Observing Microorganisms through a Microscope

Chapter 3

Basic techniques needed to study Bacteria

- 1. Grow Bacteria
- 2. Isolate Bacteria
- 3. Grow Bacteria in pure culture
- 4. Observe Bacteria
- 5. Identify Bacteria

Microscope

- Resolving Power - ability to distinguish two distinct points
  - absolute limit of the Resolving Power is about 1/2 the wavelength of light that is used to illuminate the specimen

Preparing smears for staining

- 1. Bacteria on slide
- 2. Air Dry
- 3. Bacteria are HEAT FIXED to the slide
- 4. Stain is applied

Staining Reaction

- Stains - salts composed of a positive and negative ion, one of which is colored (chromophore)

- Basic Dyes - chromophore is the positive ion
  - dye+ Cl-
- Acid Dyes - chromophore is the negative ion
  - Na+ dye-

Bacteria are slightly negative, so are attracted to the positive chromophore of the BASIC DYE

- Common Basic Dyes
  - crystal violet
  - methylene blue
  - safranin
  - basic fuchsin

Acid Dyes - used for **Negative Staining**
(background is stained)

Mordant - intensifies the stain or coats a structure to make it thicker and easier to see after it is stained

Example:

Flagella - can not normally be seen, but a mordant can be used to increase the diameter of the flagella before it is stained

Salmonella typhosa

Differential Stains

- React differently with different types of bacteria
- 2 Most Common
  - Gram Stain
  - Acid-Fast Stain

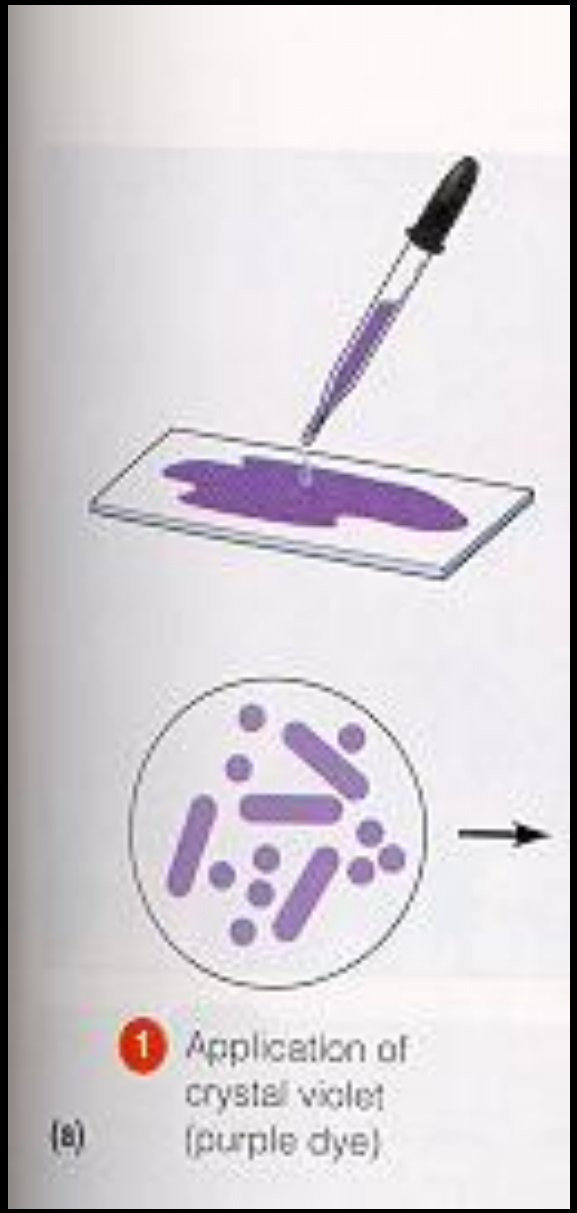
Gram Stain

- 1884 Hans Christian Gram
- most important stain used in Bacteriology

- Divides all Bacteria into 2 groups:
  - Gram (+)
  - Gram (-)

