



# General Microbiology Lab

## Bacterial Staining

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### Lab 3

# 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)

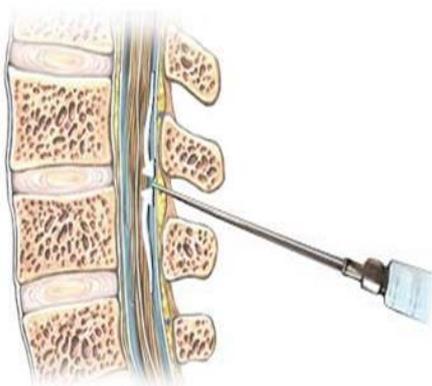
Do not use them near an open flame

Be careful of your clothes

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

Wear suitable protective gloves.

CSF



Synovial fluid



Swab



Throat is swabbed in the area of the tonsils

Tonsil



**Clinical samples**

## Laboratory methods of diagnosing bacterial infections

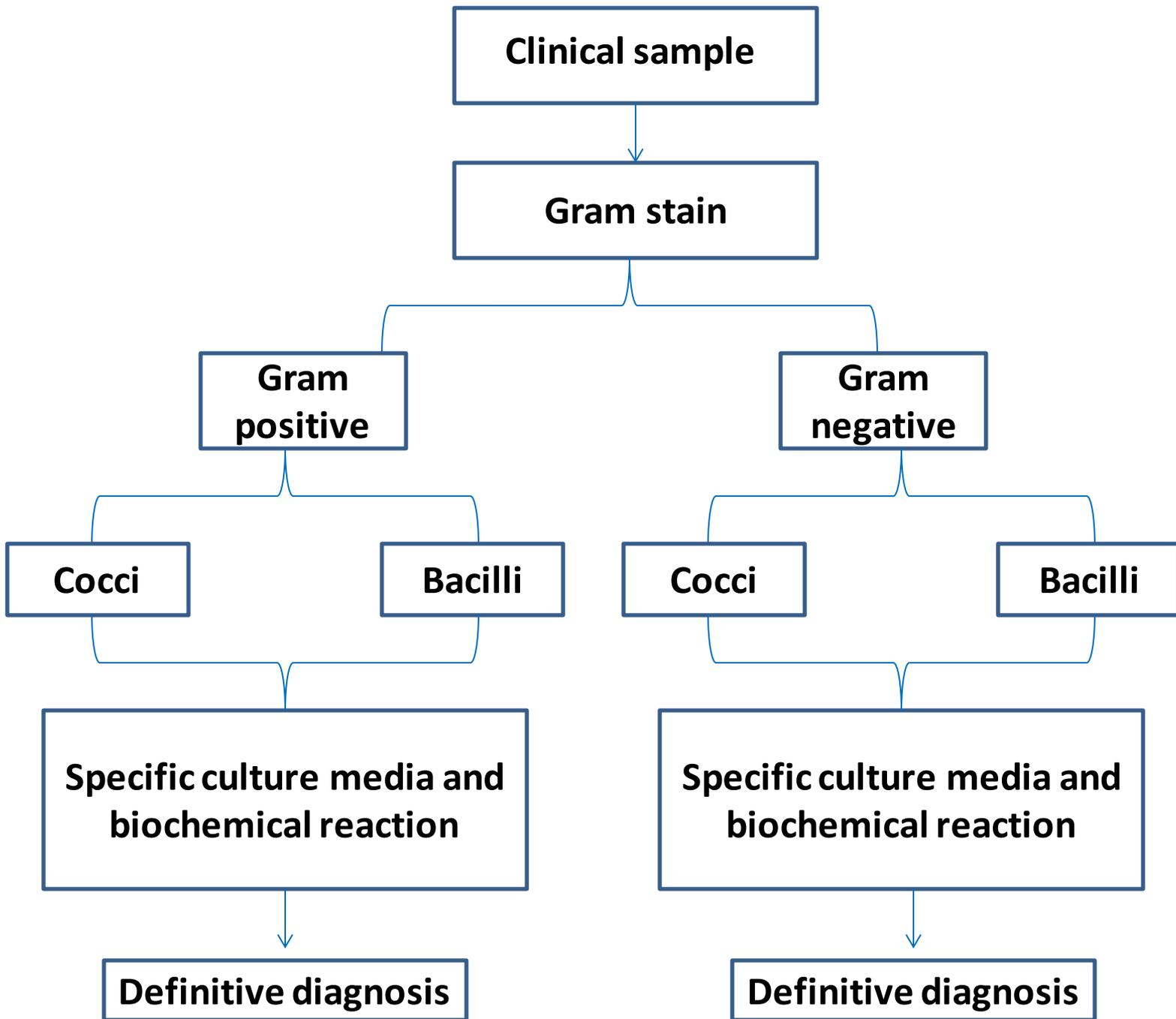
Staining

Growth pattern on culture media

Biochemical reactions

Antibiotic sensitivity

**Diagnosis**



# 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

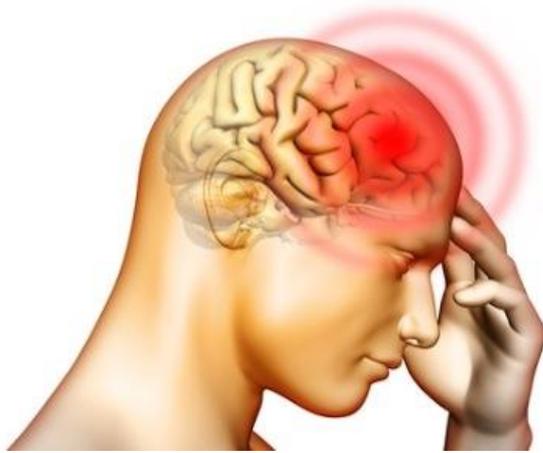
1. Direct body samples (Blood, CSF, synovial fluid, swabs, ...etc)
2. From cultured bacteria (Broth, agar)

