General Microbiology

Lab4

Bacterial Staining

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Staining preparations

Objectives

To understand the **medical importance** of bacterial staining

To understand the **theoretical basis** for staining techniques

Understand the **meaning of differential staining**

To explain the **procedure** for selected bacterial staining techniques

Safety considerations

Be careful with the Bunsen burner flame

Volatile and flammable liquids (ethanol, isopropanol-acetone)

Do not use them near an open flame

Be careful of your clothes

Hold all slides with forceps or a clothespin when heat-fixing

Wear suitable protective gloves.

Why should we stain bacteria?

Bacteria have nearly the same refractive index as water, therefore, when they are observed under a microscope, they are opaque or nearly invisible to the naked eye. Different types of staining methods are used make cells visible under light microscope

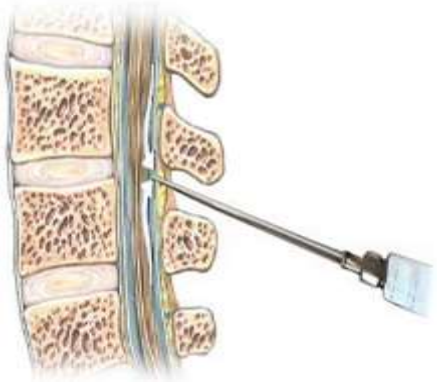
Source of samples for staining

1. Direct body samples (Blood, CSF, synovial fluid, swabs, …etc)

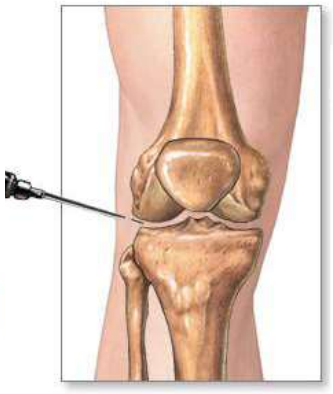
2. From cultured bacteria (Broth, agar)