Gram Stain

1. Crystal violet

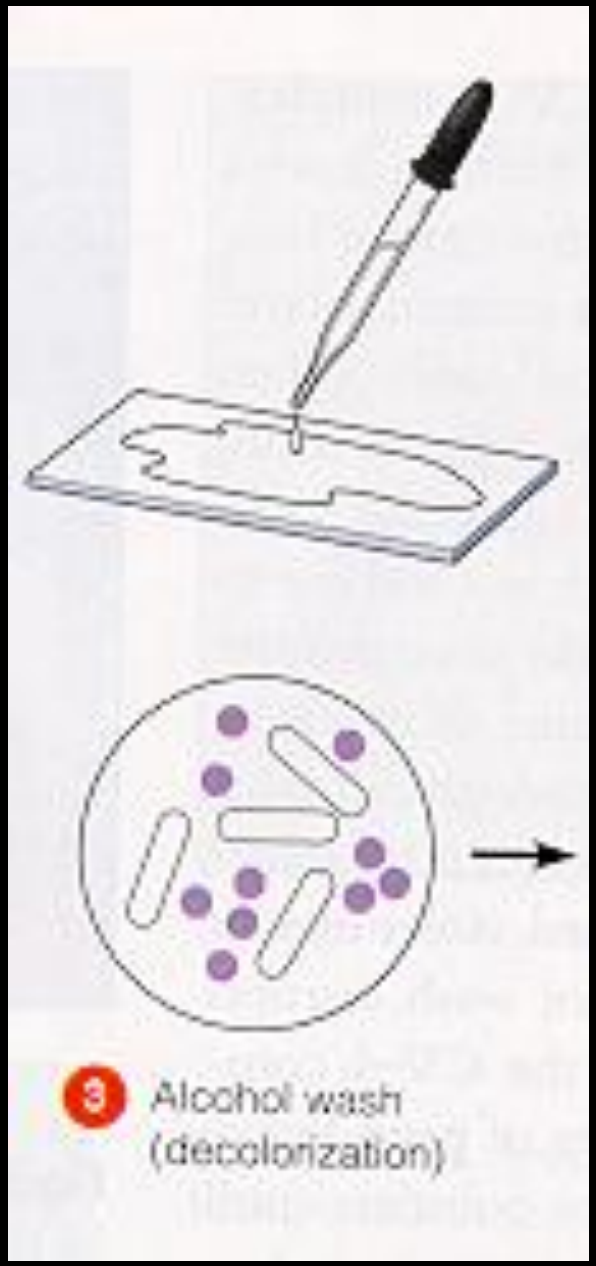


Gram Stain

2. Grams Iodine (mordant)

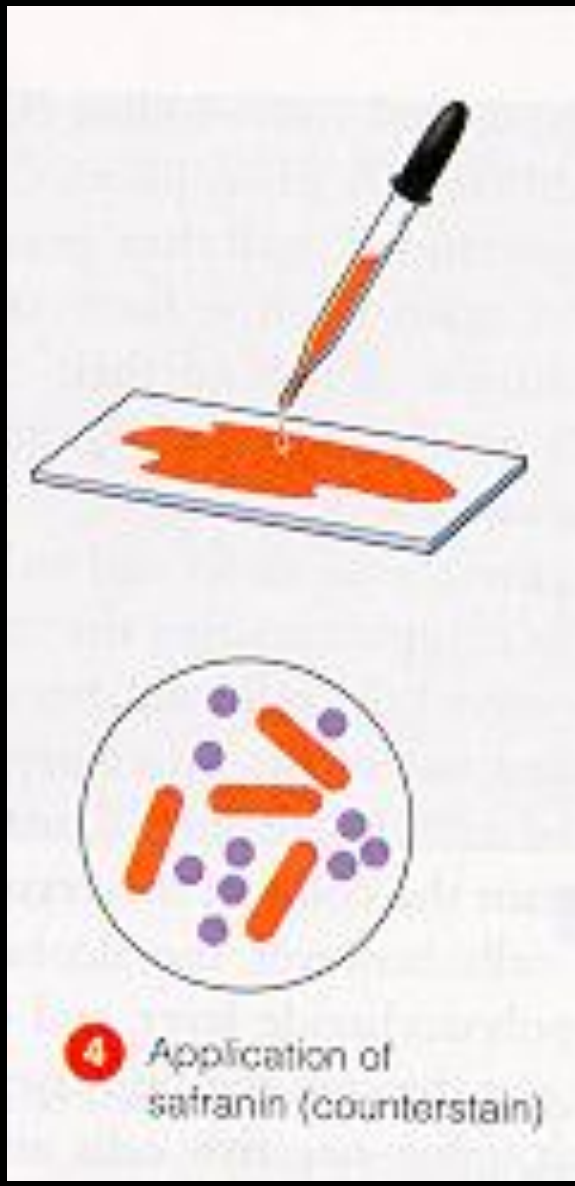
Gram Stain

3. Alcohol



Gram Stain

4. Safranin (Counterstain)



Results

- Gram (+) Purple
- Gram (-) Red
- Difference - due to structure of cell wall
  - Gram (+) Thick cell wall
  - Gram (-) Thin cell wall

