222 MBIO
Microbial Fine Structure

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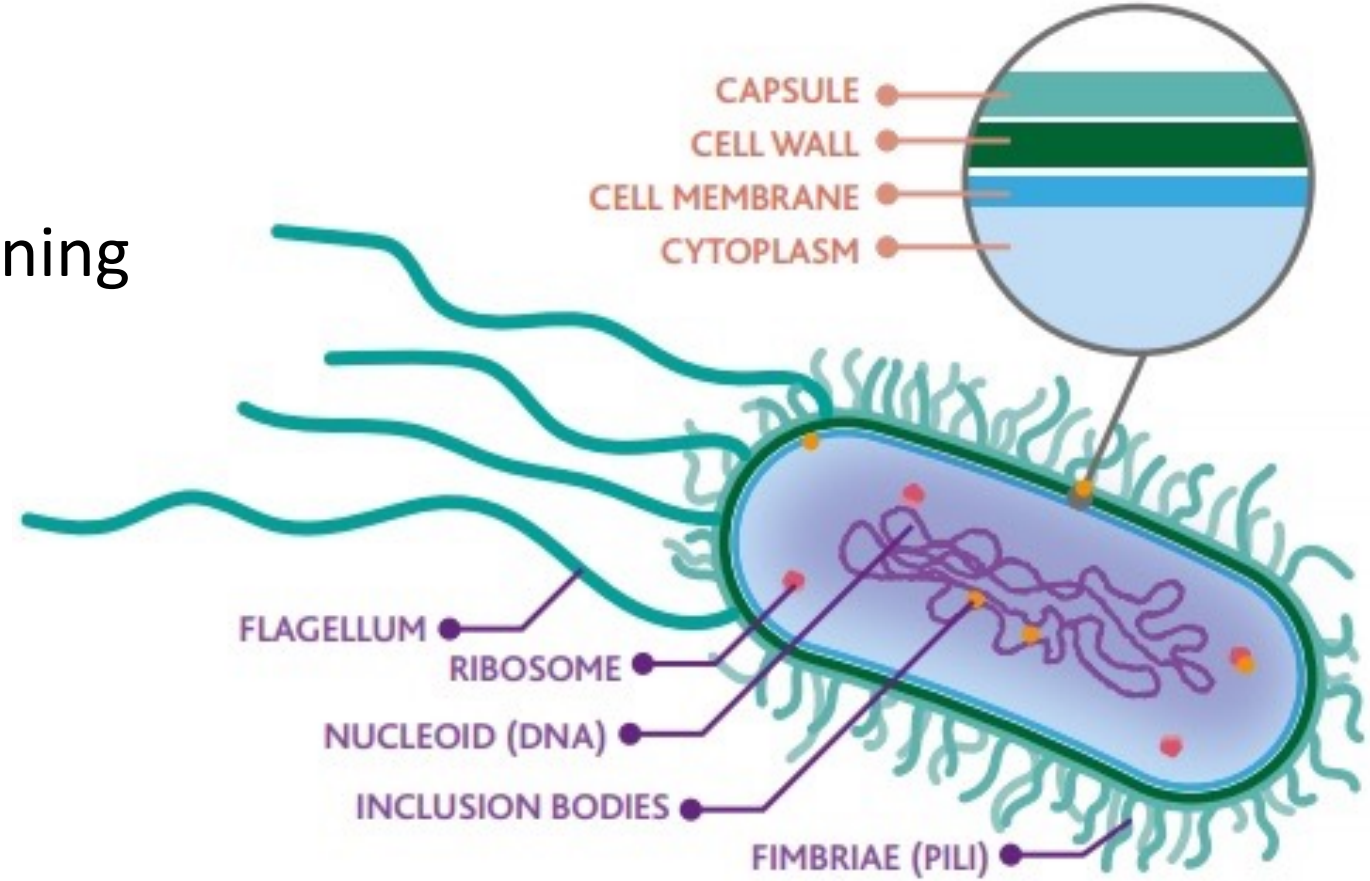
Bacterial Capsule and Capsule Staining