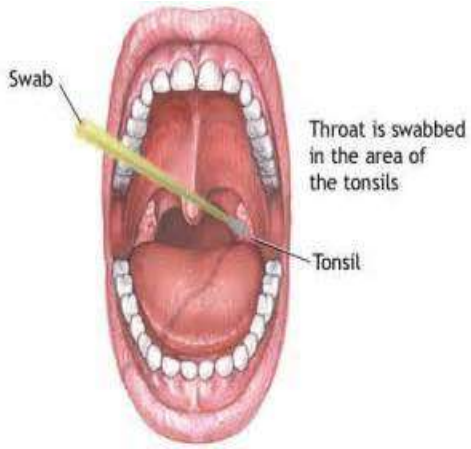
Clinical samples

CSF



Synovial fluid

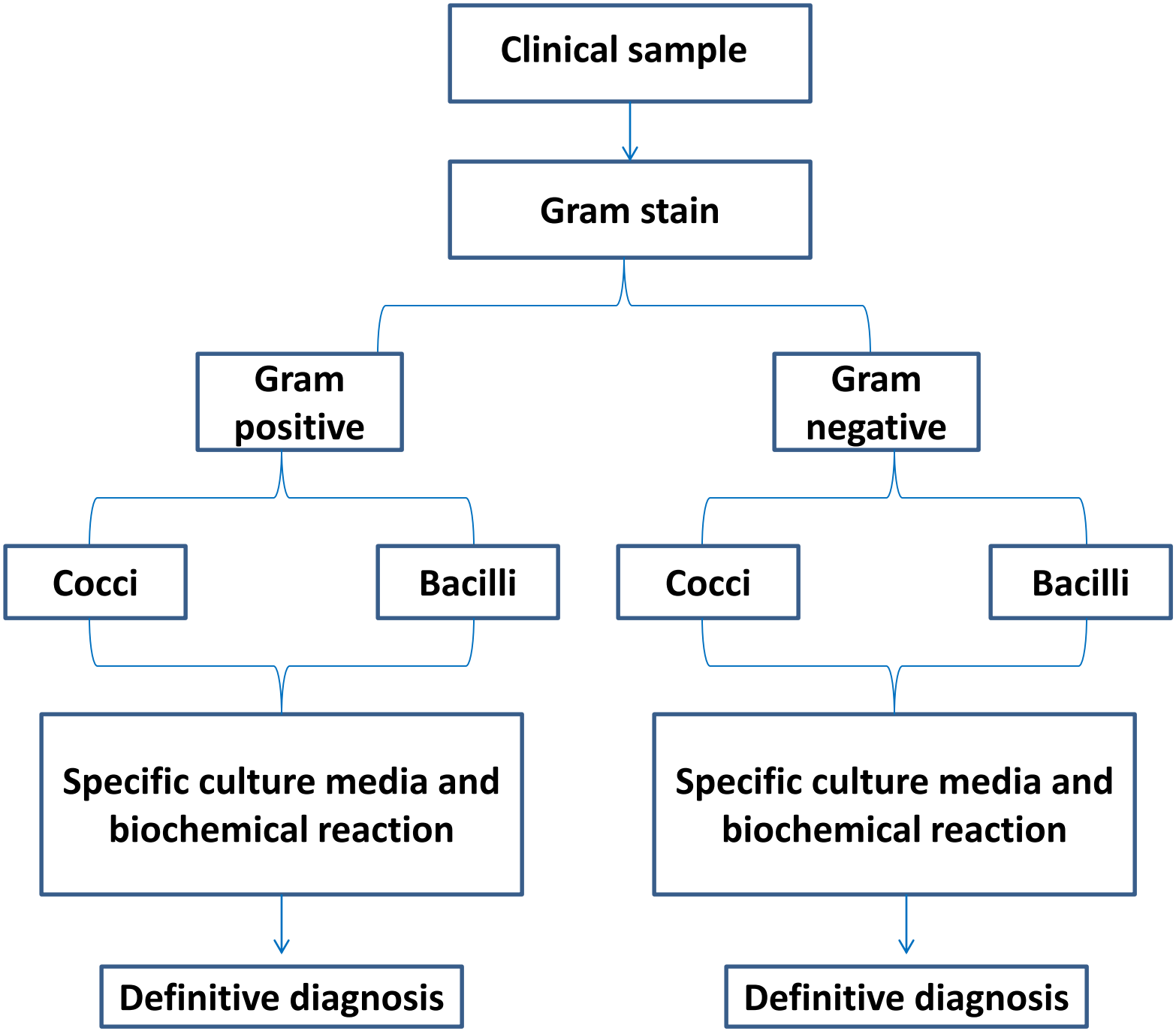




Laboratory methods of diagnosing bacterial infections

- Staining
- Growth pattern on culture media
- Biochemical reactions
- Antibiotic sensitivity

Diagnosis


Clinical sample
Gram stain
Gram positive
Gram negative
Cocci
Bacilli
Cocci
Bacilli
Specific culture media and biochemical reaction
Specific culture media and biochemical reaction
Definitive diagnosis
Definitive diagnosis

3 Types of Staining Procedures

- Simple Staining (shapes and arrangements)
- Differential Staining
- Special Staining (Capsule, flagella, spores)

