



محاضرة رقم: 8

Lecture No 8:

# DIAGNOSTIC MICROBIOLOGY

Sawa University

College of health and medical techniques

Department of Medical Laboratories

4 Stage

جامعة ساوة الاهلية

كلية التقنيات الصحية والطبية

قسم تقنيات المختبرات الطبية

المرحلة/الرابعة

تدريسي المادة : م.م عذراء هادي

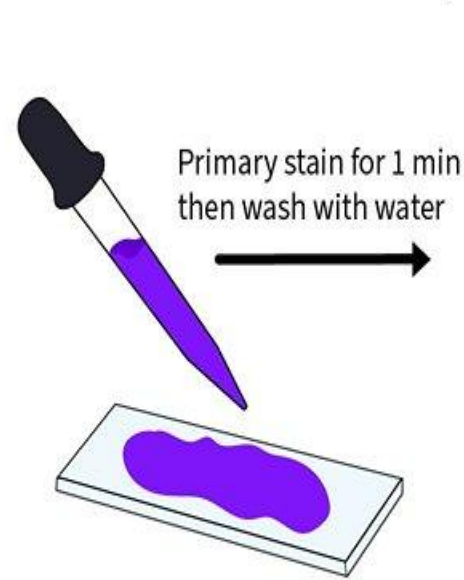
# Staining



Staining: A specimen must contain at least 10<sup>5</sup> organisms per milliliter before it is likely that organisms will be seen on a smear. Liquid medium containing 10<sup>5</sup> organisms per milliliter does not appear turbid to the eye. Specimens containing 10<sup>2</sup>–10<sup>3</sup> organisms per milliliter produce growth on solid media, .there are many types of stains, each with specific applications

1. Crystal violet

All bacteria will be stained  
bluish or bluish-purple



Gram +ve: Purple  
Gram - ve: Purple

2. Gram's Iodine

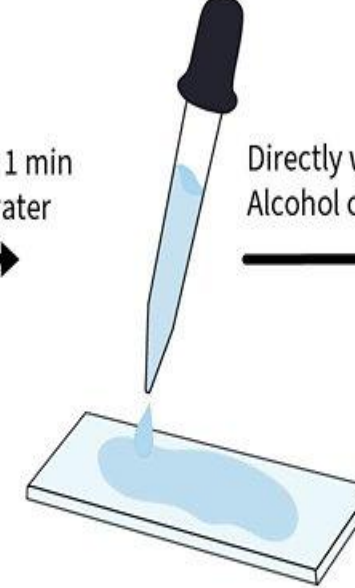
Enhance the Crystal violet  
staining by forming  
Crystal violet - Iodine complex



Gram +ve: Purple  
Gram - ve: Purple

3. Alcohol/Acetone

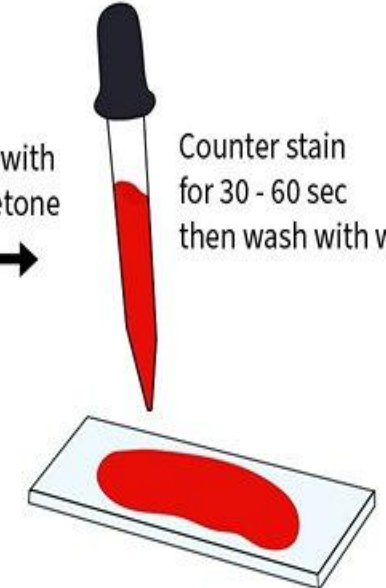
Decolorizer washes away  
Primary stain from  
gram -ve bacteria



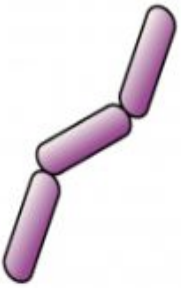
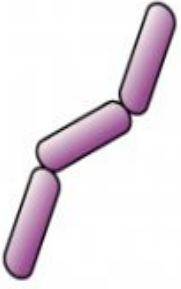
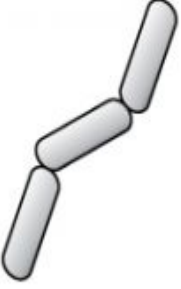
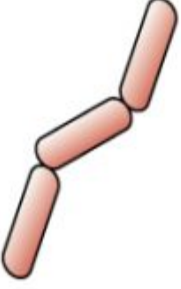
Gram +ve: Purple  
Gram - ve: Colorless