## 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



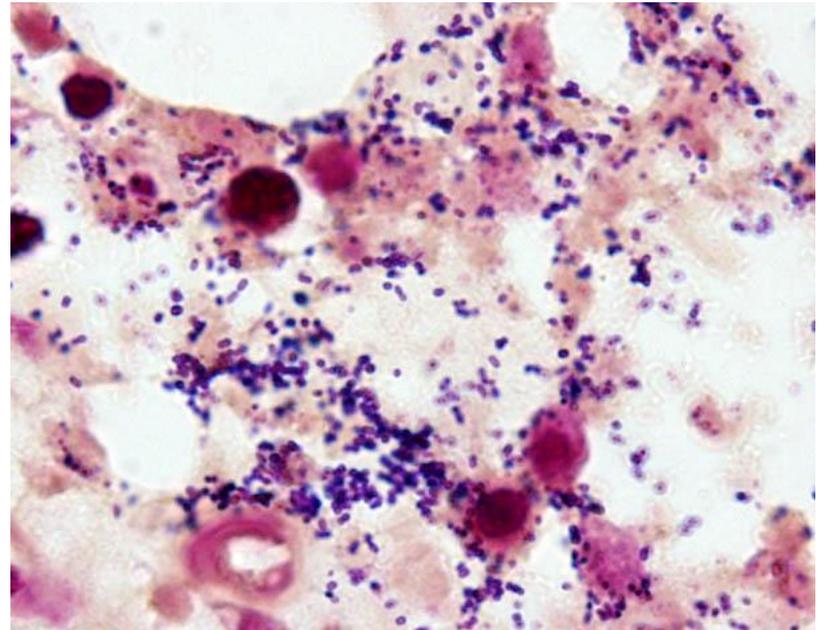
Lab diagnosis

**(2 days)**

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

# Differential Stains

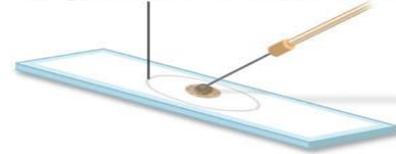
- Two or more reagents
- Distinguish
  - Bacterial groups
- Example
  - Gram stain
  - Acid Fast Stain



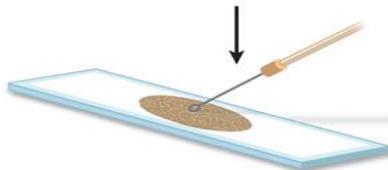
# Staining Procedure

## Slide Preparation

**From Liquid Media**  
“Target circle” on bottom of slide.



Two loopfuls of liquid containing organisms are placed in the center of the “target circle”.

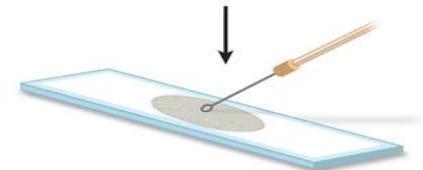


Organisms are dispersed over entire area of the “target circle”.

**From Solid Media**

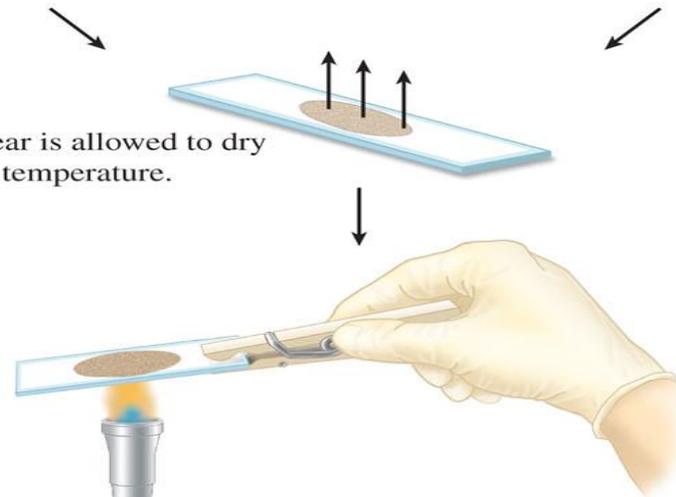


Two loopfuls of water are placed in center of “target circle”.



A very small amount of organisms is dispersed with inoculating loop in water over entire area of “target circle”.

The smear is allowed to dry at room temperature.



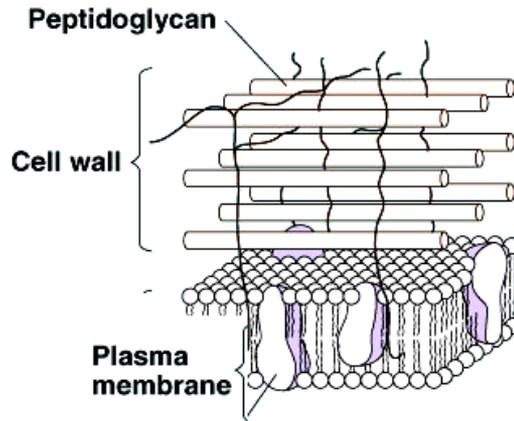
Slide is passed through flame several times to heat-kill and fix organisms to slide. Use of clothespin is suggested.