Identification of a Bacteria Unknown

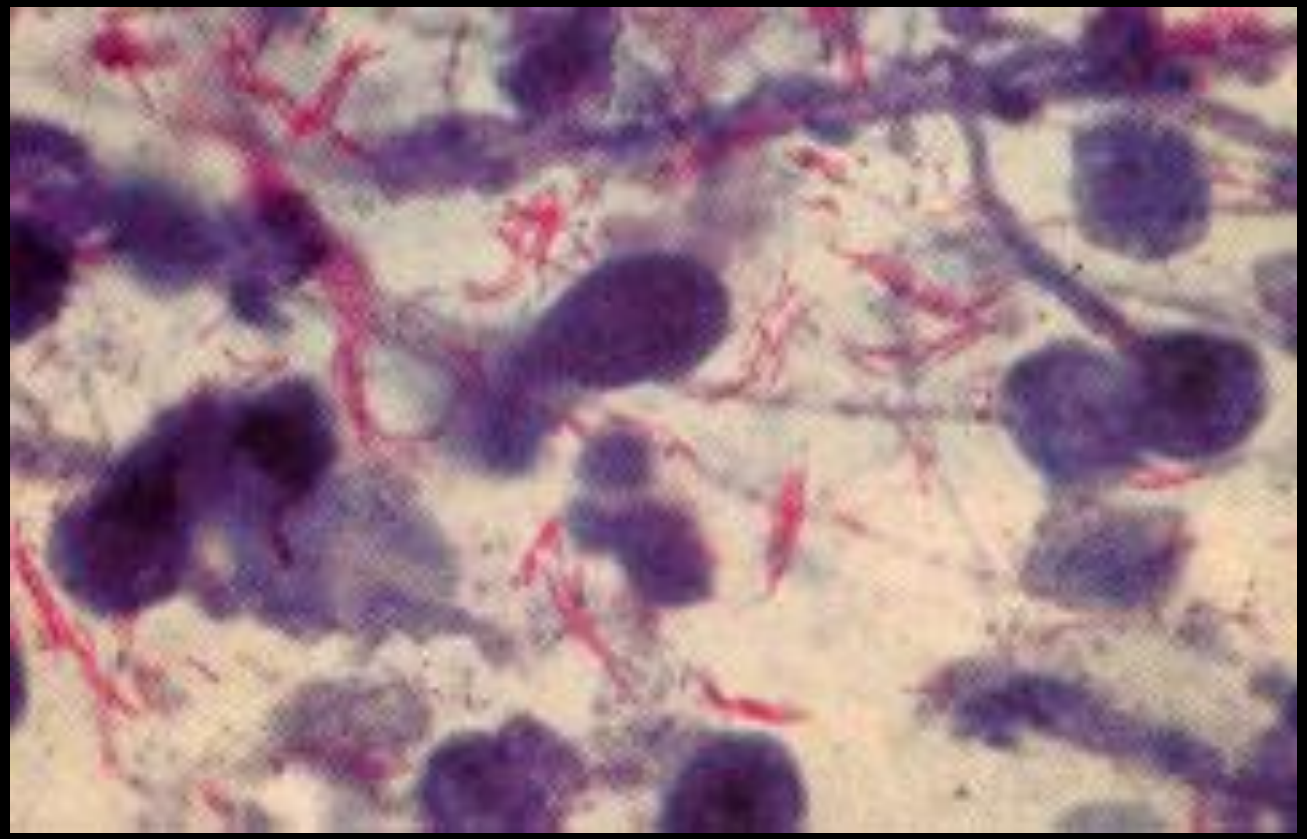
- 1. Gram Reaction
- 2. Morphology

Acid - Fast Stain

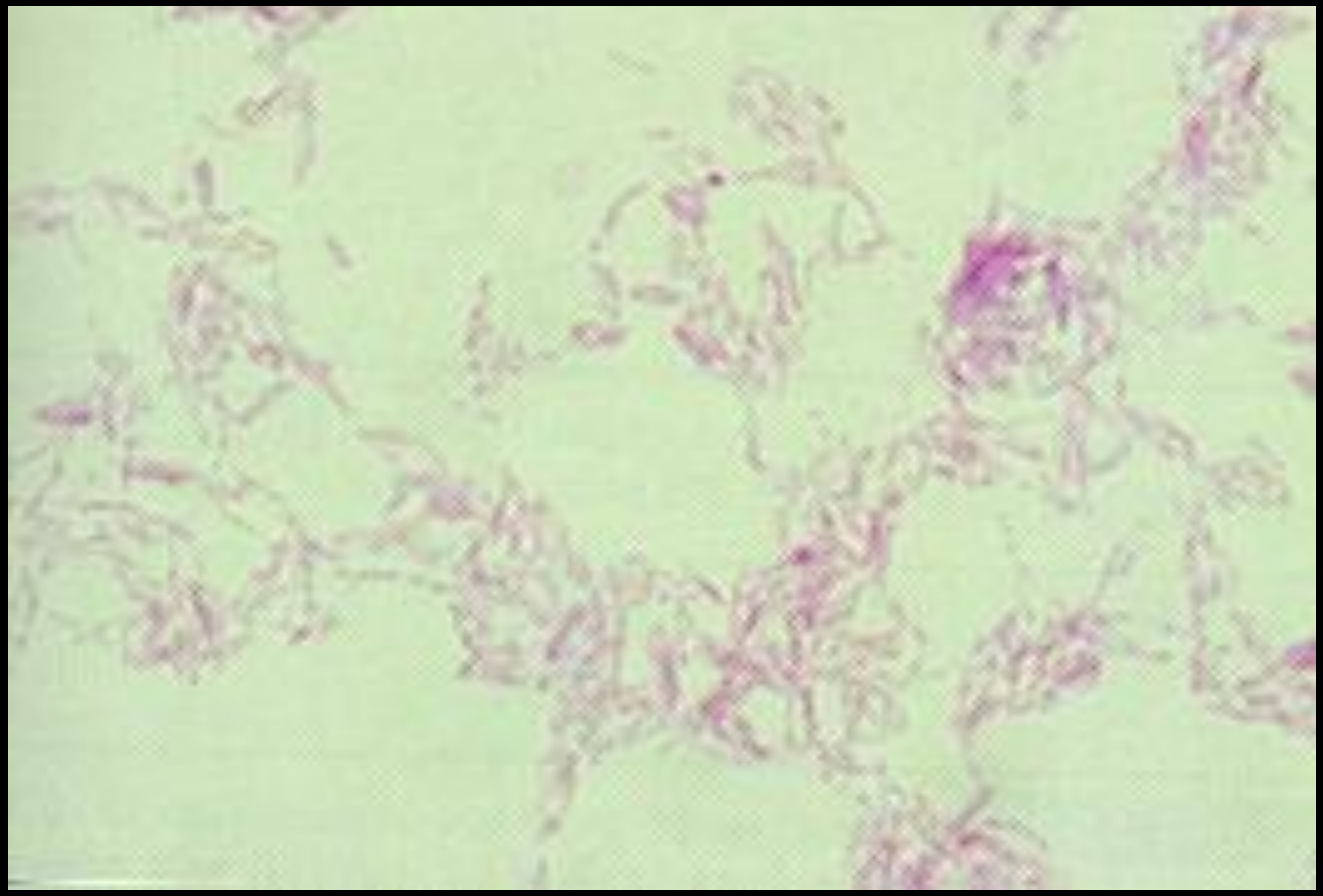
- Differential Stain - divides bacteria into 2 groups
- Acid - Fast
- Non Acid - Fast
- Used to identify organisms in the Genera *Mycobacterium* (high lipid and wax content in cell wall)