- Lab 3 -

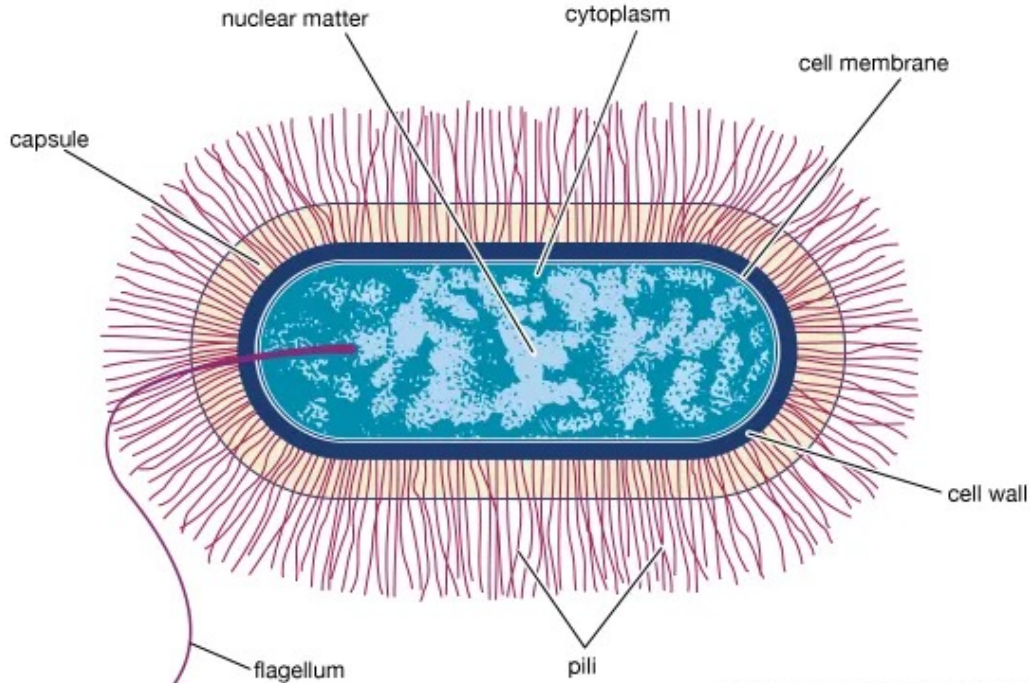
Aim

- To **demonstrate the presence of a bacterial capsule** using capsule staining techniques.