Medical Application of bacterial staining

- It is the **first step** to determine the identity of a particular bacterial sample
- **Performed on body fluid** when infection is suspected
- It yields **results much more quickly** than culture
- Important for **empirical therapy**

CSF

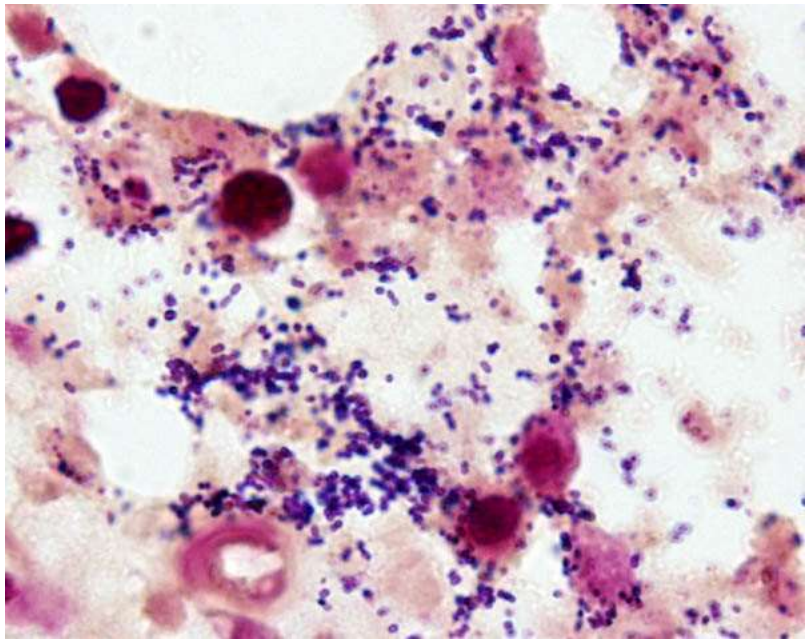
Lab diagnosis

(2 days)

Treatment is prescribed before the definitive lab diagnose is achieved (called Empirical therapy)

Differential Stains

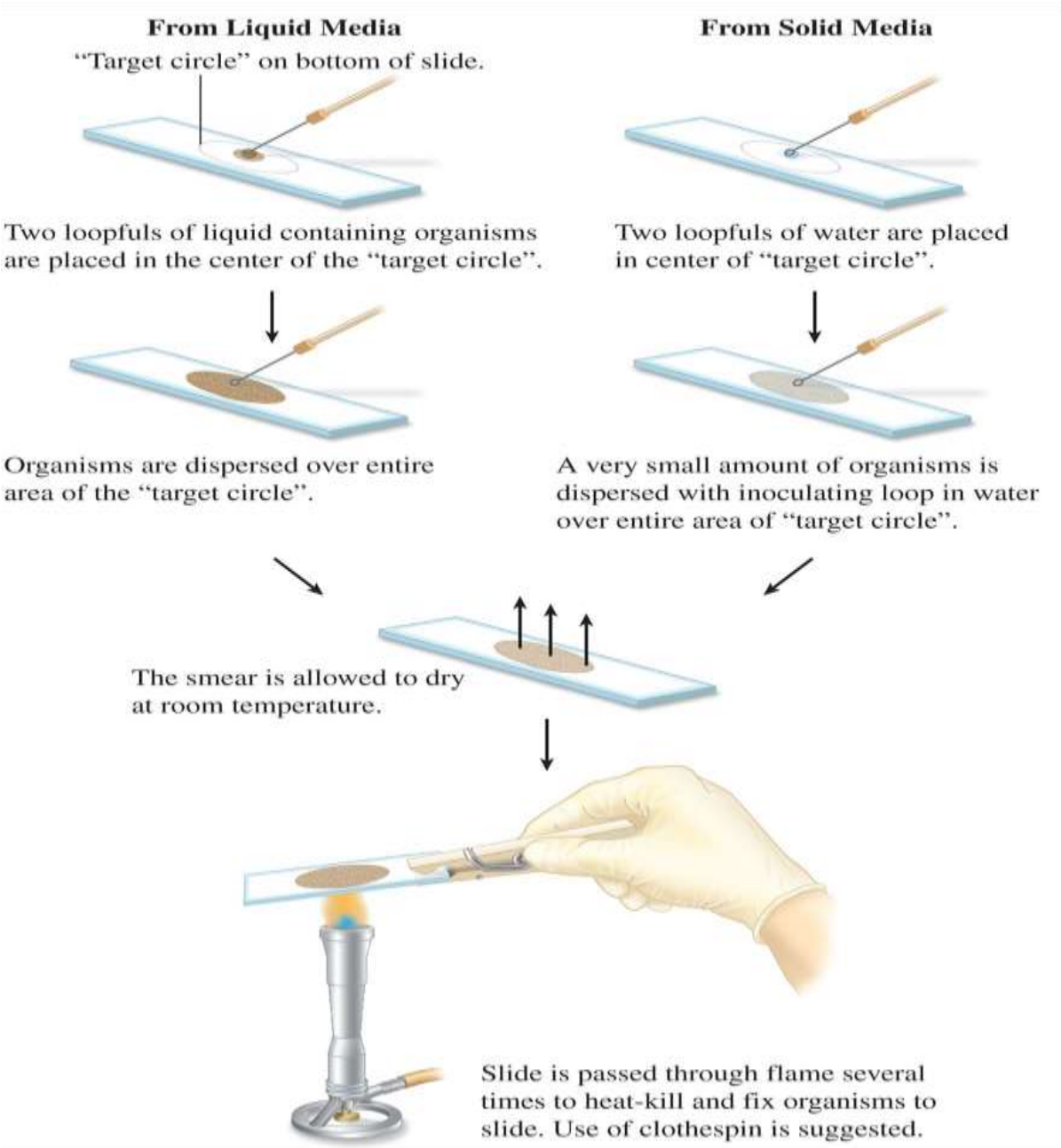
- Two or more reagents
- Distinguish
  - Bacterial groups
- Example
  - Gram stain
  - Acid Fast Stain



