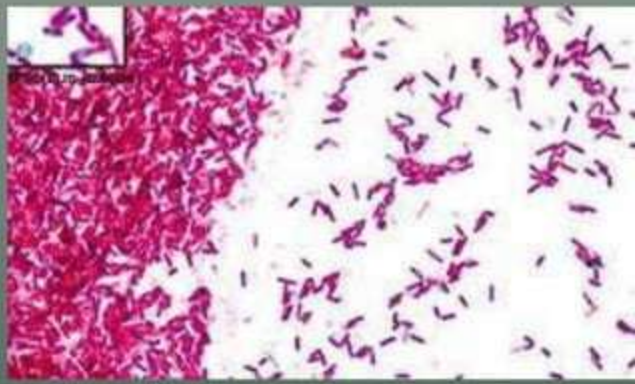


Gram positive cocci in chains



Gram negative bacilli

ACID FAST STAINING

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- This is also known as ziehl-neelsen staining.
- This method is a modification of Ehrlich's(1882)original method for the differential staining of tubercle bacilli and other acid fast bacilli.
- Stain used consists of basic fuschin with phenol added.

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ACID FAST STAINING

Acid-fast staining is another widely used differential staining procedure in bacteriology. This stain was developed by Paul Ehrlich in 1882. Ziehl and Neelsen independently proposed acid fast stain in 1882-1883, which is commonly used today. Some bacteria resist decolourisation by both acid and alcohol and hence they are referred as acid-fast organisms.

ACID-FAST STAINING

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Acid-alcohol (3% HCI95% ethanol) is a very intensive decolouriser. This staining technique divides bacteria into two groups.
1) Acid-fast and
2) Non-acid-fast

This procedure is extensively used in the diagnosis of M. tuberculosis and M. leprae.

ACID-FAST STAINING

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Prepare a smear of given bacterial suspension.
Allow smears to air dry and then heat fix it,
Flood the smear with carbol fuchsin stain and heat the slide from below till steam rises for 5 minutes.
Do not boil the stain and ensure that stain does not dry out.
Allow the slide to cool for 5 minutes toprevent the breakage of slide in the subsequent step.

ACID-FAST STAINING

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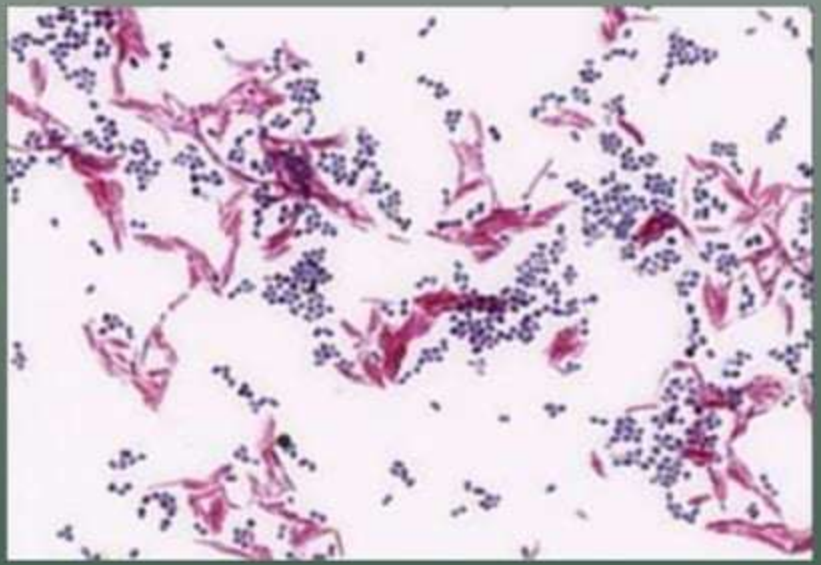
Wash with tap water,decolorise the slide by using acid-alcohol or 20% sulphuric acid until carbol fuchsin fails to wash from smear.

Wash with water and counter stain with 1% aqueous solution of malachite green or methylene blue for 1 to 2 ninutes.

Wash smear with tap water, dry and examine under oil- immersion objective.

OBSERVATION




THANK
YOU