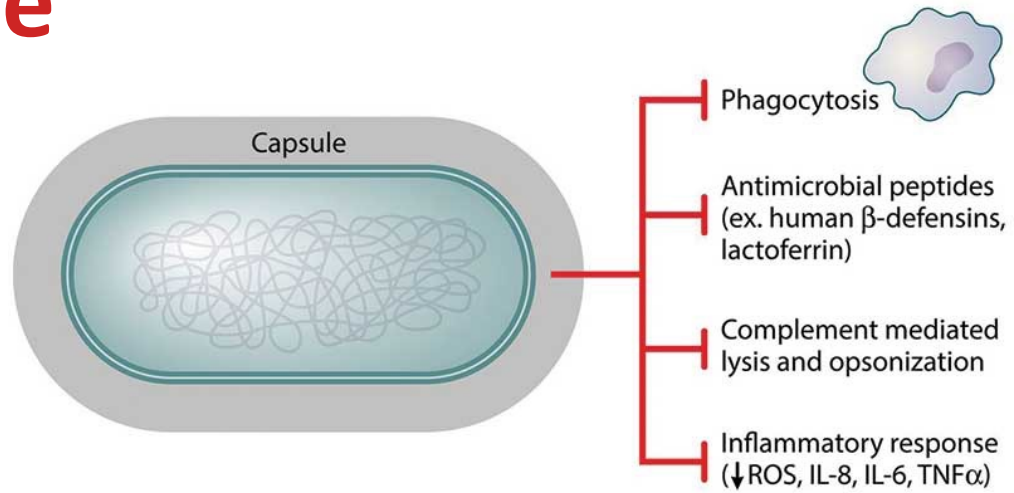
Introduction

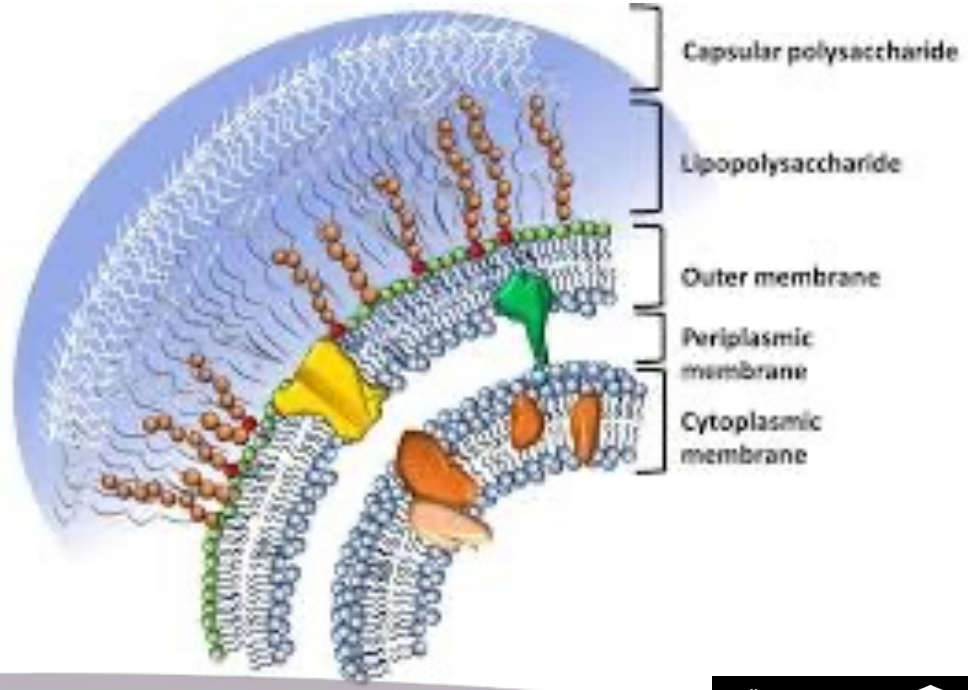
- Many bacteria, including Gram-positive and Gram-negative species, are **surrounded** by an **outer polysaccharide layer** known as **the capsule** (or **glycocalyx**).
- The capsule is **located outside the cell wall** and forms a **distinct protective layer**.