Staining Procedure

Slide Preparation

- Clean slide
- LABEL !!!
- Heat fix (usually)
  - Kill organism
  - Adhere to slide
  - Accepts dye
- Problems
  - Too thick
  - Wash off specimen

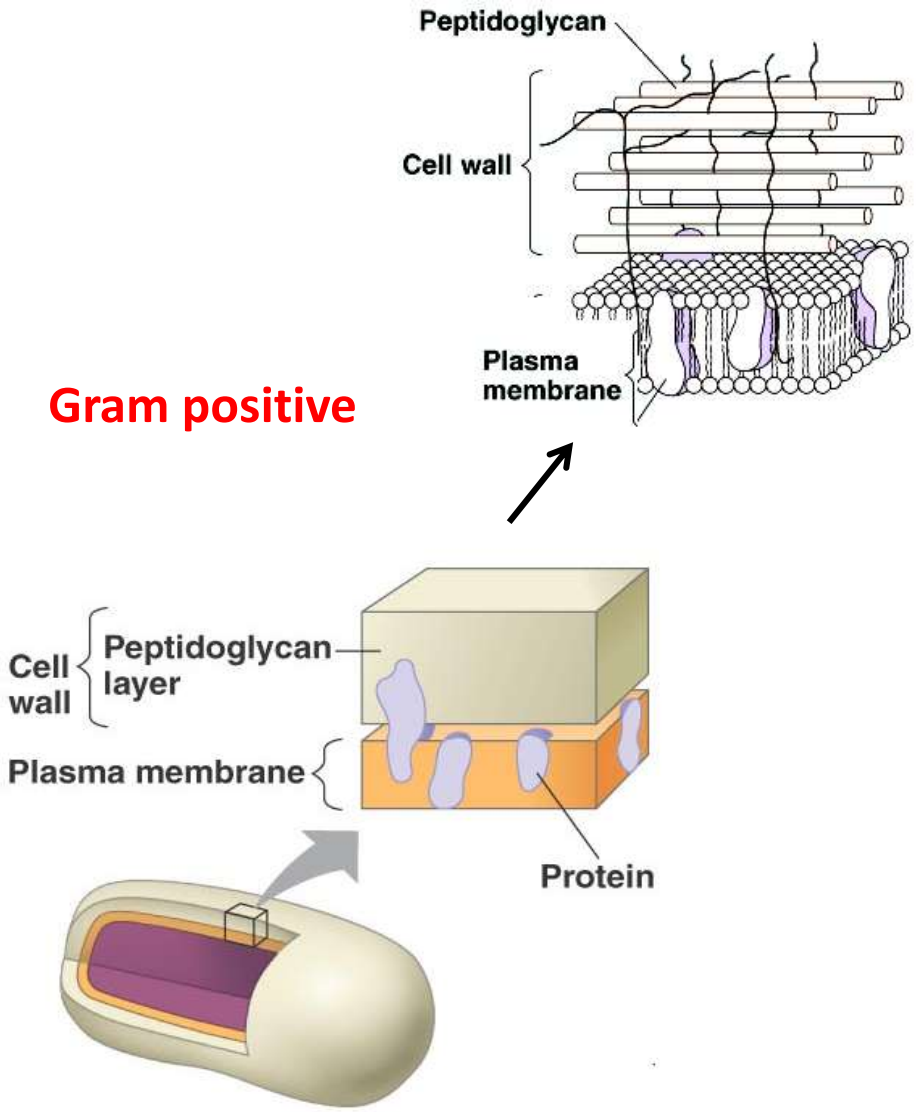


Gram staining

Gram positive vs. Gram negative bacteria

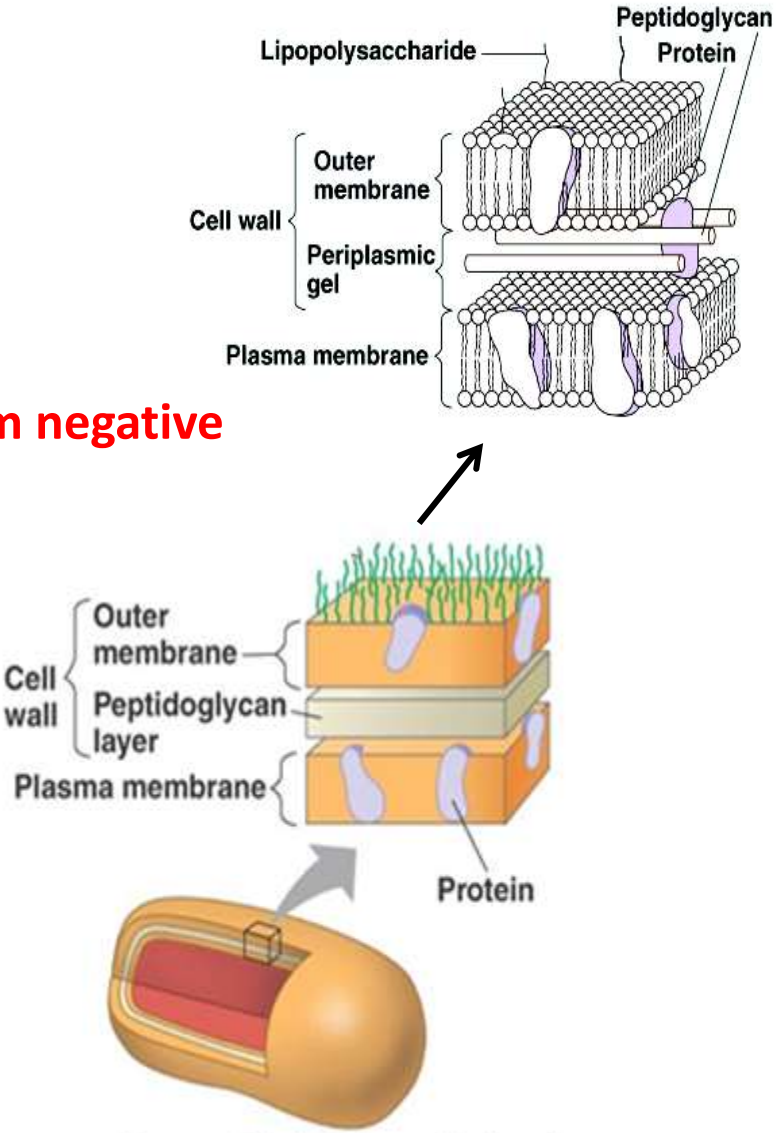
Staining Principle

Gram positive



(a) Gram-positive: peptidoglycan traps crystal violet.

Gram negative

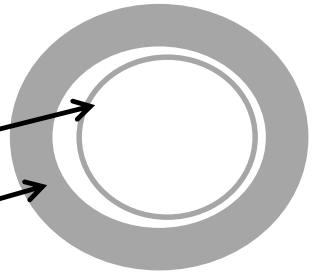


(b) Gram-negative: crystal violet is easily rinsed away, revealing red dye.