4. Safranin/Carbol fuchsin

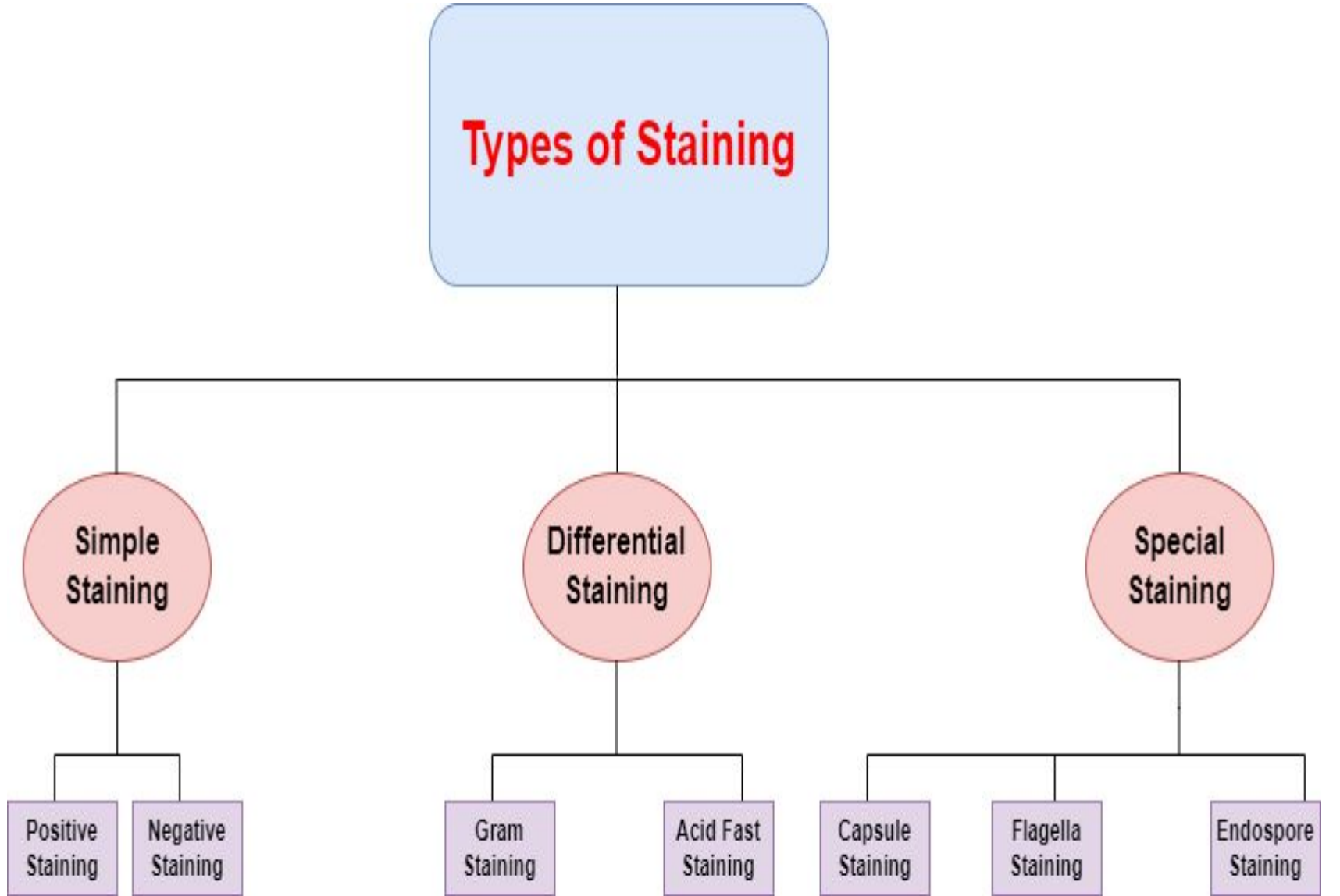
Counterstain stains the colorless  
gram -ve bacteria