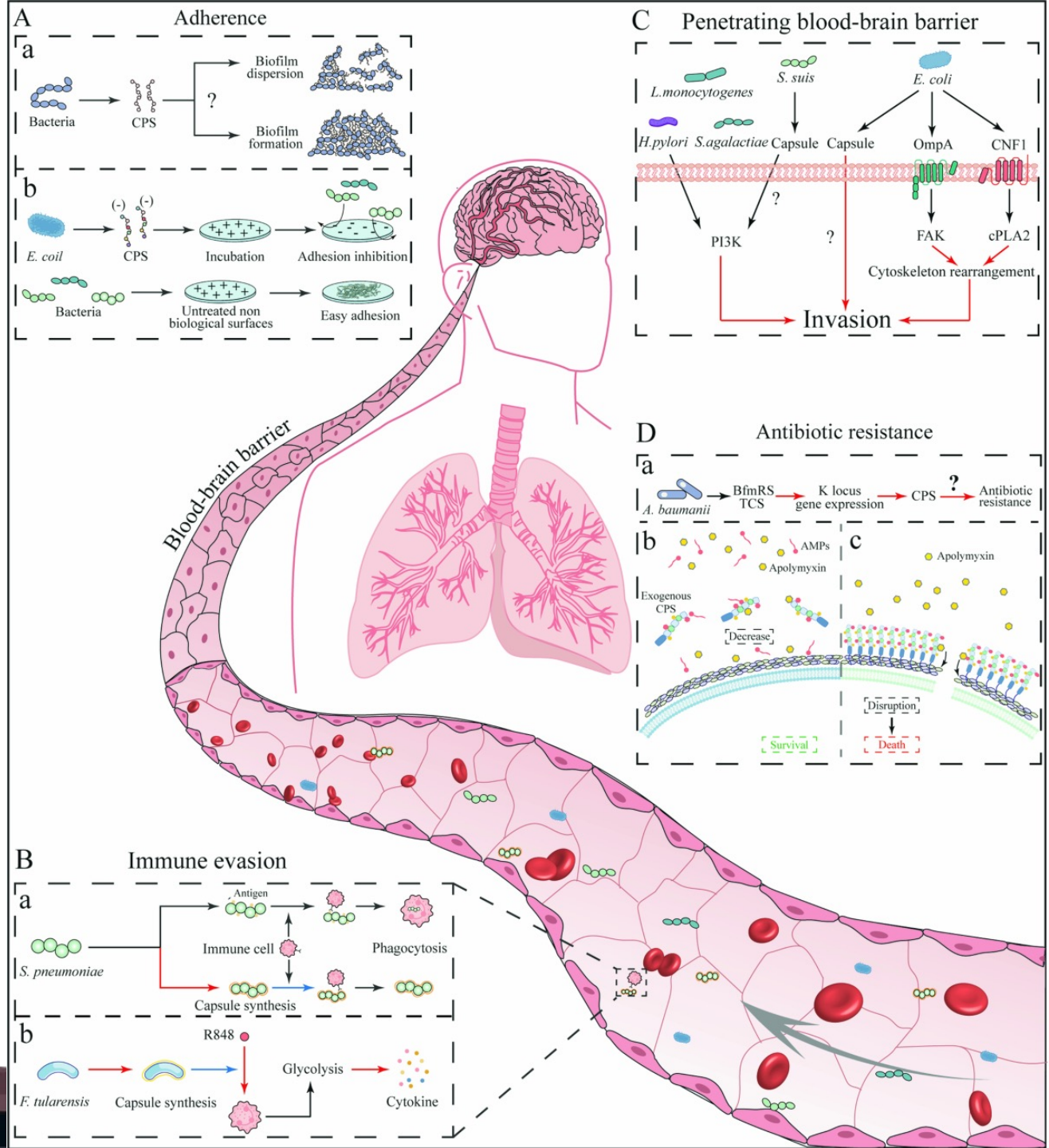
Importance of the Bacterial Capsule

- Acts as a **major virulence factor**
  - **Protects** bacteria from **host immune defenses** (e.g., **phagocytosis**)
- **Enhances adherence and biofilm formation**
- **Prevents** dehydration by **retaining water**
- **Identification** of encapsulated strains is clinically important



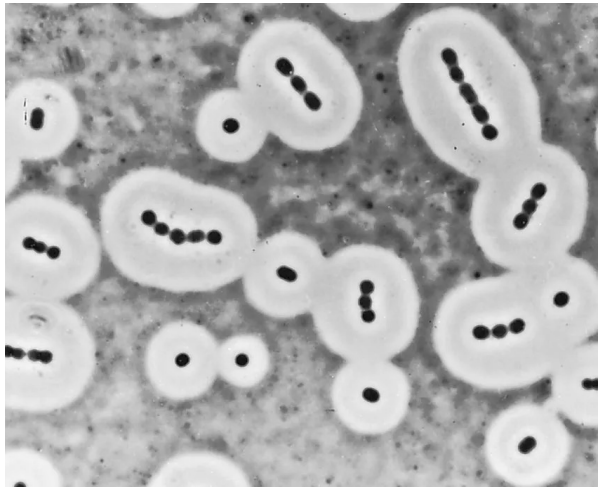


The figure is for illustration only!

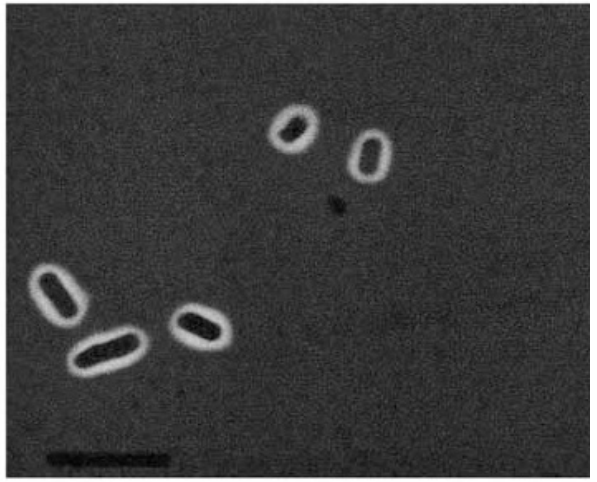


❖ Examples of encapsulated bacteria:

- *Streptococcus pneumoniae*
- *Klebsiella pneumoniae*
- *Bacillus anthracis*
- *Neisseria meningitidis*
- *Escherichia coli*



Streptococcus pneumoniae



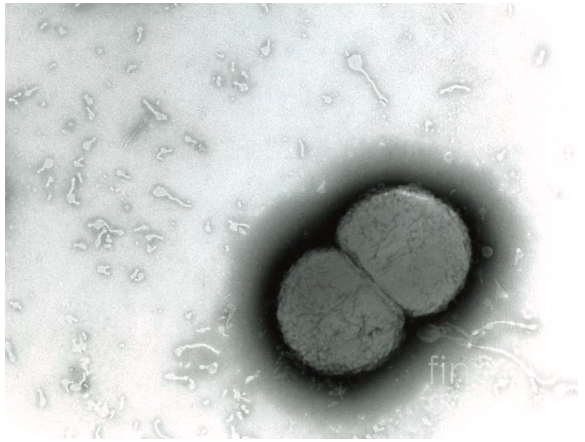
Klebsiella pneumoniae

- **Note:**

→ **Older cultures** are more likely to produce capsules.

→Use cultures that are at **least 5 days old.**

Bacillus anthracis



Neisseria meningitidis

Principle of Capsule Staining

- Because the capsule is a **key virulence determinant**, accurate **identification** of encapsulated strains is clinically important.
- Capsules are **water-soluble and non-ionic**
- Neither acidic (-) nor basic (+) stains will **penetrate or adhere** to the capsule.

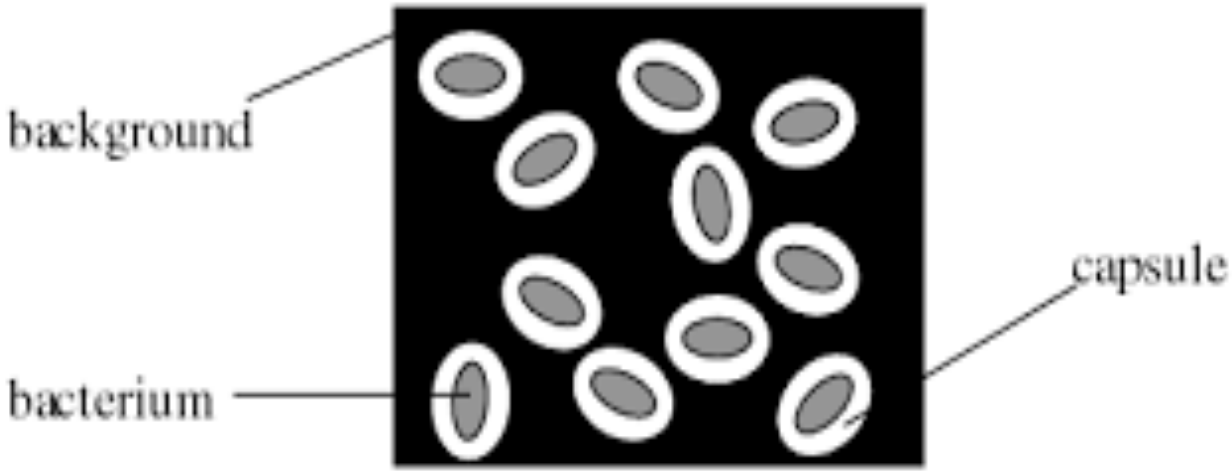
- **SO, WE NEED SPECIAL WAY TO STAIN CAPSULES!**

- **Capsule staining** therefore **relies on indirect visualization, usually by**:

  - Staining the **cell**

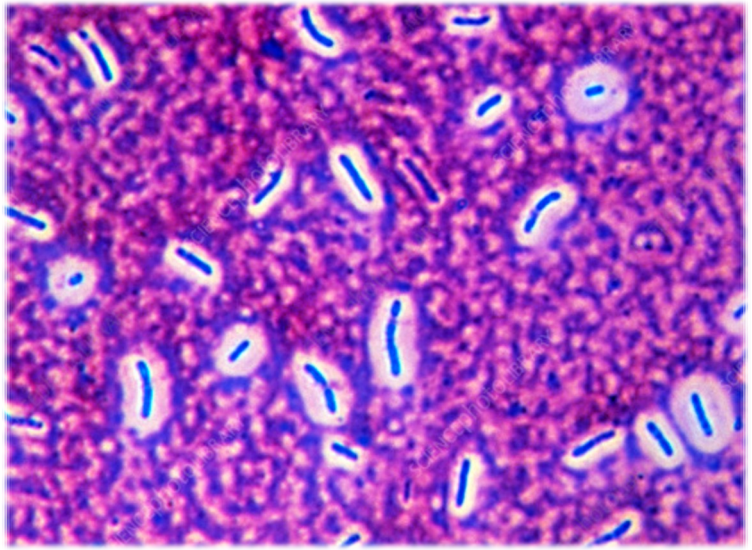
  - Staining the **background**

  - Leaving the **capsule unstained** as a clear zone



Anthony's Capsule Stain

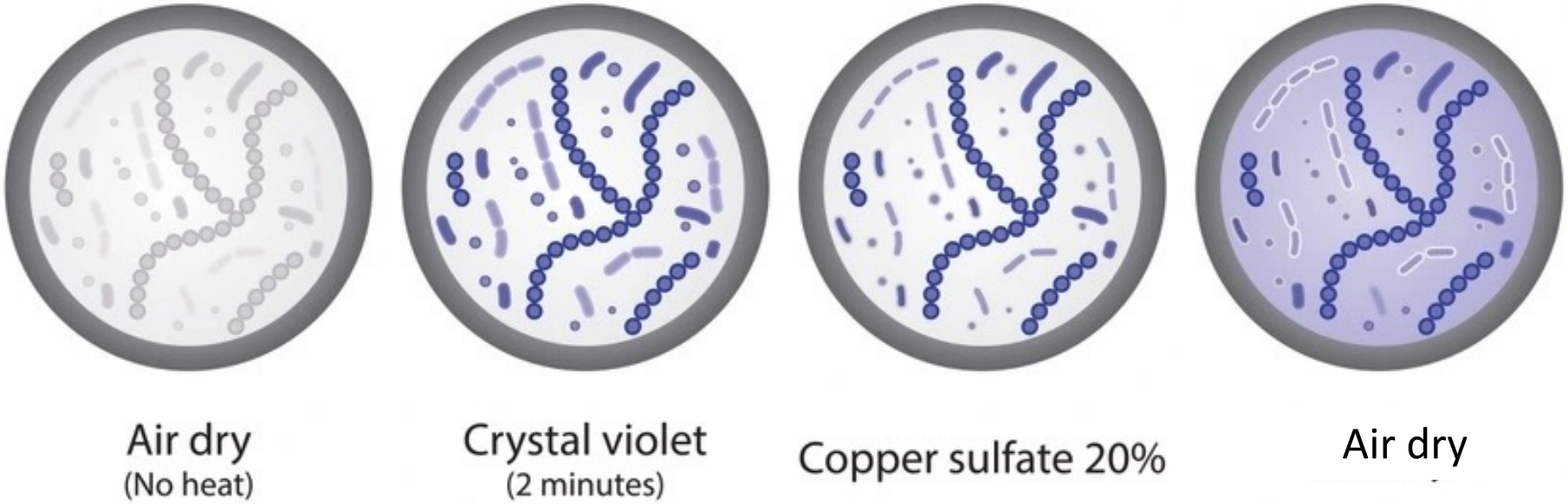
- **Crystal violet** → is used as the primary stain
- **Copper sulfate** → acts as a mordant, to **fix or intensify** dyes
- **No** additional negative stain is used
- **At the end of staining:**
  - Cells and background appear **purple**
  - **Capsules** remain **unstained** (white/clear)