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

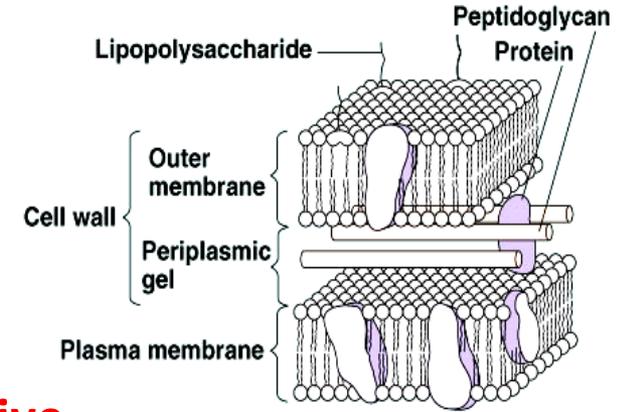
# Gram staining

# Gram positive vs. Gram negative bacteria

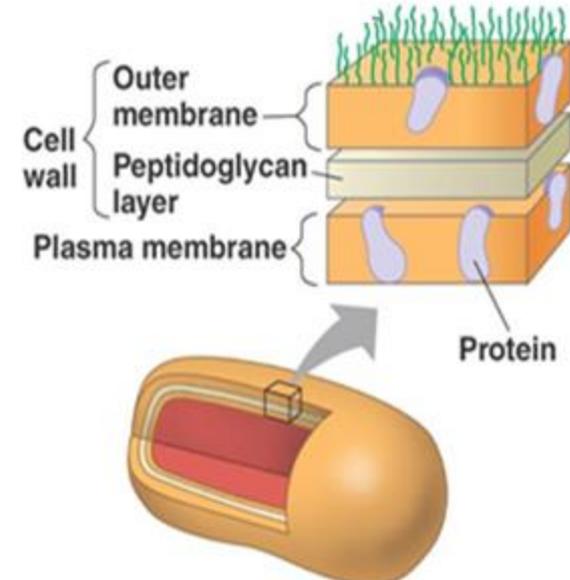
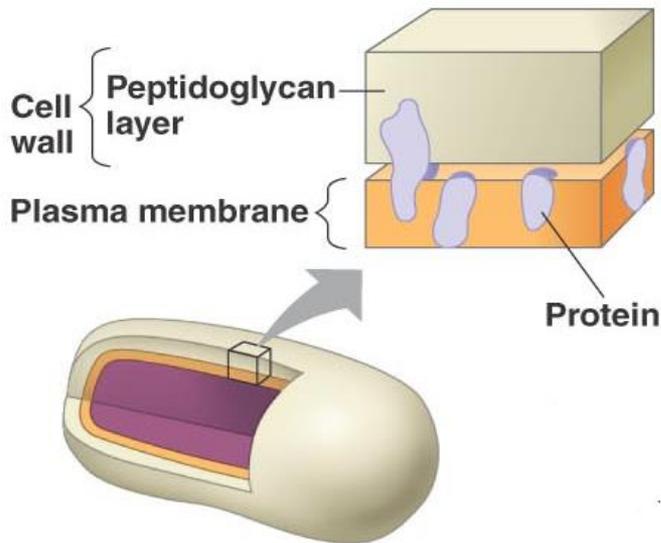
## Staining Principle



**Gram positive**



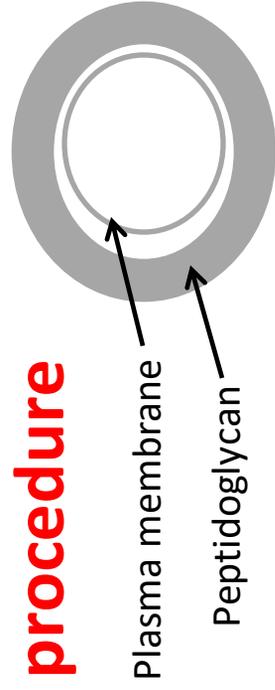
**Gram negative**



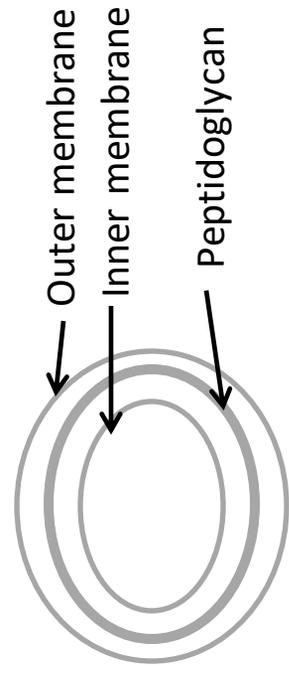
(a) Gram-positive: peptidoglycan traps crystal violet.