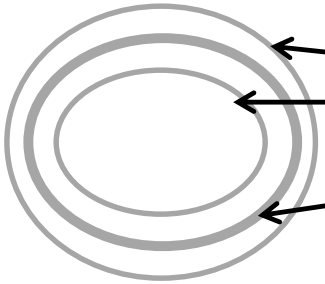
procedure

Plasma membrane

Peptidoglycan



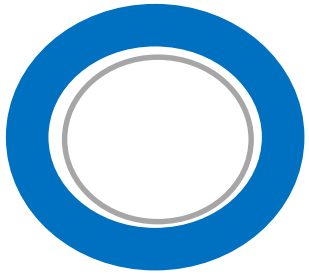
Fixation



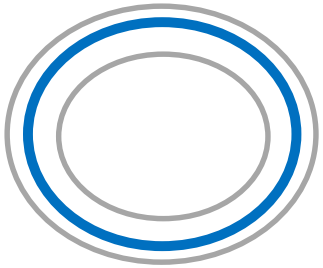
Outer membrane

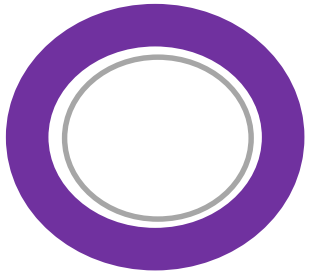
Inner membrane

Peptidoglycan

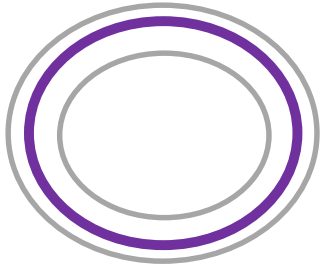


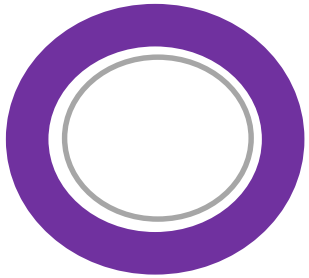
Crystal violet



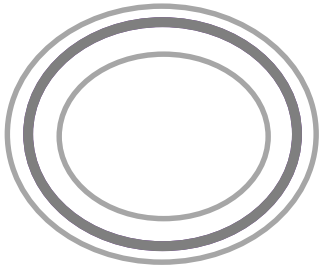


Iodine treatment

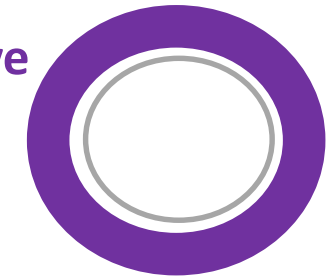