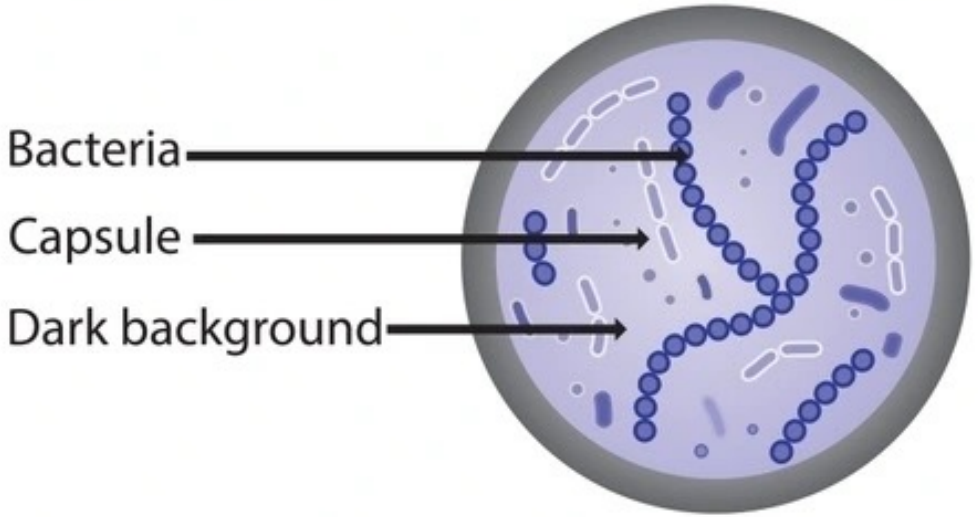


Experiment 2:
Capsule Staining Protocols

First Protocol

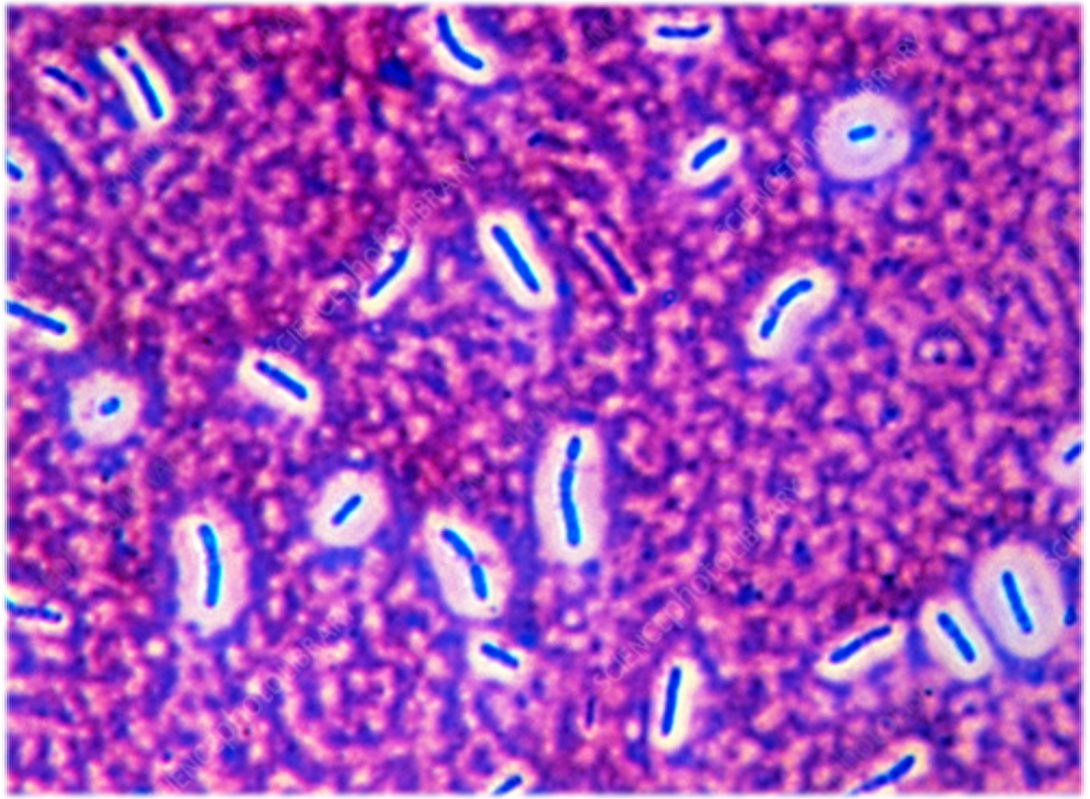
1. Prepare a bacterial **smear**
2. **Air dry only** — **DO NOT heat fix** (Heat may destroy or shrink the capsule)
3. Flood slide with **1% crystal violet for 2 minutes**
4. **Gently rinse** with 20% copper sulfate
5. Air dry — **DO NOT blot** (Blotting will remove the unheated-fixed bacteria from the slide and/or cause disruption of the capsule.)
6. Examine under oil immersion (100×)





