## OUTLINE

#### PRELAB:

- 1. Culture Media Preparation to Analysis
- 2. Preparation of Bacterial Smear
- Eixation.

Heat Fixation

Chemical Fixation

Preparation & Fixation of Smear

4. Dyes

Basic Dyes

Acidic Dyes

Staining

LAB INSTRUCTIONS:

- 6. Introduction
- 7. Making smears
- 8. Simple Stain
- 9. Gram Stain

10. Endospore Stain (Schaeffer-Fulton Method)

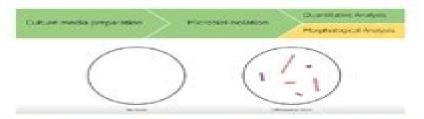
11. Acid Fast Stain

POST-LAB

#### Legend:

Remember Lecturer Book

#### **CULTURE MEDIA PREPARATION TO ANALYSIS**



#### PREPARATION OF BACTERIAL SMEAR

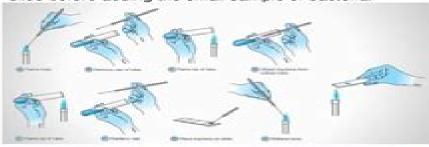
### A. Steps

- Flame loop
- Remove cap of tube
- Flame tip of tube
- Obtain bacteria from culture tube
- · Flame tip of tube
- Beplace cap

- Place bacteria on slide.
- Re-flame loop

#### B. Important steps in the preparation of bacterial smear from broth culture

- If using a broth culture, the liquid is placed directly on the slide.
- If a slant is used, a loopful of water is placed on the slide before adding the small sample of bacteria.



#### FIXATION

- Is the process by which the internal and external structures of specimens are preserved and fixed in position.
- Inactivates the enzymes that might disrupt cell morphology and toughens cell structures so that they can do not change during staining and observation

#### A. Heat Fixation

- Firm of cells (smear) is gently heated as a slide is passed through a flame
- Preserves overall morphology but also destroys proteins that may be part of subcellular structures

#### B. Chemical Fixation

- Chemical Preservatives (e.g., ethanol, acetic acid, mercuric chloride, formaldehyde mixture) penetrate cells and react with cellular components
- Used to protect fine cellular substructure renders protein and lipids to be inactive, insoluble and immobile.

## C. Preparation and Fixation of Smear & Heat

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