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

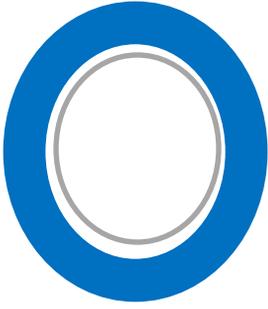
**procedure**



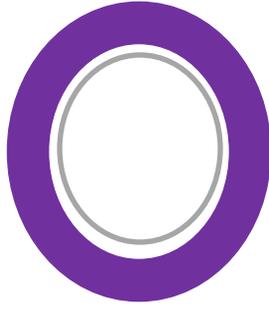
**Fixation**



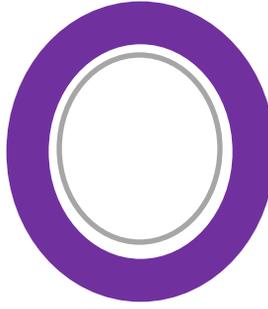
**Crystal violet**



**Iodine treatment**

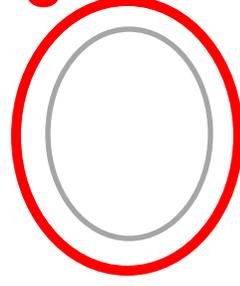


**Decolorization**

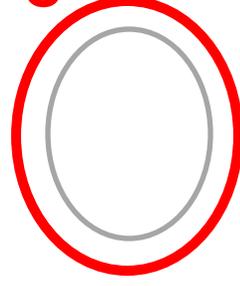
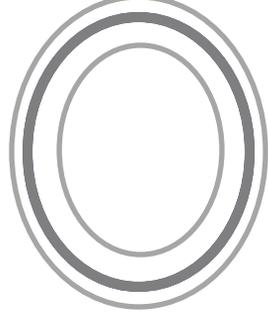


**Gram positive**

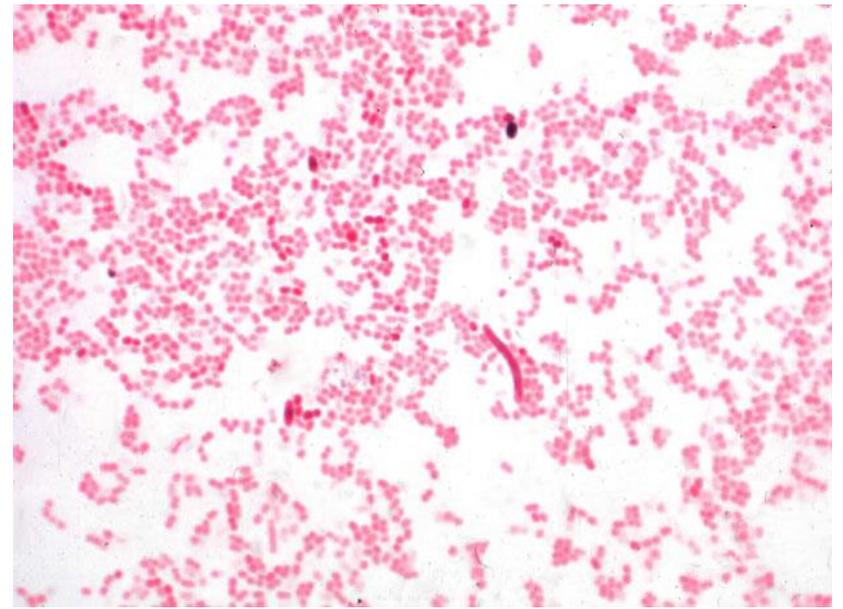
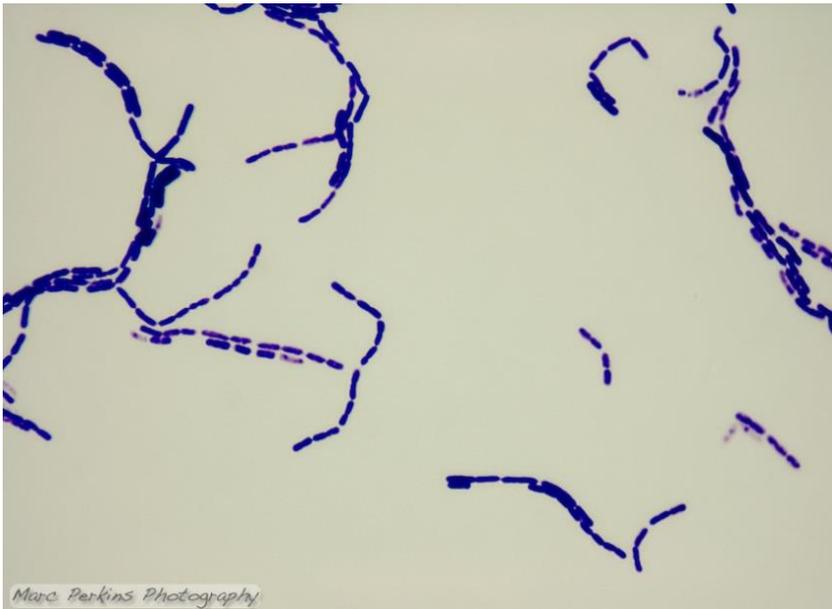
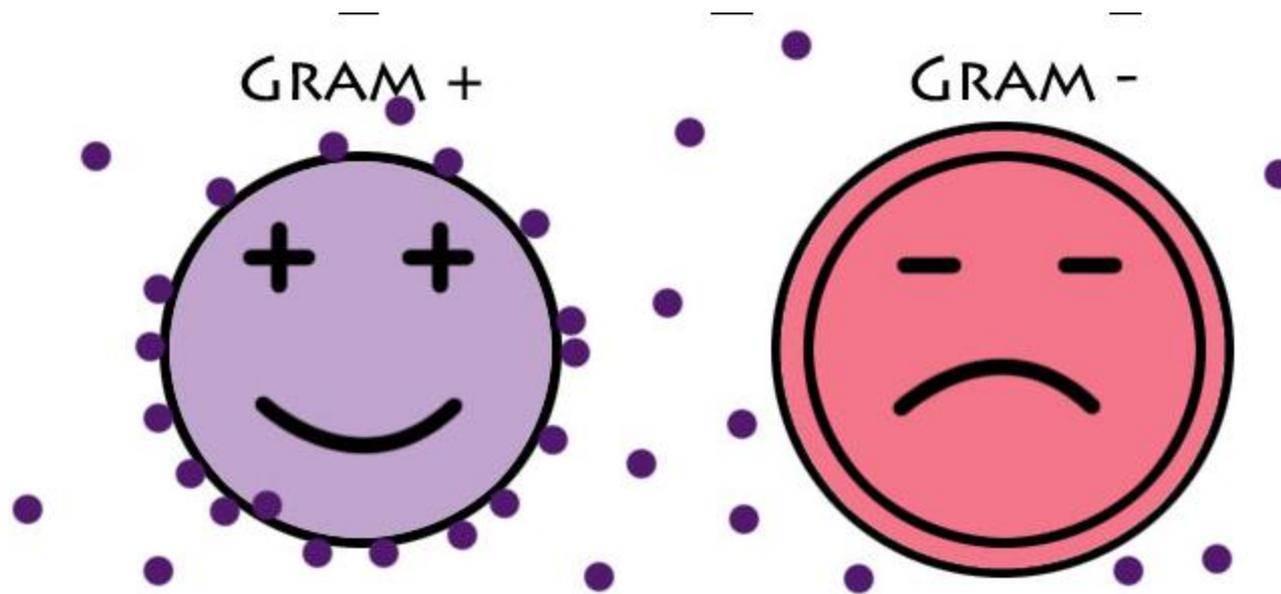
**Counter stain safranin**



