Salahaddin University- Erbil
College of Agriculture
Plant Protection Department
2nd Class

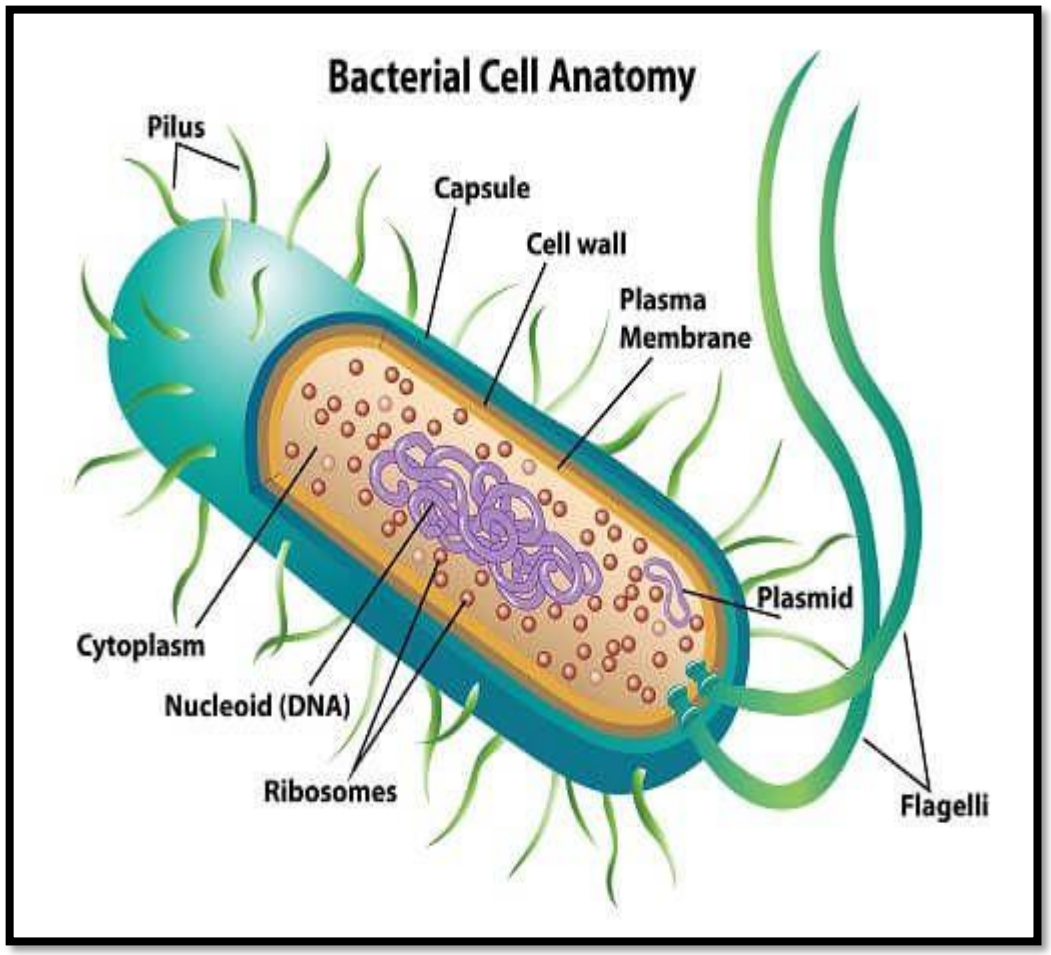
Bacterial Staining

5th lecture

Outline

- Importance of satin
- What is stain?
- Why to stain?
- Methods of staining
- Simple staining
- Differential staining
- Preparing microscopic slides

Bacterial cell



Importance of stain

- The microscopic examination of stained smear enables the **morphology**, **relative sizing** and **arrangement** of microorganisms to be seen clearly.
- It also assists in the **detection of the cells**.
- **Bacteria** can be differentiated by their staining reaction e.g. **gram positive** from **gram negative.**

What is stain and staining

Stain: These are chemical substances used to stain cells, giving the cell colour.

- They are used to differentiate different types of organisms or to view specific parts of organisms.

- **Staining:** Artificial colouration of a substance which uses selected dyes(stains) to colour biological specimens such as **cells**, **cell products** or **microorganisms** to assist in examination and identification under the microscope.

Why to stain?

- Bacteria are microscopic organisms, they are also colourless for the most part.

- To conceive them to study their structure, shape, and other structural characteristics, it becomes necessary to make them more easily visible under a light microscope.

Methods of staining

Simple stain

-look at morphology and arrangement good for observing morphology Result-all bacterial cells stain the same color Stains might include methylene blue, and crystal violet.