2 Important Pathogens

Mycobacterium tuberculosis



Mycobacterium leprae



Acid - Fast Stain

- 1. Carbolfuchsin (Red)
- 2. Acid Alcohol
- 3. Counterstain with Methylene Blue

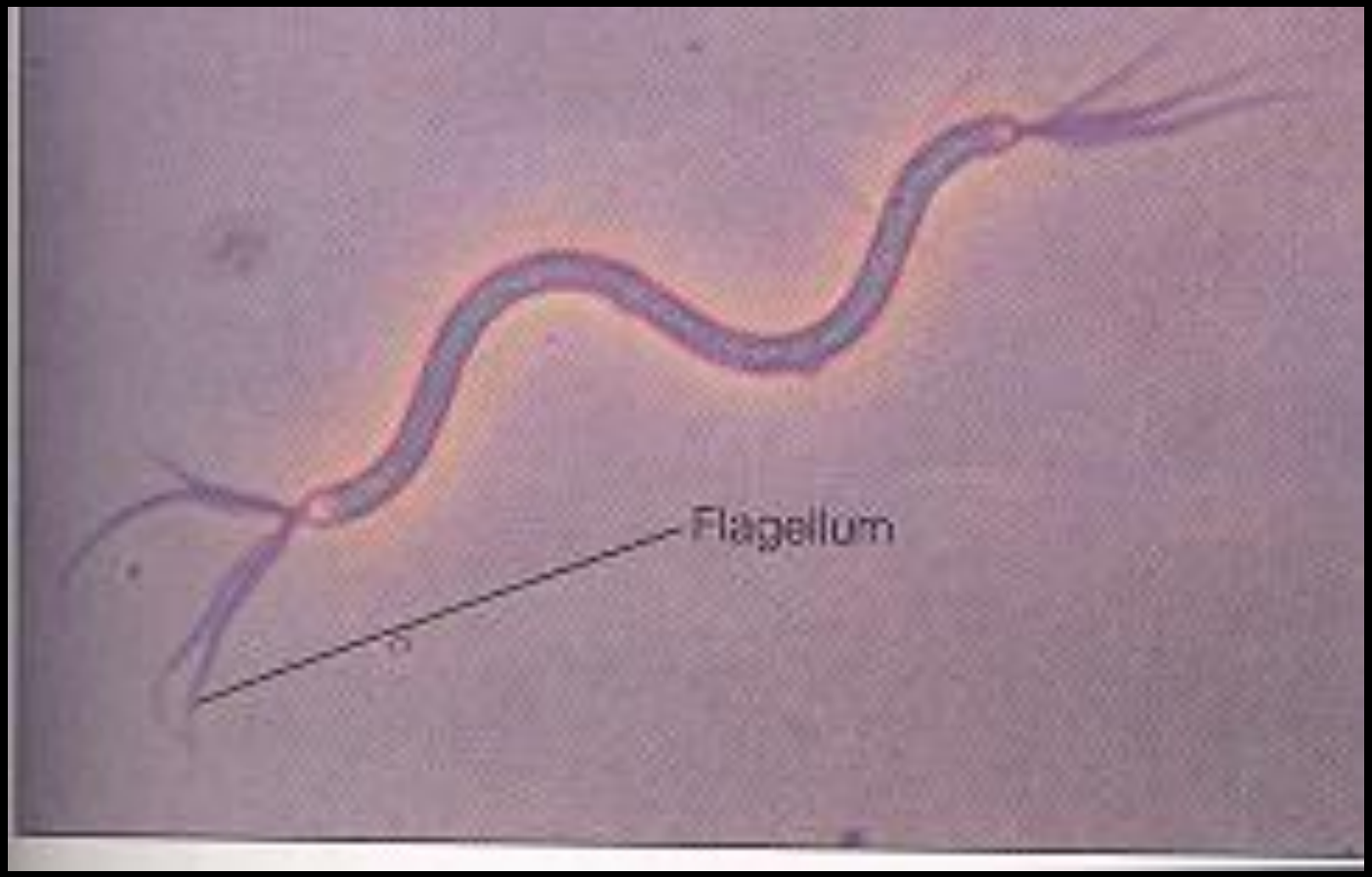
- Acid - Fast Cells Red
- Non Acid - Fast Blue