**Gram negative**



# Results of Gram staining



Come

**R  
Y  
S  
T  
A  
I**

In

**O  
D  
I  
N  
E**

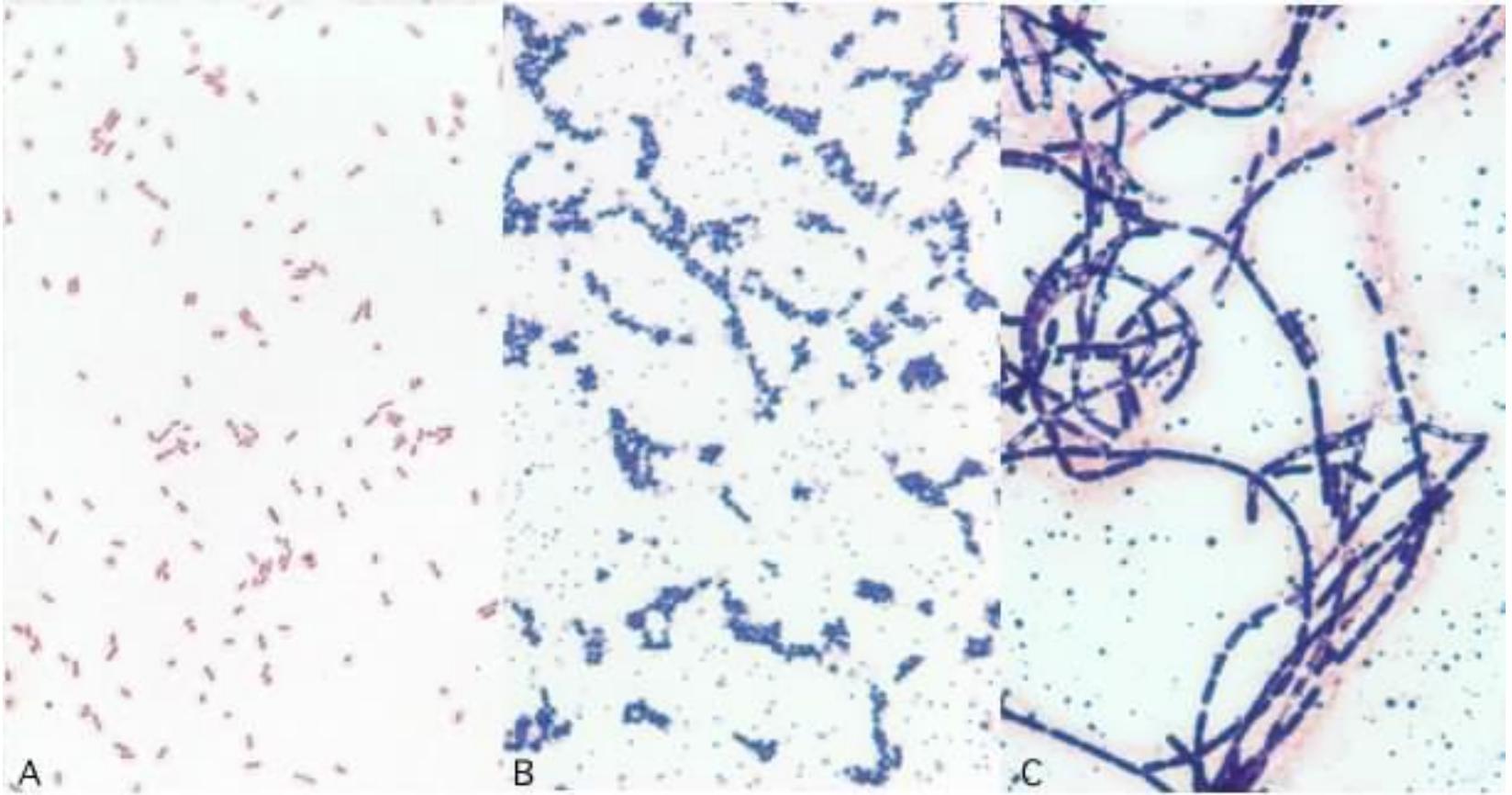
And

**L  
C  
H  
O  
L**

Stain

**A  
F  
R  
A  
N  
I  
N  
E**