Differential stain

Used to identify different types of organisms. Involves exposing cells to more than one stain Ex. Gram Stain

Special-stain

specific structures of bacteria Capsule stain, Endospore , flagella stain

Differences between Simple and Differential staining

Simple staining	Differential staining
1. This method uses only one stain.	This method uses more than one stain.
2. It imparts only one colour to all bacterial cells.	It imparts two or more different colours to bacterial cells.
3. It reveals the size, shape and arrangement of bacterial cells.	It reveals the size, shape and arrangement. In addition, it differentiates two groups of bacteria.
Example: Methylene blue staining method.	Example: 1. Gram's staining method 2. Acid Fast staining method

Types of staining techniques

Simple staining
(use of a single stain)

For visualization of morphological shape & arrangement.

Differential staining
(use of two contrasting stains separated by a decolorizing agent

Identification
- Gram stain
- Acid fast stain

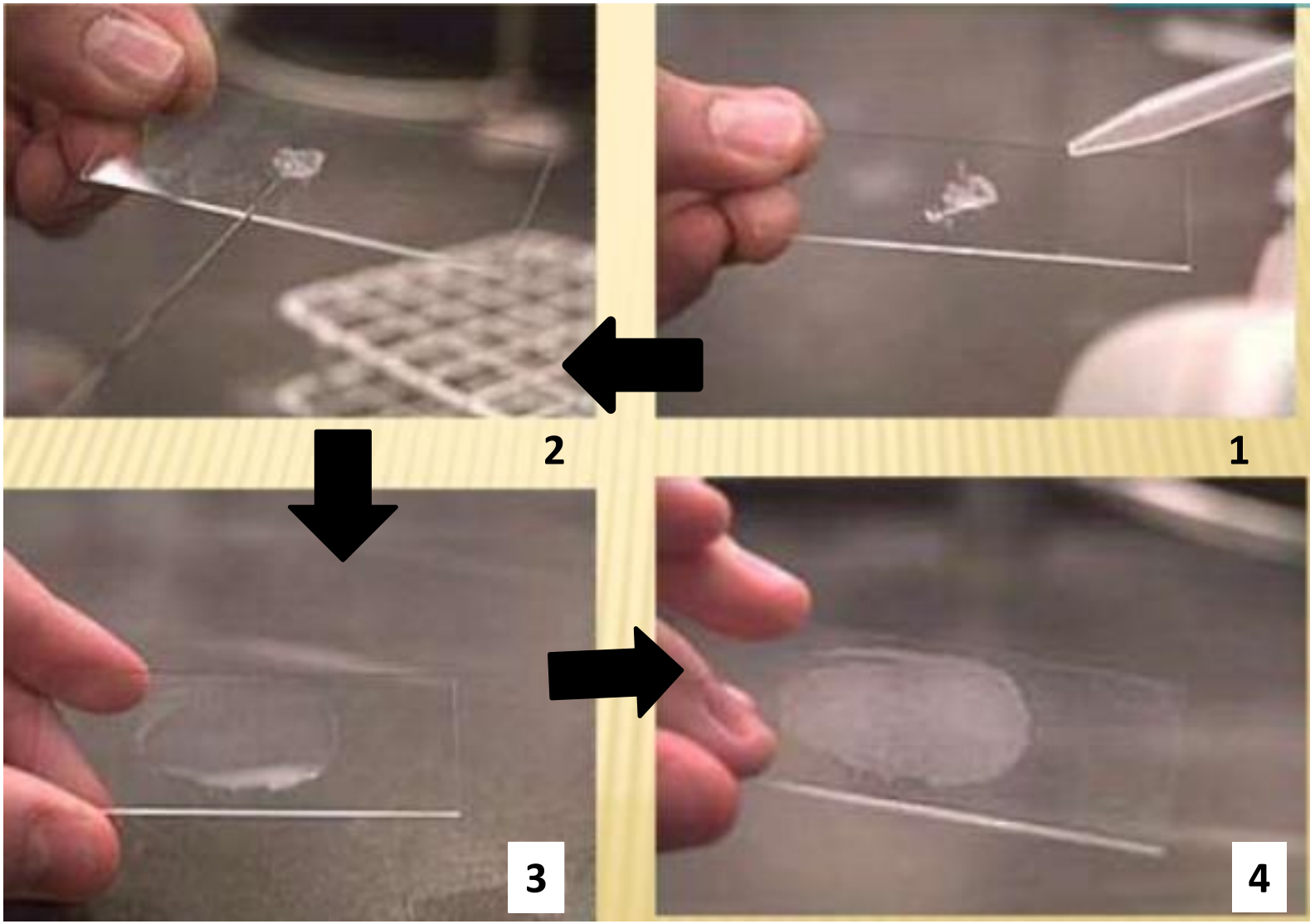
Visualization of structure
- Spore stain
- Capsule stain

Bacterial smear preparation

- ✓ **Smear:** is a distribution of bacterial cells on a slide for the purpose of viewing them under the microscope.
- ✓ **How to prepare smear:**

- Aseptically a small sample of the culture is spread over a slide surface.
- This then is allowed to air dry.
- The next step is **heat fixation** to help the cells adhere to the slide surface.
- Finally the smear is ready for staining.