Gram +ve: Purple  
Gram - ve: Red

|                                                                                                                   |                                                                                           |                                                                                       |                                                                                       |
|-------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| <p><b>Step 1</b><br/> <b>Crystal violet</b><br/> <i>primary stain added to specimen smear.</i></p>                | <p>Stains cells purple or blue.</p>                                                       |     |     |
| <p><b>Step 2</b><br/> <b>Iodine</b><br/> <i>mordant makes dye less soluble so it adheres to cell walls.</i></p>   | <p>Cells remain purple or blue.</p>                                                       |    |    |
| <p><b>Step 3</b><br/> <b>Alcohol</b><br/> <i>decolorizer washes away stain from gram-negative cell walls.</i></p> | <p>Gram-positive cells remain purple or blue. Gram-negative cells are colorless.</p>      |   |   |
| <p><b>Step 4</b><br/> <b>Safranin</b><br/> <i>counterstain allows dye adherence to gram-negative cells.</i></p>   | <p>Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.</p> |  |  |

# Types of Staining



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graph TD; A[Types of Staining] --> B((Simple Staining)); A --> C((Differential Staining)); A --> D((Special Staining)); B --> E[Positive Staining]; B --> F[Negative Staining]; C --> G[Gram Staining]; C --> H[Acid Fast Staining]; D --> I[Capsule Staining]; D --> J[Flagella Staining]; D --> K[Endospore Staining];
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**Simple  
Staining**

Positive  
Staining

Negative  
Staining

**Differential  
Staining**

Gram  
Staining

Acid Fast  
Staining

**Special  
Staining**

Capsule  
Staining

Flagella  
Staining

Endospore  
Staining



☐ Simple stains are directed toward coloring the forms and shapes

,present

☐ differential stains are directed toward coloring specific

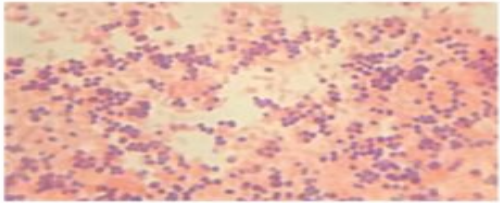
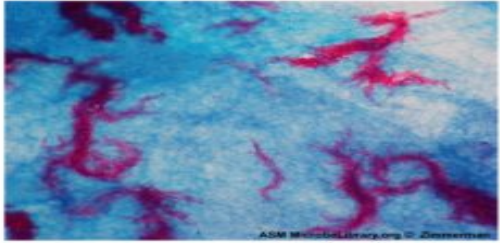
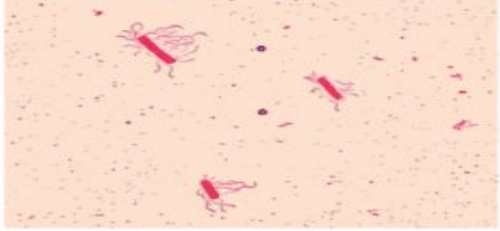
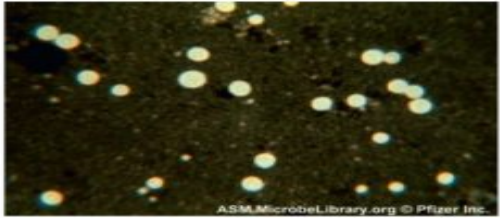
,components of the elements present

☐ diagnostic antibody or DNA probe-mediated stains are directed

.specifically at identification of an organism

## SIMPLE STAINS

| Stain Type             | Specific Dyes                                                                           | Purpose                                                                               | Outcome                                                                        | Sample Images                                                                         |
|------------------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| <b>Basic stains</b>    | Methylene blue, crystal violet, malachite green, basic fuchsin, carbolfuchsin, safranin | Stain negatively charged molecules and structures, such as nucleic acids and proteins | Positive stain                                                                 |    |
| <b>Acidic stains</b>   | Eosin, acid fuchsin, rose bengal, Congo red                                             | Stain positively charged molecules and structures, such as proteins                   | Can be either a positive or negative stain, depending on the cell's chemistry. |    |
| <b>Negative stains</b> | India ink, nigrosin                                                                     | Stains background, not specimen                                                       | Dark background with light specimen                                            |  |