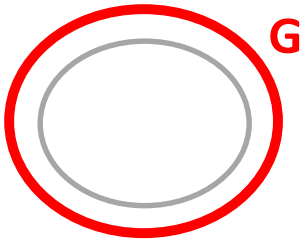
Decolorization



Gram positive

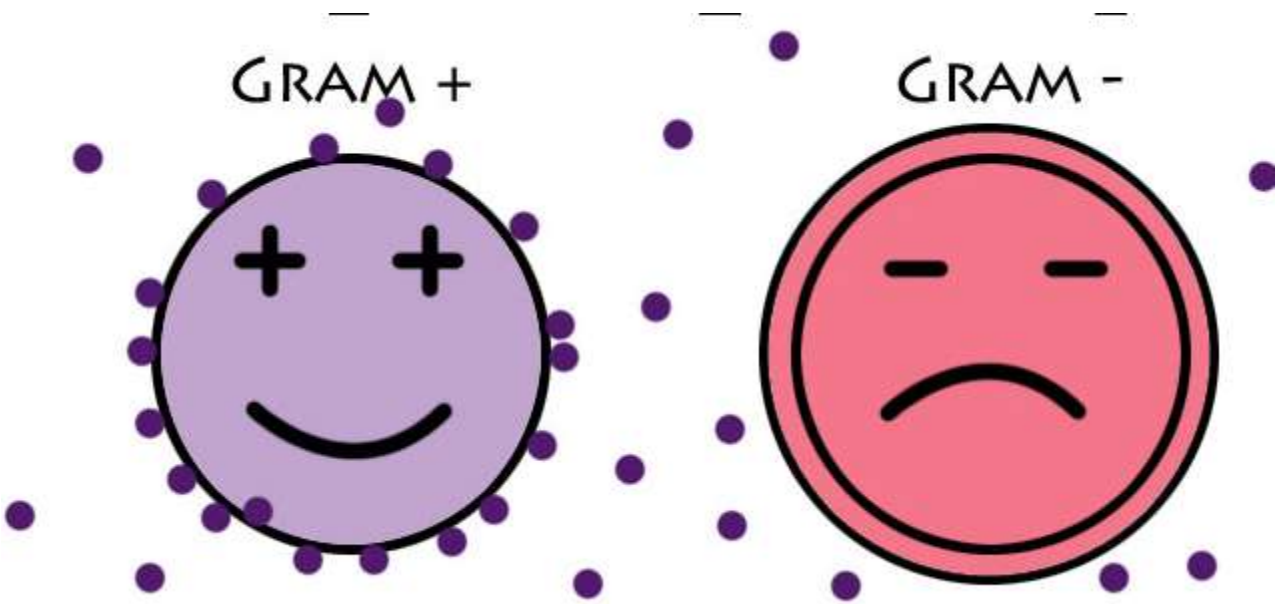


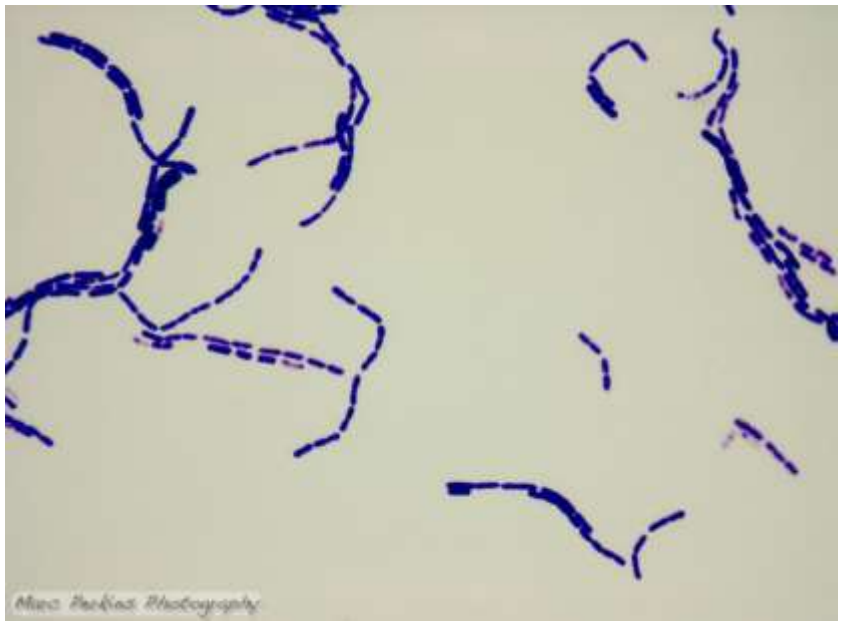
Counter stain safranin

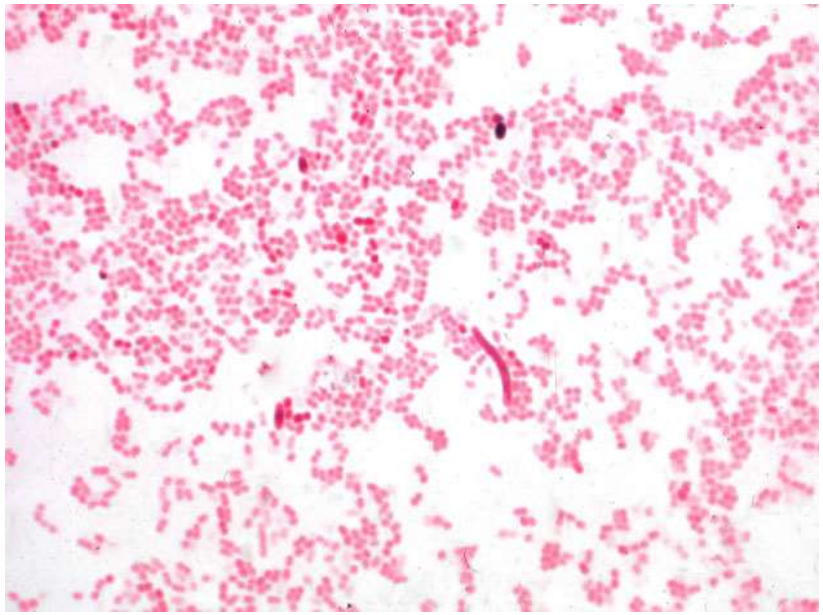


Gram negative

Results of Gram staining







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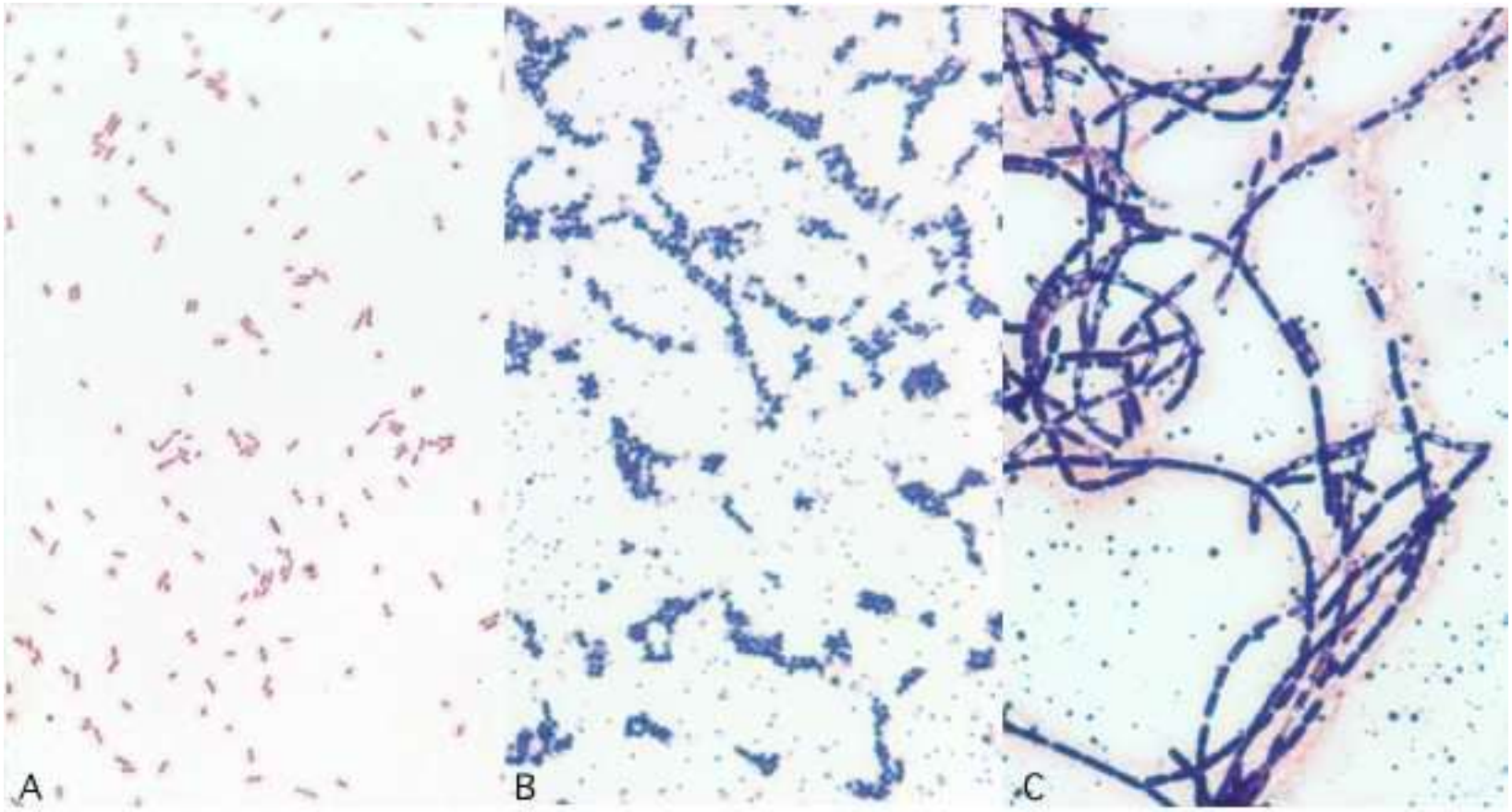
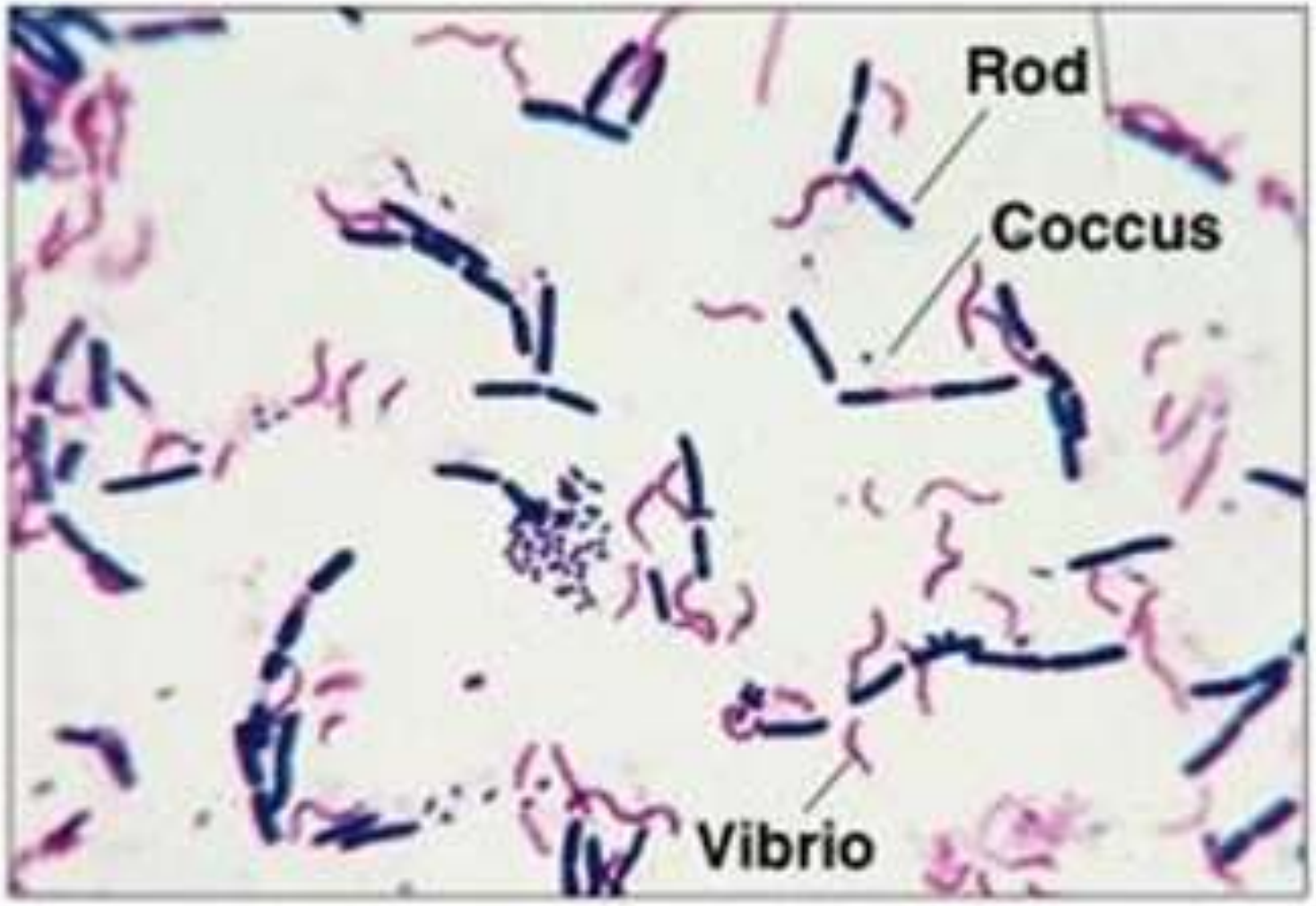
Results of Gram staining