Bacterial smear preparation



Preparing microscopic slides

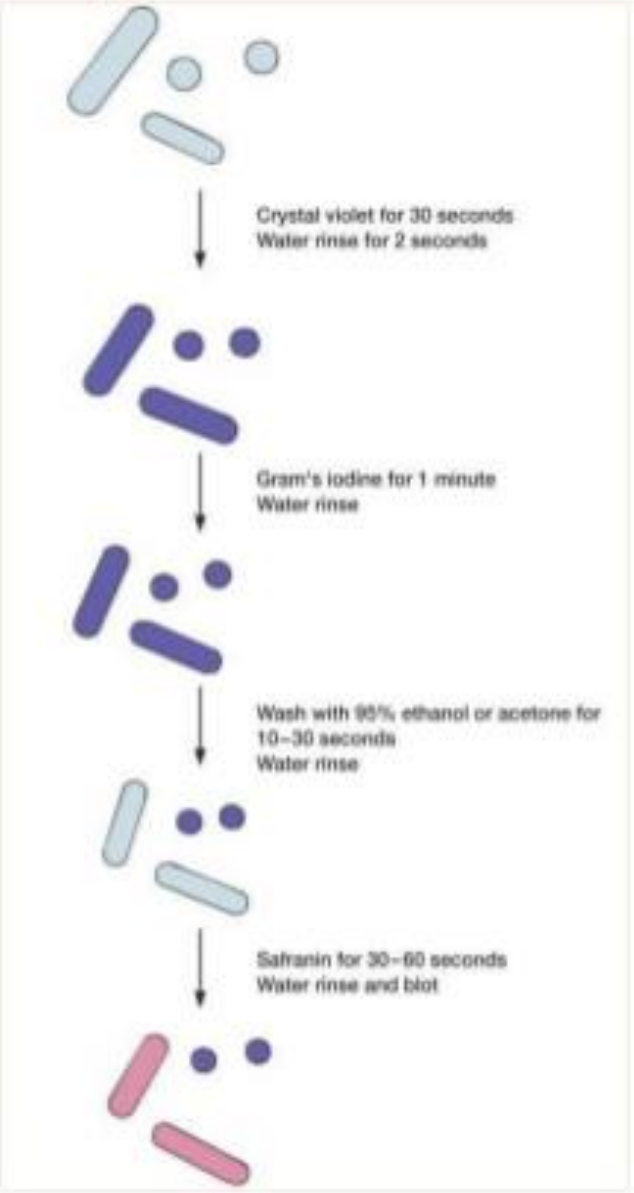
Procedure for staining

Reagents:

- Crystal violet (primary stain)
- Iodine solution/Gram's Iodine(mordant that fixes crystal violet to the cell wall)
- Decolorize (e.g. ethanol)
- Safranin (secondary stain)
- Distilled Water

GRAM STAIN TECHNIQUES

- Prepare bacterial smear on the clean slide.
- Pass the slide through over the flame 2–3 times. (**Heat fixing**)
- Apply Crystal Violet (**Primary stain**) on smear for 1 minutes & rinse with water.
- Apply Gram's iodine (**Mordant**) for 1 minute & wash with water.
- Then wash with 95% alcohol (**Decolouriser**) for 10-20 seconds & rinse with water.
- Apply Safranin (**Seconday stain**) for 1 minute & wash with water.
- Air dry, Blot dry & Observe under Microscope.