# Results of Gram staining

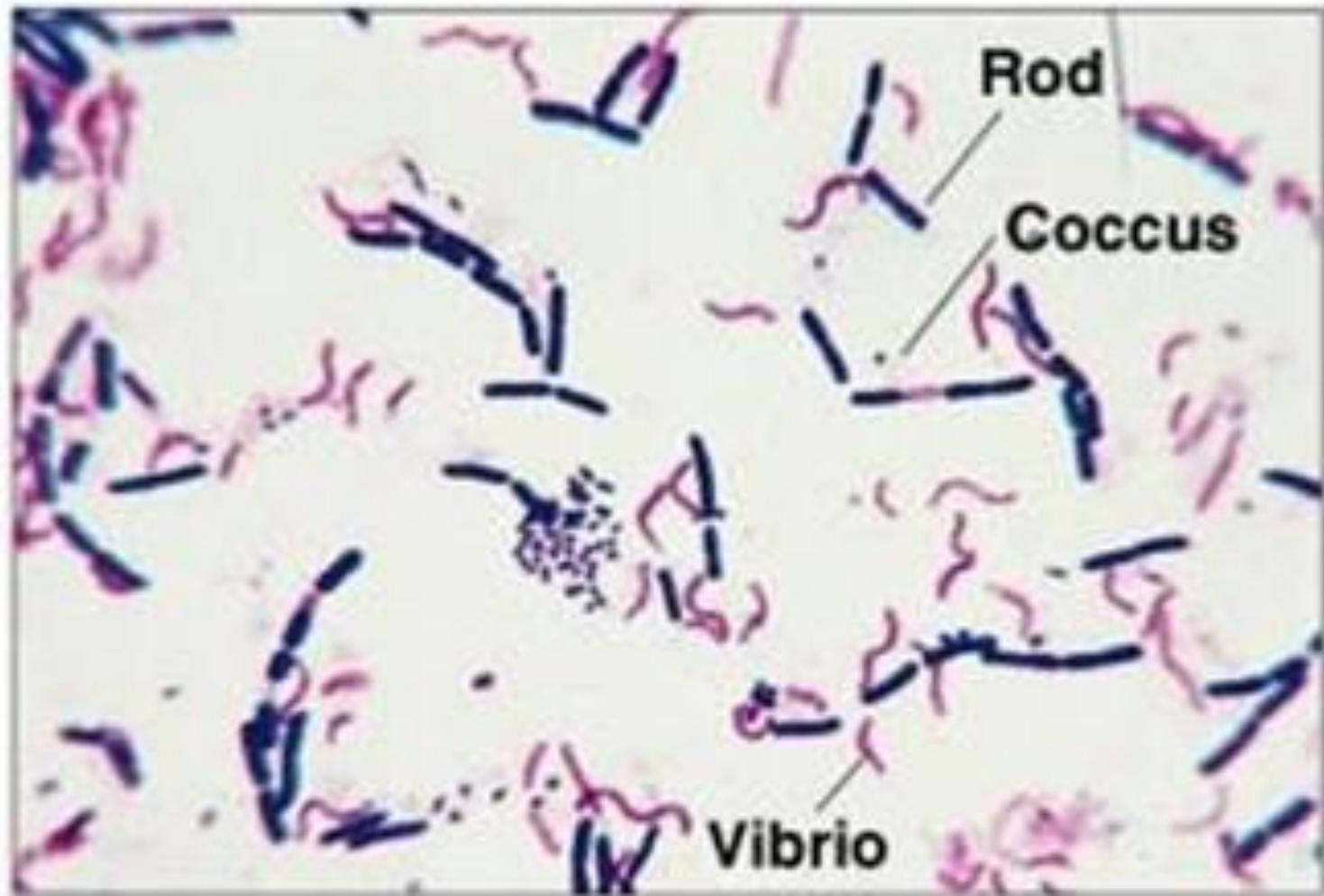


*Proteus*

*S. aureus*

*B. cereus*

# Results of Gram staining



# Acid fast staining

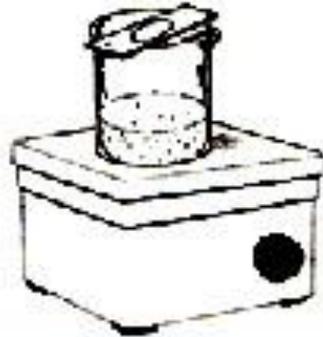
## Medical Application

Important in identifying bacteria and parasites such as:

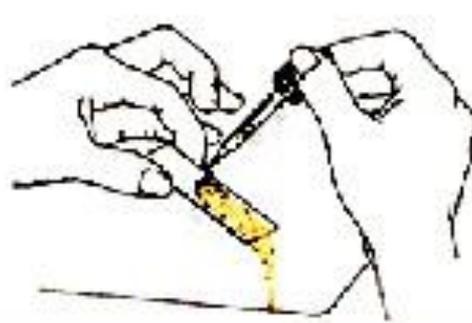
- *Mycobacterium*; specifically *M. leprae* (leprosy) and *M. tuberculosis*.
- The aerobic actinomycete genus *Nocardia*; specifically, the opportunistic pathogens *N. brasiliensis* and *N. asteroides* that cause the lung disease nocardiosis.
- The protozoan parasite *Cryptosporidium* that causes diarrhea in humans (cryptosporidiosis)

# Acid fast staining

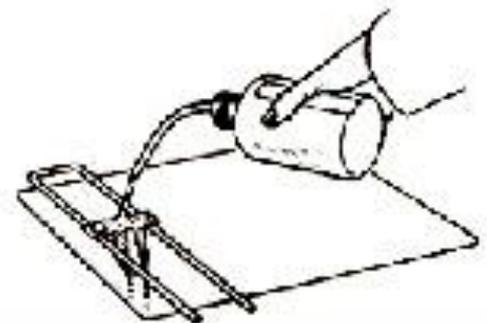
## Procedure



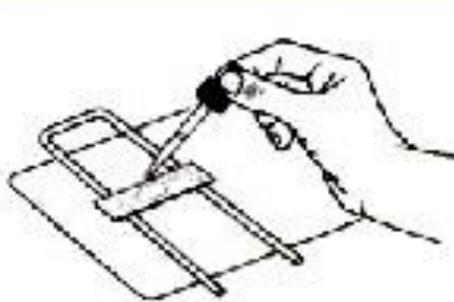
- 1** Cover smear with carbolfuchsin. Steam over boiling water for 8 minutes. Add additional stain if stain boils off.



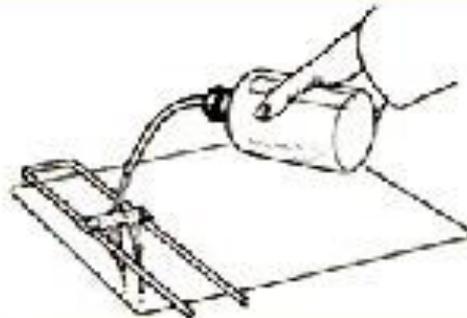
- 2** After slide has cooled decolorize with acid-alcohol for 15 to 20 seconds.



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



- 4** Counterstain with methylene blue for 30 seconds.



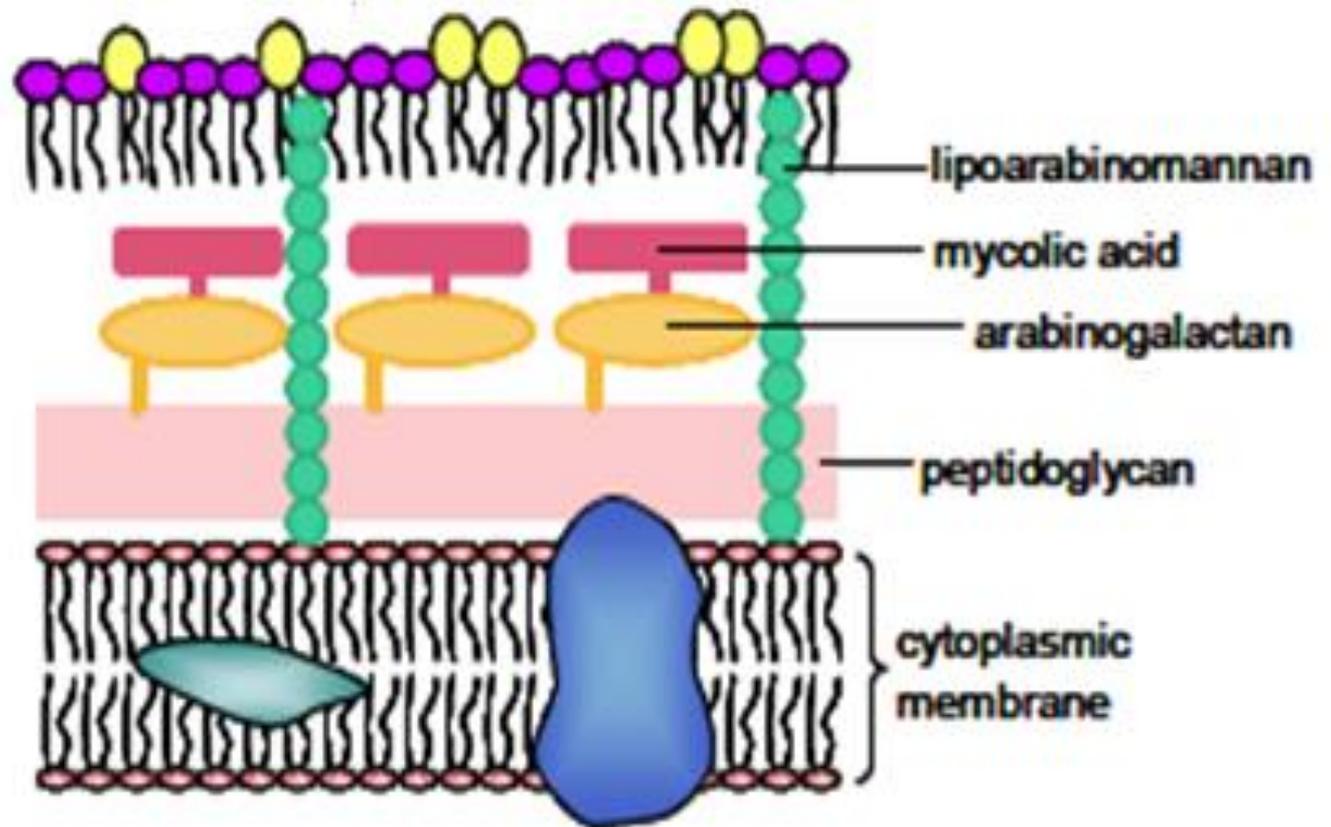
- 5** Rinse briefly with water to remove excess methylene blue.