Proteus ***S. aureus*** ***B. cereus***

Results of Gram staining



Acid fast staining

Medical Application

Important in identifying bacteria and parasites such as:

- *Mycobacterium;* specifically *M. leprae* (leprosy) and *M. tuberculosis*.

- The aerobic actinomycete genus *Nocardia;* specifically, the opportunistic pathogens *N. brasiliensis* and *N. asteroids* that cause the lung disease nocardiosis.

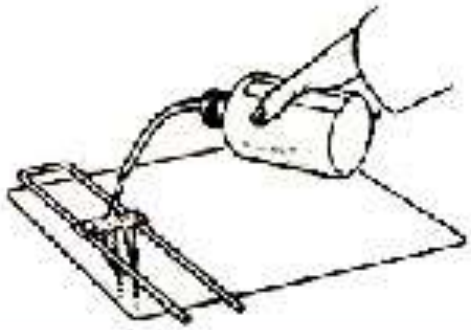
- The potozoan parasite *Cryptosporidium* that causes diarrhea in humans (cryptosporidiosis)

Acid fast staining

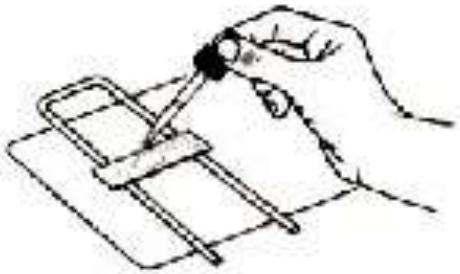
Procedure

1 Cover smear with carbolfuchsin.Steam over boiling water for 8 minutes. Add additional stain if stain boils off.

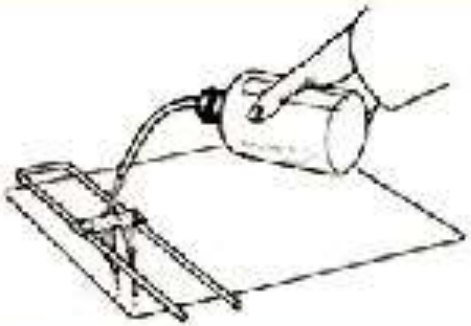
2 After slide has cooled decolorize with acid-alcohol for 15 to 20 seconds.