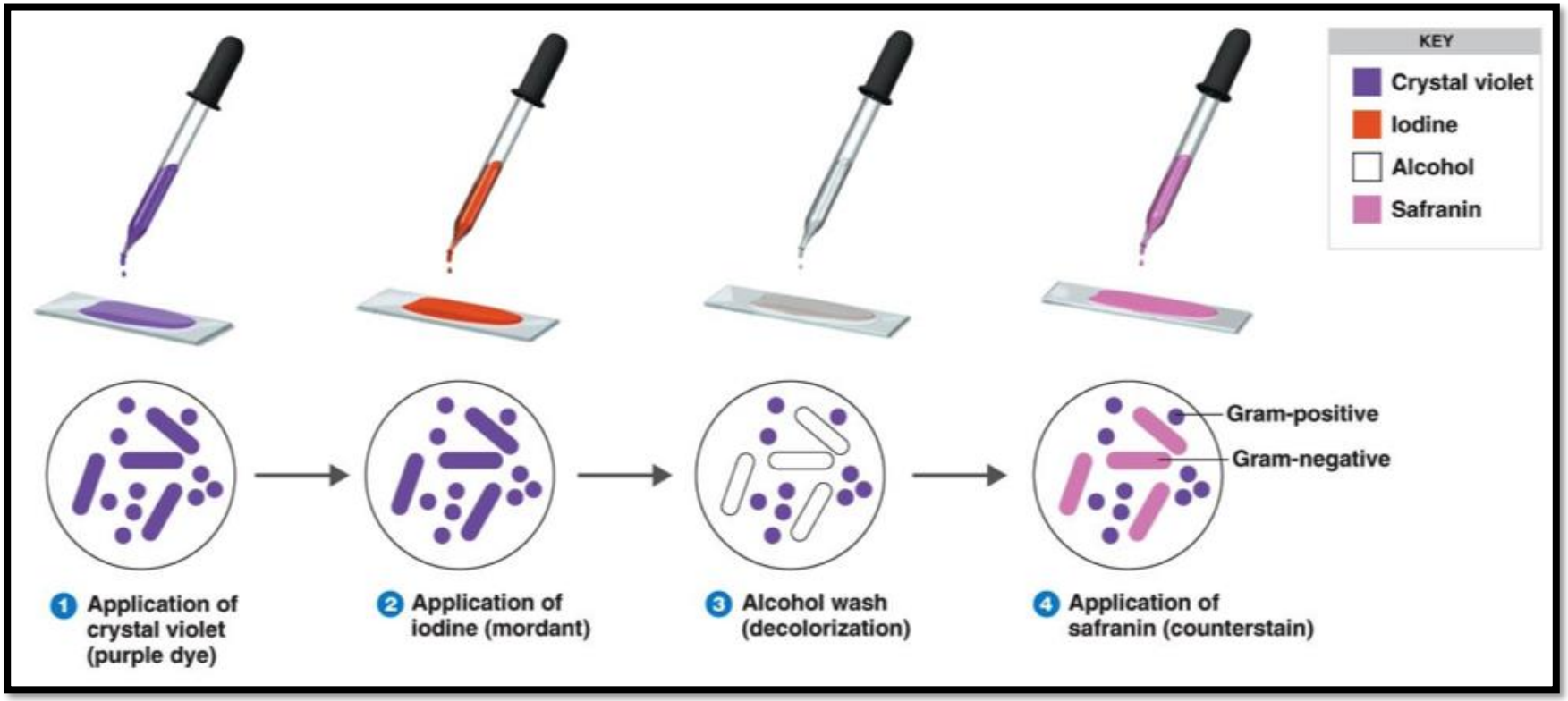
Summary of Gram stain

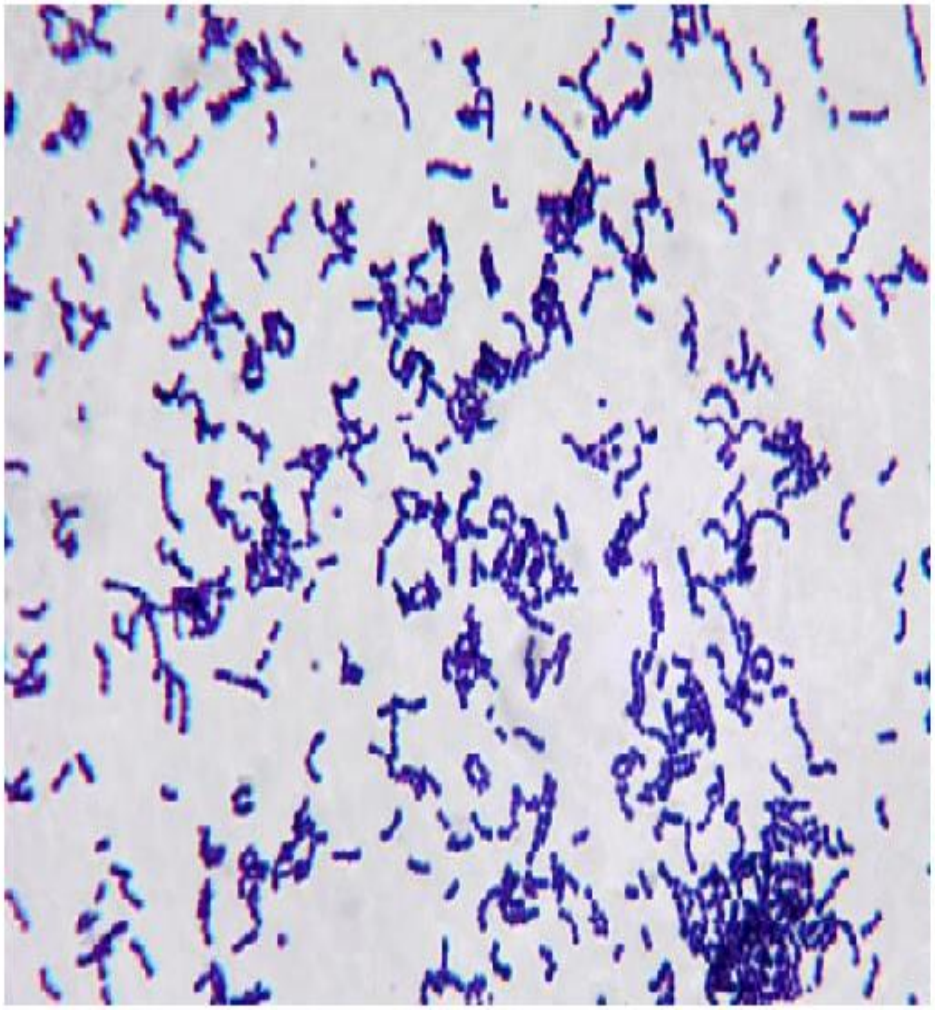
Application of	Reagent	Cell color	
		Gram-positive	Gram-negative
Primary dye	crystal violet	purple	purple
mordant	iodine	purple	purple
Decolorizer	alcohol/acetone	purple	colorless
Counter stain	safranin/carbol fuchsin	purple	pink or red