Special Stains

Capsule Stain

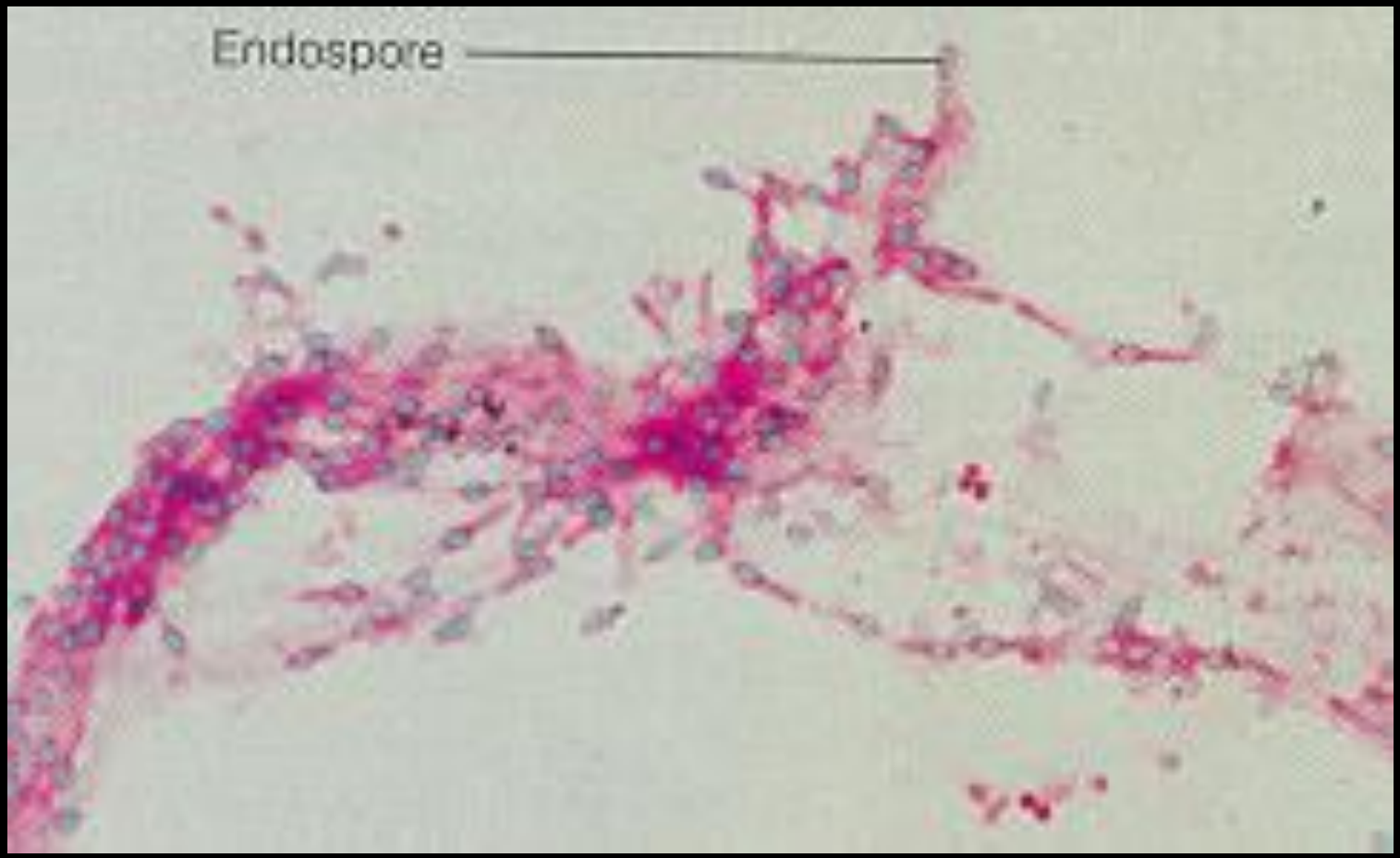
Klebsiella pneumoniae

Flagella Stain