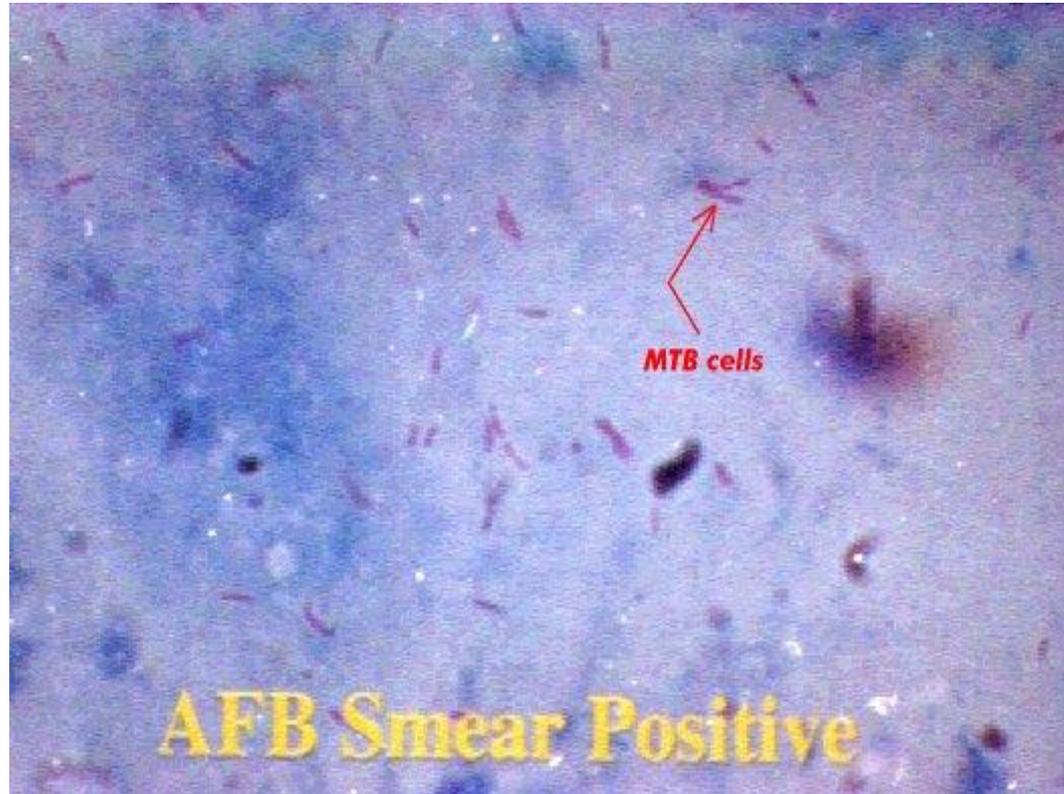
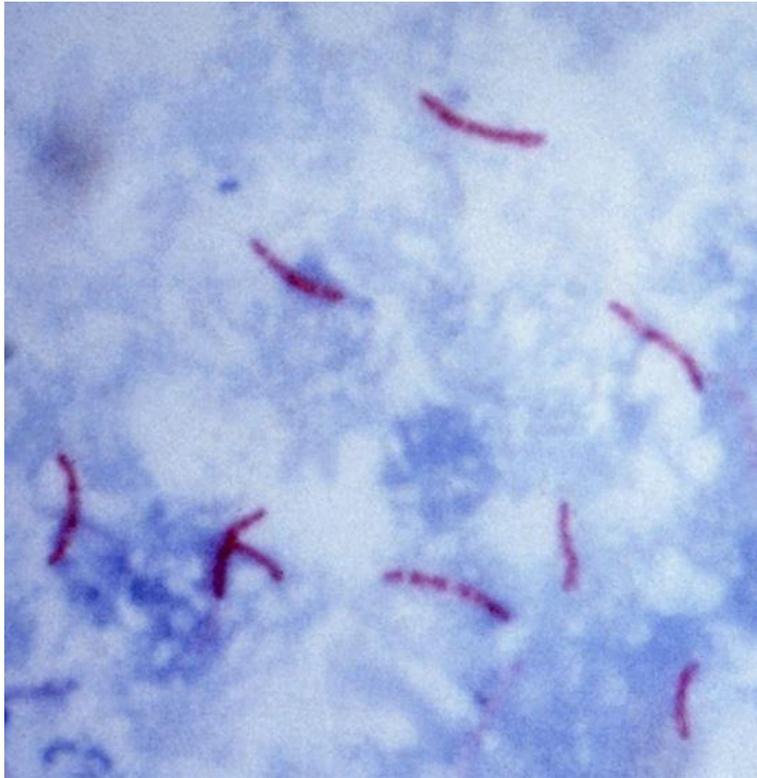
- 6** Blot dry with bibulous paper. Examine directly under oil immersion.

# Acid fast staining

## Principle



# Results of acid fast staining



**TB bacteria**