Gram staining – **used for identifying bacteria.**

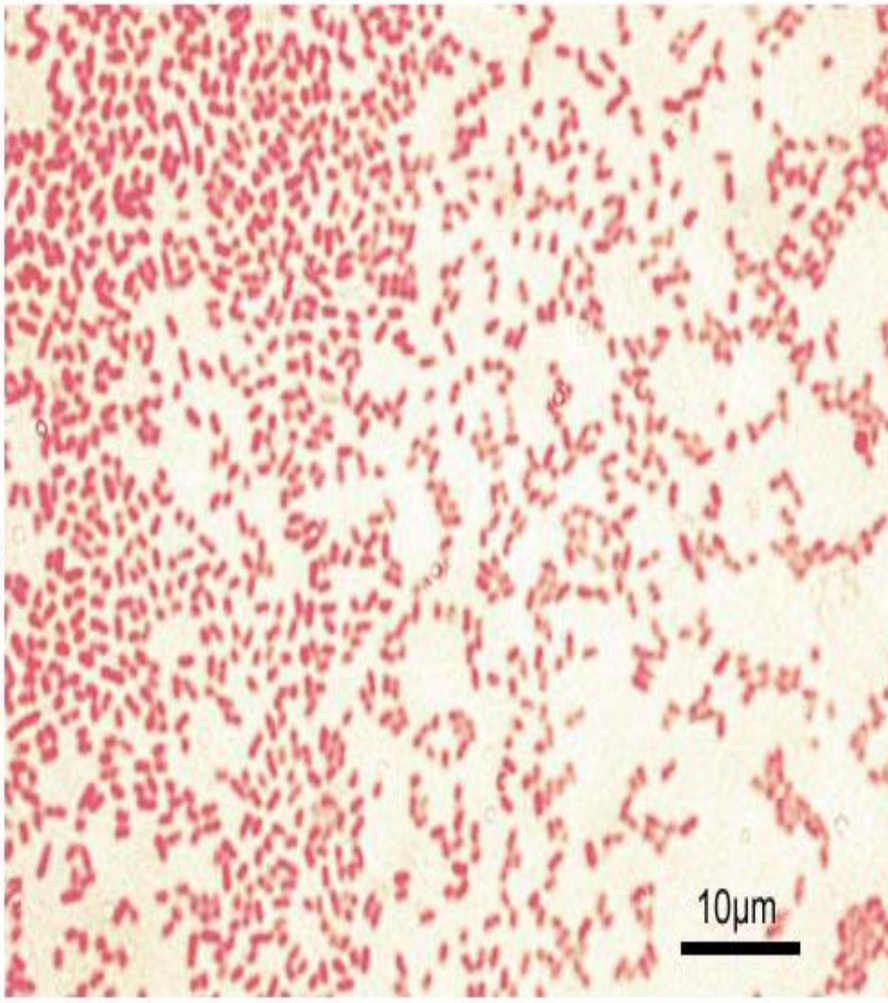
Composed from :

