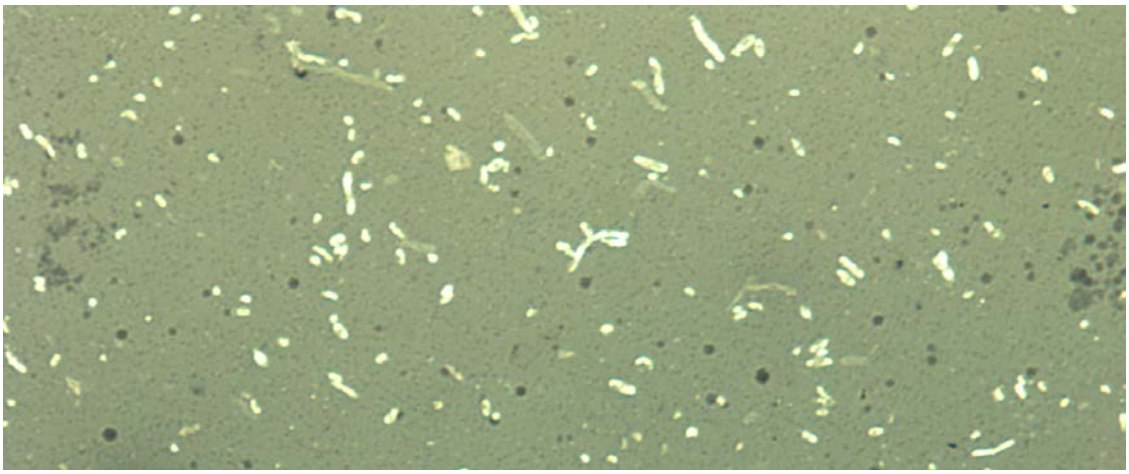
PREPARATION OF BACTERIAL SMEAR AND STAINING : SIMPLE & NEGATIVE STAINING



Negative Staining

Bacteria consists 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 see them in unstained conditions.
Fixation and staining, therefore are of prime importance to increase visibility, accentuate specific morphological features and preserve them for future study.

Fixation:
Fixation is the process by which the internal and external structures of microorganisms and cells are preserved and fixed in position.

The stained cells should resemble living cells as closely as possible.
Fixation inactivates the enzymes that might disrupt cell morphology and toughens cell structures, so that they donot change during staining and observation.
During fixation microorganisms are usually killed and attached firmly to the microscope slide.
Fundamentally, there are two different types of fixation:

1. **Heat Fixation &**
2. **Chemical Fixation.**

1. **Heat Fixation**
   Routinely used to observe bacteria.
   Film of cells on a glass slide is gently heated by passing through a flame.
   Preserves overall morphology but not structures within the cell.

2. **Chemical Fixation**
   Used to protect fine cellular substructures and the morphology of larger or more delicate microorganisms or cells.

Chemical fixative penetrate cells and react with cellular components, usually proteins and lipids and render them inactive, insoluble and immobile.
Chemical fixative mixture contains ethanol, acetic acid, mercuric chloride and glutaraldehyde.

Dyes/Stain:

Many types of dyes are used to stain microorganisms have two features in common:

Chromophore groups, groups with conjugated double bonds that give the dye its colour, and bind with cesls by ionic, covalent, or hydrophobic bonding and stain the cells /microorganisms directly.

Some stains such as Indian Ink , Nogrosin Black stain the background instead of the cells, known as **Negative staining** in which the unstained cells appear bright against dark background.

Important terms:

Chromogen: The portion of the stain that is the colored molecule (often a benzene derivative).

Chromophore: The portion of the chromogen that gives it its color. A chromogen may have multiple chromophores, with each adding intensity to the color.

Auxochrome: The charged portion of the chromogen that allows it to act as a dye through ionic or covalent bonds between the chromogen and the cell.

Dyes that can bind cells by ionic interactions are probably the most commonly used dyes. These ionisable dyes are divided into two classes based on their nature of their charged group:

1. **Basic Dyes:** Methylene Blue, basic fuchsin, crystal violet, safranin and malachite green have positively charged groups (salts). Basic dyes bind to negatively charged molecules like nucleic acids, proteins and the surfaces of prokaryotic cells.
2. **Acidic Dyes:** Eosin, rose Bengal, and acid fuchsin-possesses negatively charged groups such as carboxyls (-COOH) and phenolic hydroxyls (-OH). Acidic dyes, because of the negative charge, bind to positively charged cell structures.

The staining effectiveness of ionisable dyes may be altered by pH, since the nature and degree of the charge on cell components change with pH. Thus acidic dyes stain best under acidic conditions when proteins and many other molecules carry a positive charge; basic dyes are most effective at higher pHs.

Dyes bind through covalent bonds or because their solubility e.g., Feulgen staining for DNA.

Simple Stain Preparation

1. Begin with a heat-fixed emulsion
2. Cover the smear with stain- methylene blue (60 sec)
3. Rinse the slide with water
4. Gently blot dry with bibulous paper - do not rub
5. Observe under oil immersion

Negative Stain Preparation

1. Begin with a drop of acidic stain at one end of a clean slide
2. Aseptically add organisms and emulsify with a loop. Do not over-inoculate and avoid spattering the mixture
3. Take a second clean slide, place it on the surface of the first slide, and draw it back into the drop
4. Do it until the drop flows across the width of the spreader slide
5. Then push the spreader slide to the other end and dispose of the spreader slide
6. Air dry and observe under the microscope

Why negative staining?

To determine morphology and cellular arrangement in bacteria that are too delicate to withstand heat-fixing. Also, where determining the accurate size is crucial, a negative stain can be used because it produces minimal cell shrinkage

Exercise:

1. Write the procedure to prepare bacterial smear.
2. Write the composition of simple stain & negative stains.

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