| DIFFERENTIAL STAINS    |                                                                                                                                                                                                                |                                                                                                  |                                                                          |                                                                                       |
|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Stain Type             | Specific Dyes                                                                                                                                                                                                  | Purpose                                                                                          | Outcome                                                                  | Sample Images                                                                         |
| <b>Gram stain</b>      | Uses crystal violet, Gram's iodine, ethanol (decolorizer), and safranin                                                                                                                                        | Used to distinguish cells by cell-wall type (gram-positive, gram-negative)                       | Gram-positive cells stain purple/violet. Gram-negative cells stain pink. |    |
| <b>Acid-fast stain</b> | After staining with basic fuchsin, acid-fast bacteria resist decolorization by acid-alcohol. Non acid-fast bacteria are counterstained with methylene blue.                                                    | Used to distinguish acid-fast bacteria such as <i>M. tuberculosis</i> , from non-acid-fast cells | Acid-fast bacteria are red; non-acid-fast cells are blue.                |    |
| <b>Endospore stain</b> | Uses heat to stain endospores with malachite green (Schaeffer-Fulton procedure), then cell is washed and counterstained with safranin.                                                                         | Used to distinguish organisms with endospores from those without; used to study the endospore.   | Endospores appear bluish-green; other structures appear pink to red.     |    |
| <b>Flagella stain</b>  | Flagella are coated with a tannic acid or potassium alum mordant, then stained using either pararosaniline or basic fuchsin.                                                                                   | Used to view and study flagella in bacteria that have them.                                      | Flagella are visible if present.                                         |   |
| <b>Capsule stain</b>   | Negative staining with India ink or nigrosin is used to stain the background, leaving a clear area of the cell and the capsule. Counterstaining can be used to stain the cell while leaving the capsule clear. | Used to distinguish cells with capsules from those without.                                      | Capsules appear clear or as halos if present.                            |  |

## Steps of Preparing a Stained Biological Specimen for Microscopic Study

Steps of staining biological specimens vary greatly depending upon the type of staining techniques being applied. Some common steps in most of the staining techniques are given below:

**Wet Mounting:** Wet mounting is the first step in simple vital staining technique where living cells are .to be studied. In this step the specimen under study is placed in a drop of water on a glass slide

**Fixation:** Fixation involves treating the specimen with fixatives like formaldehyde and ethanol. This .kills the specimen and maintains its structure by preventing decay

**Embedding:** Some cells and tissues need embedding in suitable medium like paraffin wax to make .sectioning easier

**Deparaffinization:** If embedding medium is used, deparaffinization is done using suitable solvent to .remove the embedding medium

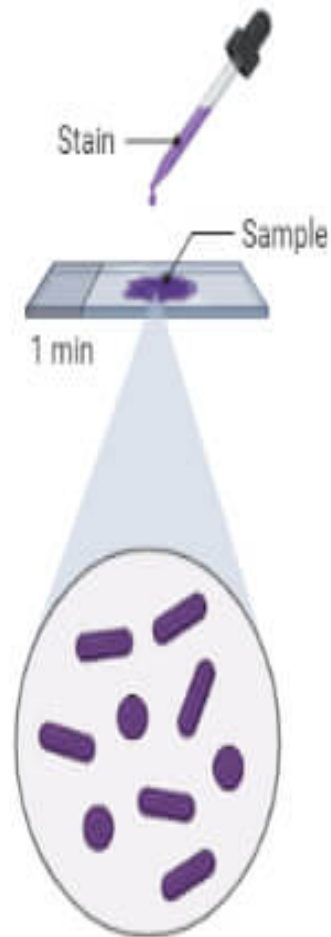
**Staining:** This step involves application of the stain to the specimen. Sometimes mordants are added with stains in order to stain the unstainable specimen. Based on the staining techniques the further steps vary. It may involve washing, heating etc, followed by application of counterstain or other .substances

**Mounting:** After staining is completed the specimen is mounted onto the slide with a suitable medium. Then a cover slip is placed on it. After mounting the specimen is ready for microscopic .studies