3 Stop decolorization action of acid-rinsing briefly with water.



4 Counterstain with methylene blue for 30 seconds.



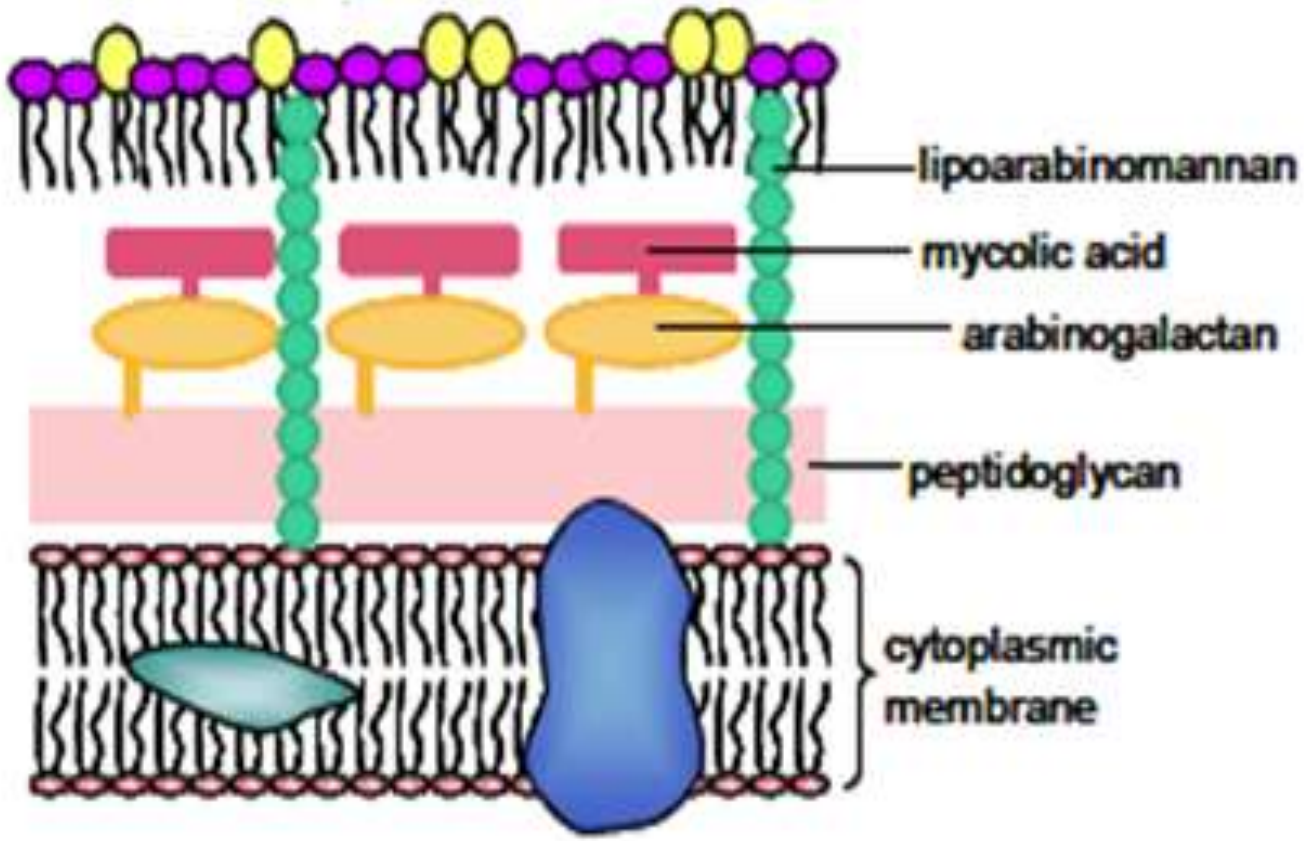
5 Rinse briefly with water to remove excess methylene blue.

6 Blot dry with bibulous paper. Examine directly under oil immersion.

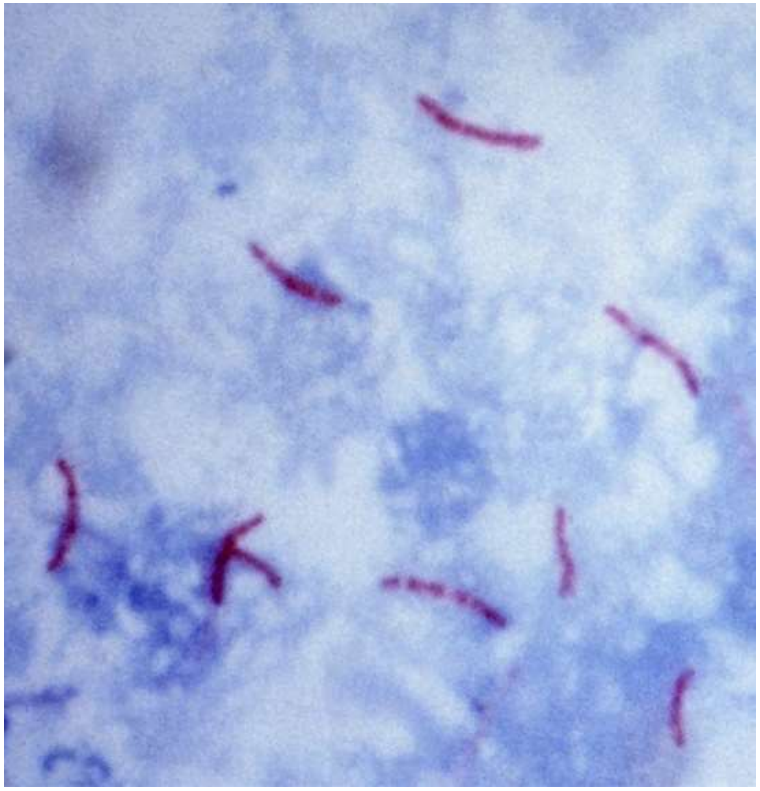
Ziehl-Neelsen acid-fast staining procedure

Acid fast staining

Principle



Results of acid fast staining



TB bacteria