1- Crystal violet. 2- iodine. 3- Alcohol 95%. 4- Safranine



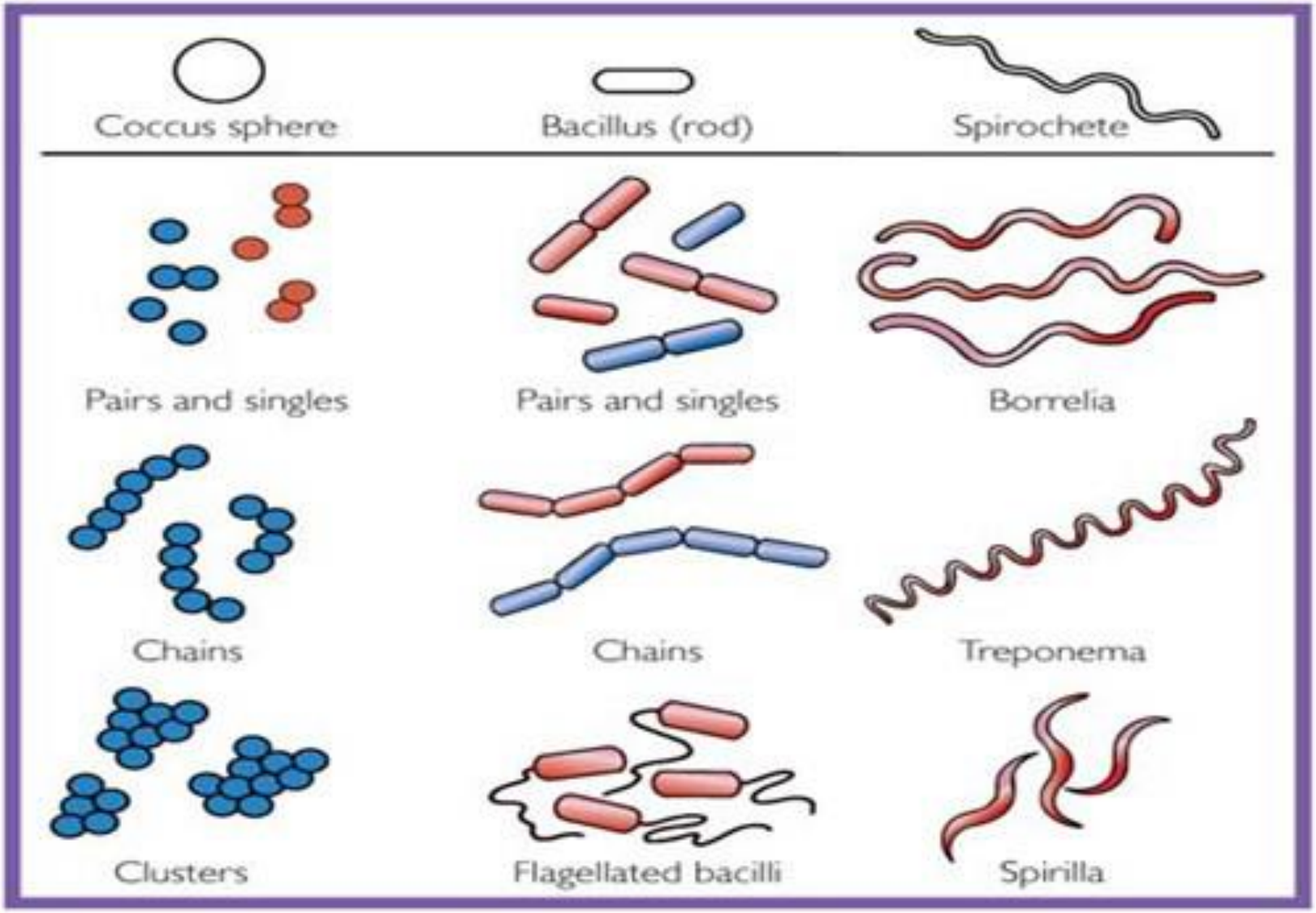


Gram Positive Bacteria



Gram Negative Bacteria

Bacterial morphology



Common errors in staining procedure

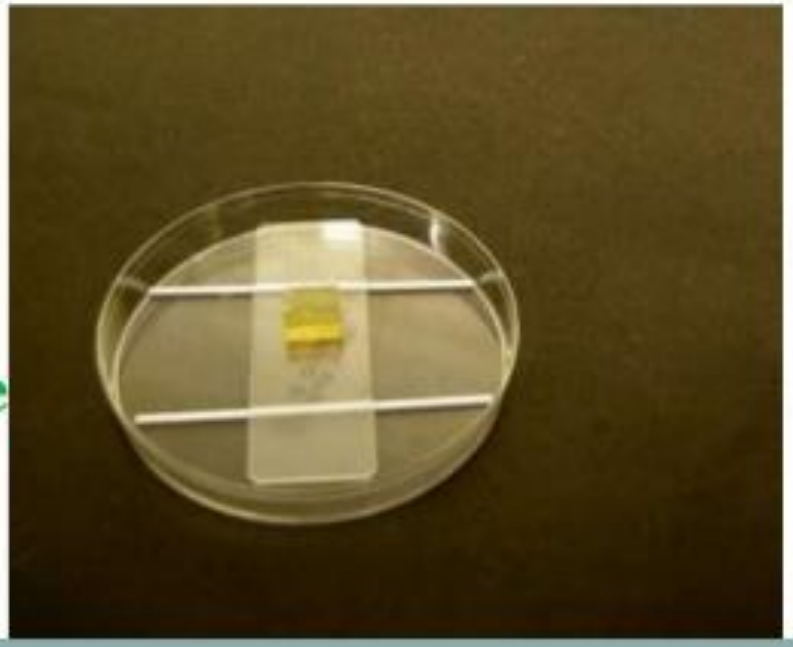
- Excessive heat during fixation.
- Low concentration of crystal violet.
- Excessive washing between steps.
- Insufficient iodine exposure.
- Prolonged decolonization.

