Unit-2

Date

Identification of Bacteria Using Staining techq: ⇒

Means Identification of Bacteria.

In our surrounding Many type of Bacteria is (+)nt (Like Cocci, Bacilus, Spirilli) & which Cause diff. types of diseases;

The bacteria size are very small (we cannot seen by our Naked eyes.

⇒ So with the Help of Microscope & dyes solution (Methylene blue, Crystal Voilet, Safranine) bacteria is Identified.

Staining is a techq. by which we Identify the structure & arrangment of Bacteria by using diff. dyes solution Under Microscope.

Types of Staining

- Simple staining
- Differential staining
  - Gram Staining
    - G.+ve
    - G.-ve
  - Acid fast staining

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① Simple Staining ⇒

Method of staining in which bacteria are stained by using a single stain/dye.

↓

Eg ⇒ Methylene blue, Safranine, malachite green

⇒ This staining is only used to examine → Cell shape, Arrangm. of Bacteria

Principle ⇒

In Simple staining, We generally use ⊕ Ve Charge dye

→ So first we develop ⊖ Ve Charge on the surface of bacteria by addn. of OH^- or Removal of H^+ ion. Because Many Carboxyl group ⊕ ist on the surface of bacteria.

↓

Then we used dye & dye contain ⊕ Ve Charge which attach with ⊖ Ve Charge & it will Make Visible of Bacteria under Microscope.

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Procedure =>

A clean Grease-free slide is taken. [DEPTH OF BIOLOGY]

(Wash करो Slide को गरम Detergent से & then dry it & then the Slide is pass out on flame 2 to 3 time rapidly.

↓

On that Slide Make Smear → sticky substance पर dirty mark. by using a sterile Wire Loop

Culture Media containing Bacteria.

made up of Nichrome

rapidly heat & cool

& Cell suspension.

Bacteria cell multiply.

[DEPTH OF BIOLOGY]

⇓

After Making Smear, the Slide is allowed to air dry.

⇓

After Air drying this slide is rapidly passed through a flame for 3 or 4 times for Heat fixation.

⇓

Now add few drop of diffo. dye / Indicator into the surface of Slide

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⇓

Now allow the Glass Slide for drying few seconds.

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↓↓

Then the slide is washed under Tap water / Running water to wash the excess stain. → धाेना

↓↓

Now Wipe the below Surface of Slide with cloth or Tissue Paper.

↓↓

Put the Cover Slip Over the Smear & Place on the Surface of Microscope

↓

Observe the Bacteria in Microscope. (Shape & Arrangement)

Differential Staining ⇒

- Two or More dyes used at a time.

Gram Staining ⇒

This Method is developed by ⟶ Han's Christian gram in 1884.

* The differential staining technique by which Gram ⊕ & ⊖ ve Bacteria is identified is Called Gram Staining.

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Gram ⊕ Ve	Gram ⊖ Ve.
① After staining it gives Purple/Blue Colour.	① Red/Pink colour.
② Cell wall 20-30 nm thick & Single layer	② Cellwall 8-12 nm thick & 2 layer.
③ Outer Membrane ⊖	③ ⊕
④ Peptidoglycan layer is Multilayer.	④ Single layer
⑤ Lipid Content (1-4%)	⑤ Lipid Content High (11-22%)
eg ⇒ Staphylococcus, Streptococcus, Bacillus.	eg ⇒ Vibrio, E-coli.

[DEPTH OF BIOLOGY]

[DEPTH OF BIOLOGY]

⇒ Procedure ⇒

Requirment for Gram Staining ⇝

- A clean grease free Slide.
- Bacteria Cell Suspension.
- Nichrome Wire Loop.
- Primary Stain — Crystal Violet.

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Mordant — Gram's Iodine

Decolorizing agent → 95% (Alcohol) (Ethanol 95%).

Counter stain → Basic Fuchsine or Safranine.

Gram Staining Procedure ⇒

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Take a clean Grease free slide

↓

Make a Smear Using Nichrome Wire Loop.

↓

Air dry & Heat fix.

↓

Flood Smear with Crystal Voilet for 2 Minute.

↓

~~Treat the Slide with decolorizing agent (95% Ethanol).~~

↓

Wash the Slide with Water (Tap Water)

[DEPTH OF BIOLOGY]

↓

Flood Smear with Gram Iodine for 2 Minute.

↓

Treat the slide with decolourising agent (95% Ethanol)

↓

Wash the slide with Water

↓

Flood the Smear with Fuschin or Safranin.

↓

Wash the slide with Water.

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↓

Observe it → Purple/Blue Colour = +ve (Gram Bacteria)

→ Red/Pink Colour = −ve

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Acid fast Staining ⇒

→ In Nature there are Variety of Microorganism. Each microorg have some special character.

Most of the Microorganism are easily stained by Simple staining Procedures.

→ But their is Some Microorganism that are Not easily stained by this techqnique because they have a waxy covering on its Surface.

↓

Such organism req. a Special staining technique Called Acid fast staining.

⇒ This staining techq. was discovered by Scientist Paul Ehrlich in 1883.

⇒ Acid fast staining technique helps us to differentiate the Organisms as → Acid-fast Organism.
→ Non-Acid fast.

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Acid fast Organism ⇒

The Organism that get stained by Acid fast staining techq but don't get decolourise even by strong acid.

Called Acid fast Organism.

Non-Acid fast Organism ⇒

The Organism that easily get stained & decolourise easily by a strong Acid are called Non-acid fast organism

Procedure ⇒

Take a clean Grease free slide & prepare a Smear using Nichrome Wire loop.

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Air dry & Heat fix the Slide.

↓

The Slide is flooded with ZNCF Strain & placed on Boiling water bath for steaming about 3-5 Minutes.

↓

During steaming the ZNCF Strained is added repeatedly on the slide to avoid drying of Smear.

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Further the Slide is treated to the decolorising agent i.e acid alcohol until the stain dissapear in Washing

↓

After decolorization wash with water. [DEPTH OF BIOLOGY]

↓

Further the Smear is flooded with Counter stain i.e 1% Malachite Green or 0.3% Methylene Blue for 2 Minute. [DEPTH OF BIOLOGY]

↓

After 2 Minute the Slide is washed with water & dried & observe it.