## Step 1

### Crystal violet

*Primary stain added to specimen smear.*

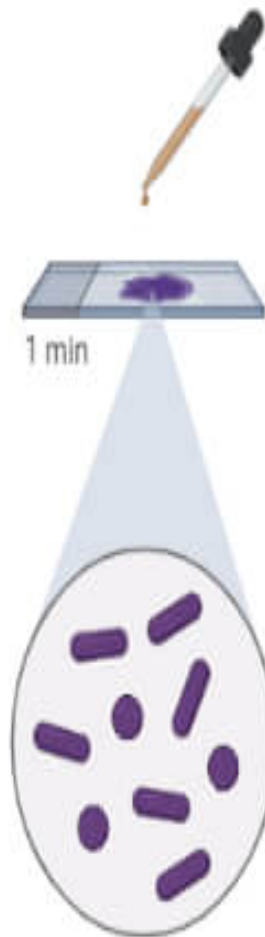


- Gram (+): purple
- Gram (-): purple

## Step 2

### Iodine

*Mordant makes dye less soluble so it adheres to cell walls.*

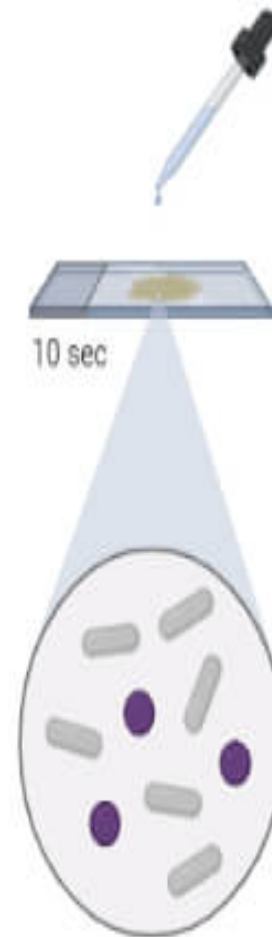


- Gram (+): purple
- Gram (-): purple

## Step 3

### Alcohol

*Decolorizer washes away stain from gram (-) cell walls.*

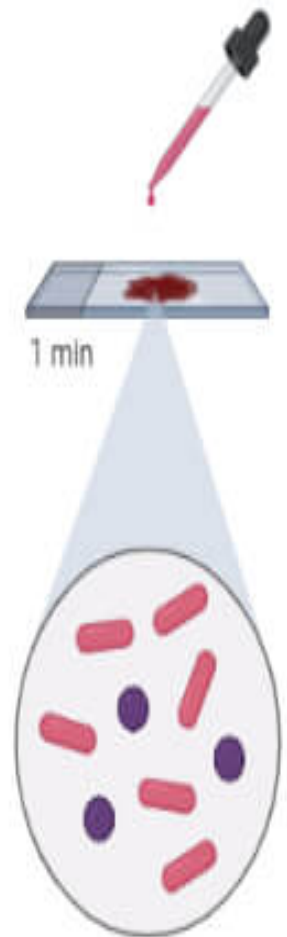


- Gram (+): purple
- Gram (-): colorless

## Step 4

### Safranin

*Counterstain allows dye adherence to gram (-) cell walls.*



- Gram (+): purple
- Gram (-): red

## Common Example of Biological Stains

The following are some of the common examples of biological stains:

**Acridine Orange:** Acridine Orange is used in fluorescence microscopy for vital staining of live cells. It is a fluorescent dye composed of acridine and phenazine moieties. It emits orange fluorescence when bound to DNA, making it useful for nucleic acid staining. Acridine Orange is used in .fluorescence microscopy for vital staining of live cells

**Eosin:** Eosin is an acidic dye composed of brominated fluorescein. Eosin stains cytoplasm and extracellular structures in tissues, imparting a pink or red color. It is often used as a counterstain in .combination with hematoxylin in H & E staining

**Hematoxylin:** Hematoxylin is a basic dye derived from the heartwood of certain trees and .undergoes oxidation to form hematein. Hematoxylin stains nuclei blue or purple

**Malachite Green:** Malachite Green is a synthetic dye belonging to the triarylmethane class. .Malachite green is used in staining bacterial endospore

**Methylene Blue:** Methylene Blue is a basic thiazine dye. It stains acidic structures in cells, such as .nucleic acids