Microscopic Observation

Using Stereo Microscope &
Compound Light Microscope

Lacto phenol Cotton Blue Staining (LPCB)

Slide Culture Technique



Preparation slide of fungi

Lacto phenol cotton blue mounting of fungi

procedure

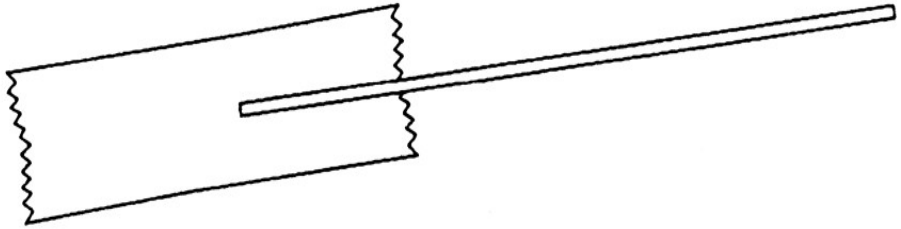
1- place a drop of Lacto phenol cotton blue on a clean slide.

2- transfer a small tuft of the fungus, preferably with spore and spore-bearing structures, into the drop using a flamed, cooled needle.

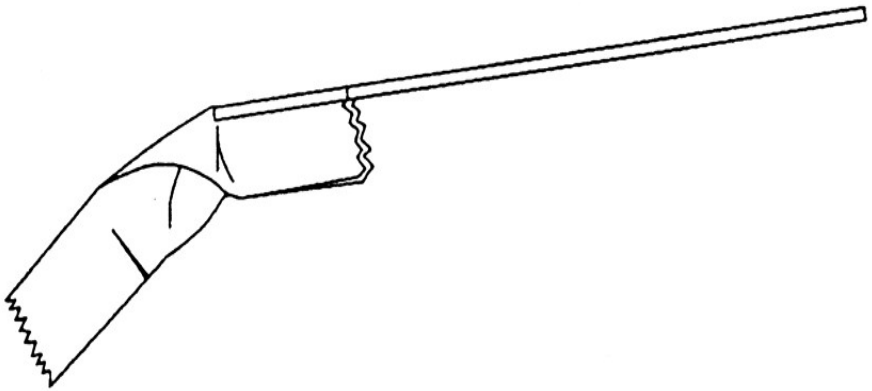
3- Gently tease the material using the two mounted needles.

4- Mix gently the stain with the mold structures.

5- place a cover-glass over the preparation taking care to avoid trapping air bubbles in the stain.



A



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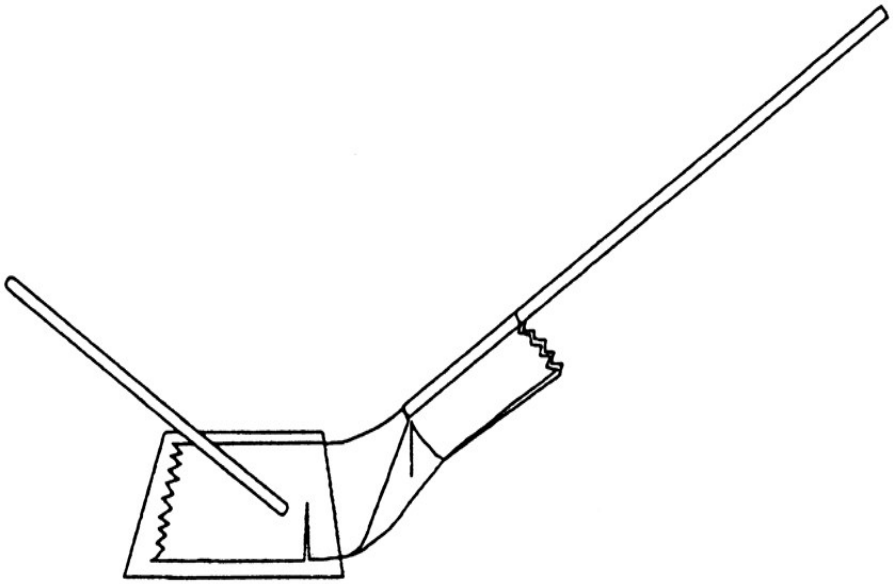