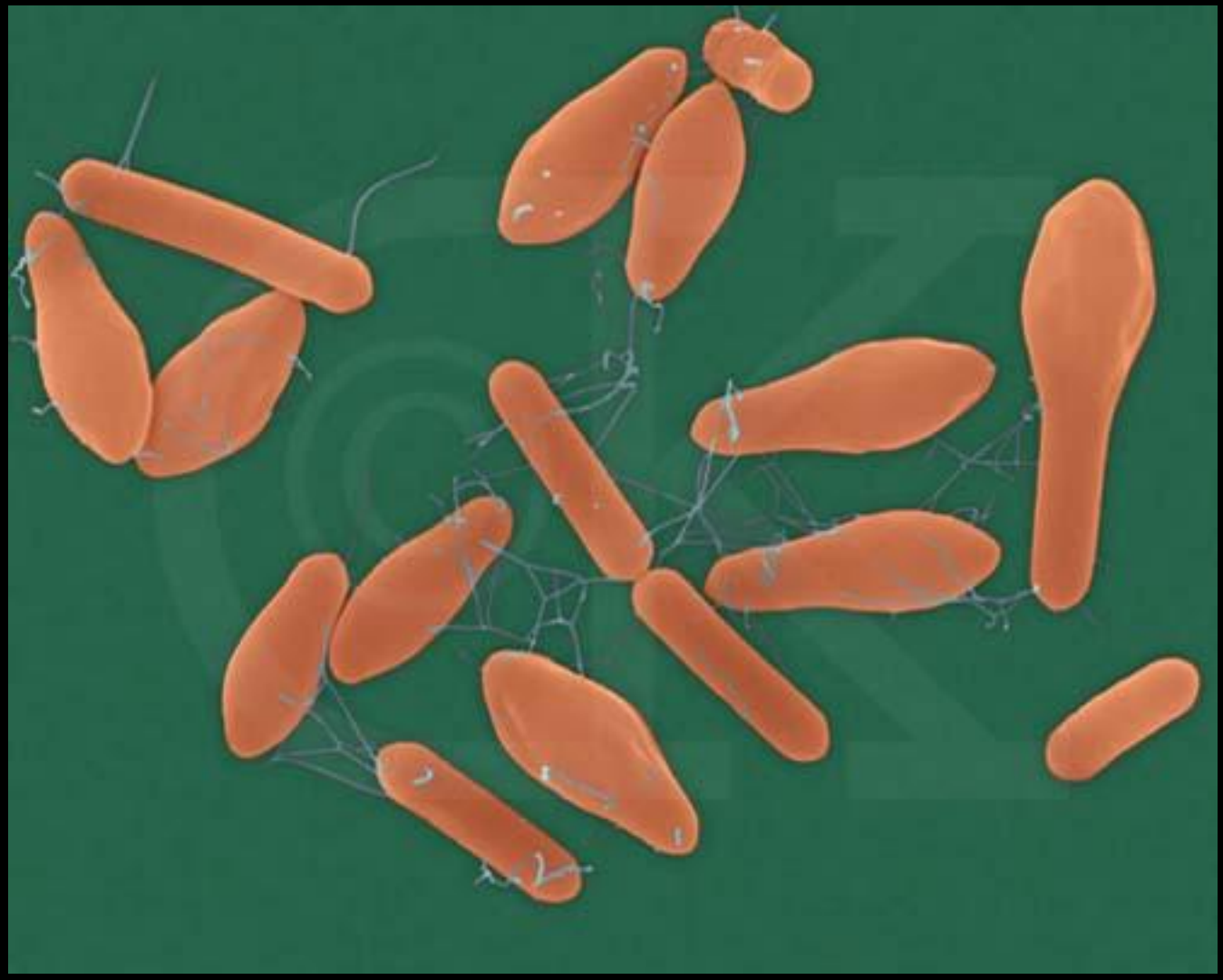


Spirillum volutans

Endospore Stain



Bacillus cereus



Clostridium botulinum