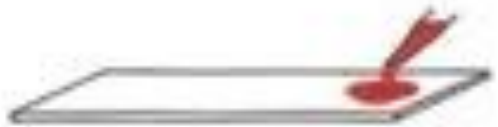
❖Results

- Bacterial cells and background: purple
- Capsule: clear, colorless halo



Second Protocol (Negative Staining)

1. Place a **drop of India ink** on a clean slide
2. Mix a **small amount** of bacterial culture into the ink
3. **Spread using a second slide at a 45◦ angle**
4. Allow to **air dry completely (no heat fixation)**
5. **Flood** with **crystal violet for 1 minute**
6. Rinse gently with distilled wate
7. Examine under oil immersion



Apply India ink
(Primary dye)

Aseptically add
bacteria

Use the spreader to make a thin layer of the dye and bacteria.
Throw the spreader slide into the slide disposal container.

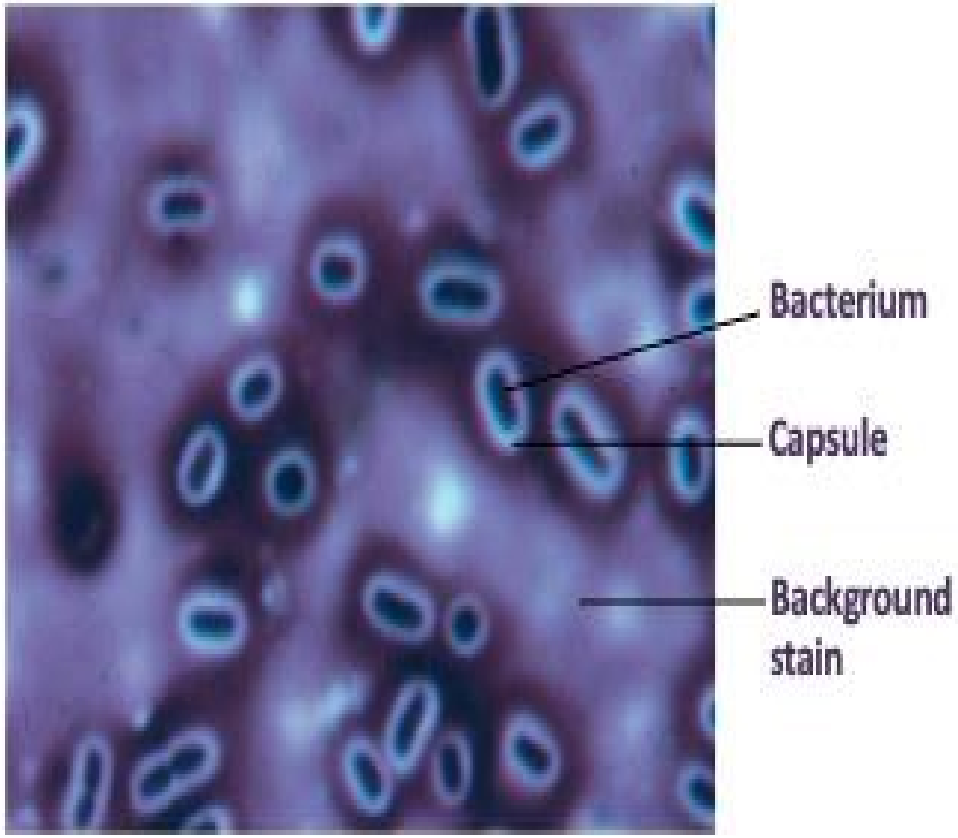
Air dry and do NOT heat
fix

Apply Crystal violet
(Secondary Dye)

Rinse with water and gently blot
with bibulous paper

❖Results

- **Background: dark**
- **Bacterial cell: purple**
- **Capsule: clear halo around the cell**



"Success in this course comes from practice, attention to detail, and responsibility in the laboratory. Engage actively and make the most of every practical session.."

End of the Lab 🤓