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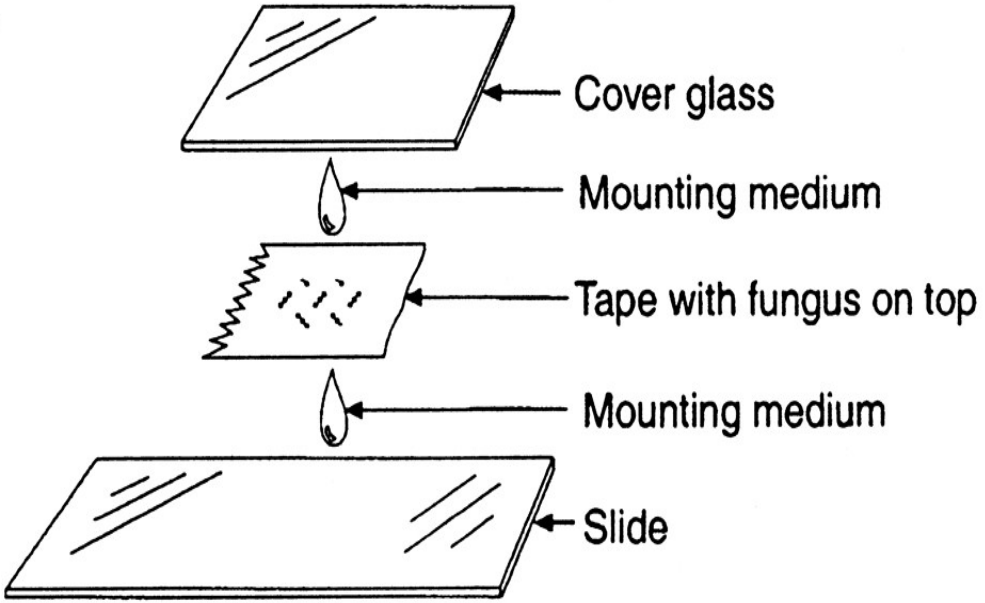
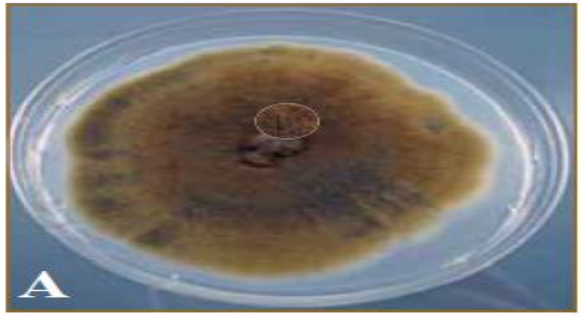
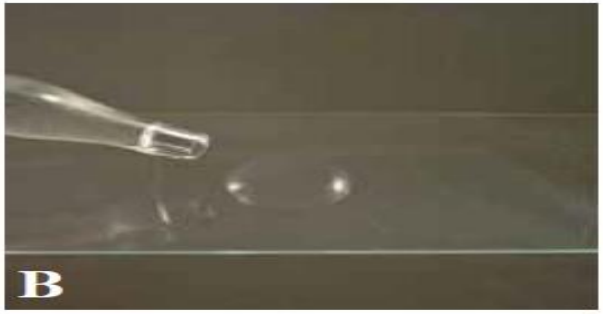
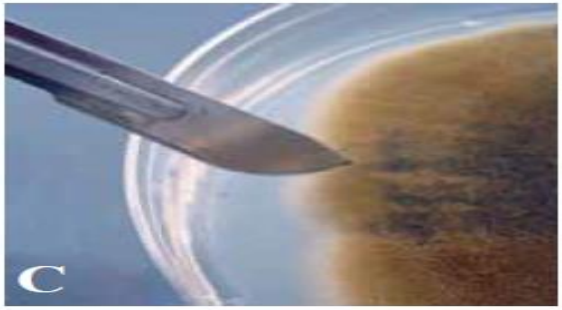

Cover glass
Mounting medium
Tape with fungus on top
Mounting medium
Slide

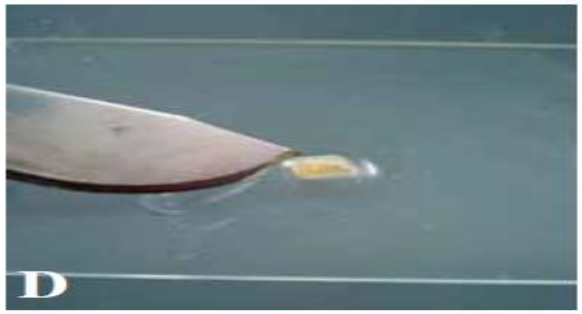
D

Preparing a slide from a fungal culture









Steps to prepare a slide mount from a fungal culture for viewing with a compound microscope: (A) Fungal culture growing on potato dextrose agar, (B) Place one drop of water on glass slide, (C) With a scalpel, cut a very small piece of agar and mycelium from the edge of the colony, (D) Place the agar piece in the drop of water, (E) Place a cover slip at a 45 degree angle over the agar piece and water to minimize air bubbles in the mount, (F) Gently flatten the agar piece with the eraser of a pencil, (G) Mycelium is dispersed and ready to view

Homework

What are the differences between **Gram-positive** bacteria and **Gram-negative** bacteria?