

# MICROBIOLOGY LAB

## Module 6: Staining Methods

### OUTLINE

#### PRELAB:

1. Culture Media Preparation to Analysis
2. Preparation of Bacterial Smear
3. Fixation

Heat Fixation  
Chemical Fixation  
Preparation & Fixation of Smear

#### 4. Dyes

Basic Dyes  
Acidic Dyes

#### 5. Staining

#### LAB INSTRUCTIONS:

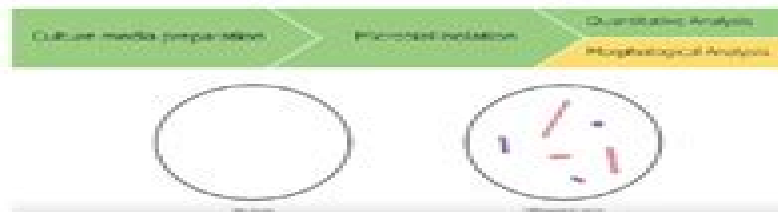
6. Introduction
7. Making smears
8. Simple Stain
9. Gram Stain
10. Endospore Stain (Schaeffer-Fulton Method)
11. Acid Fast Stain

#### POST-LAB

#### Legend:

Remember	Lecturer	Book
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### CULTURE MEDIA PREPARATION TO ANALYSIS



### PREPARATION OF BACTERIAL SMEAR

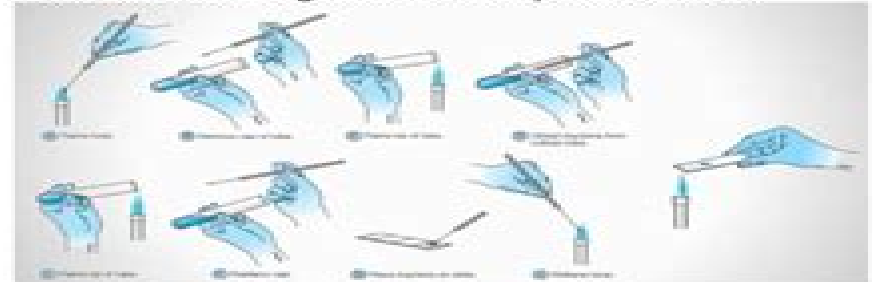
#### A. Steps

- Flame loop
- Remove cap of tube
- Flame tip of tube
- Obtain bacteria from culture tube
- Flame tip of tube
- Replace 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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**L Reisser**



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