222 MBIO
Microbial Fine Structure

Dr. Aljawharah Fahad Alabbad
Dr. Haya Mohammed Aldosari

2026

Gram Staining - Part 2
Clinical Applications & Interpretation

- Lab 5 -

Introduction

- **Gram stain** is a rapid and **essential diagnostic tool in microbiology**
- Provides **early information** about bacterial infection
- Helps in **initial classification** of bacteria (Gram + / Gram −)
- Guides **early antibiotic selection** before culture results

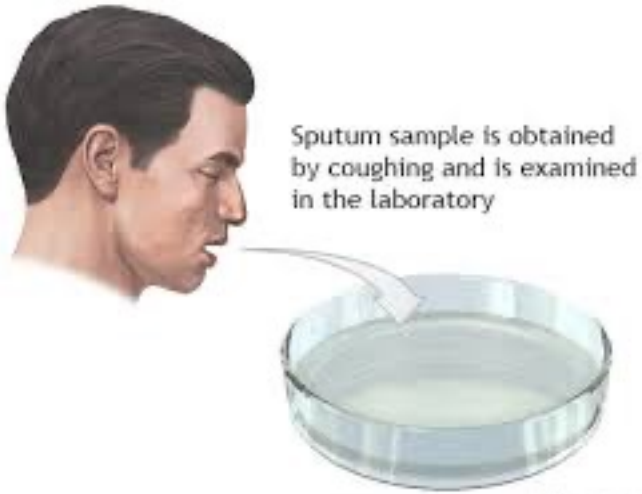
Specimen Collection

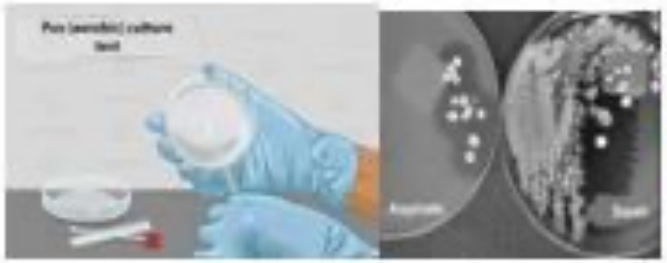
- **Clinical specimens for Gram stain:**
  - Sputum
  - Blood
  - Cerebrospinal fluid (CSF)
  - Ascitic fluid
  - Synovial fluid
  - Pleural fluid
  - Urine
  - Swabs: nose, throat, rectum, wound, cervix
- Must be collected in **sterile containers**

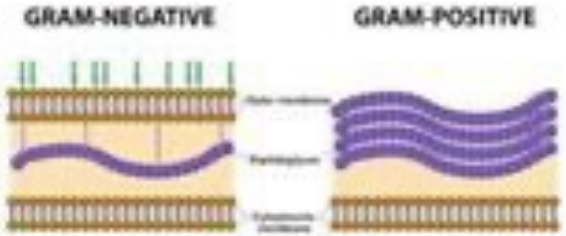

Pus swab Specimen collection

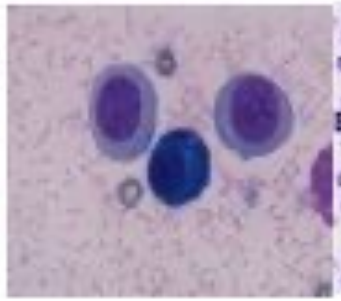

Wound swab Specimen collection

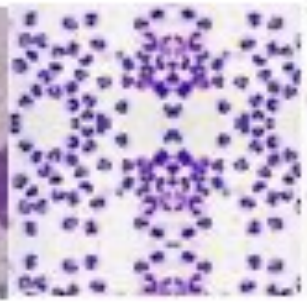

Pus aspiration Specimen collection

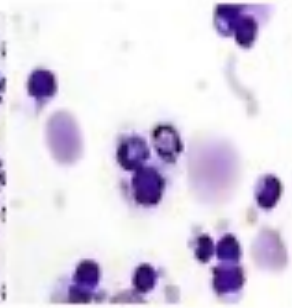

Sputum sample is obtained by coughing and is examined in the laboratory

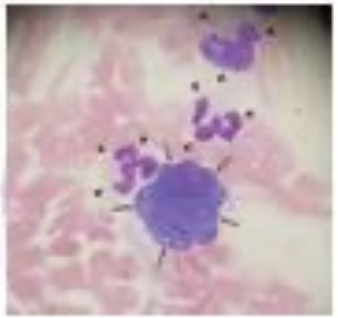



GRAM-NEGATIVE
GRAM-POSITIVE









Diagnostic Value

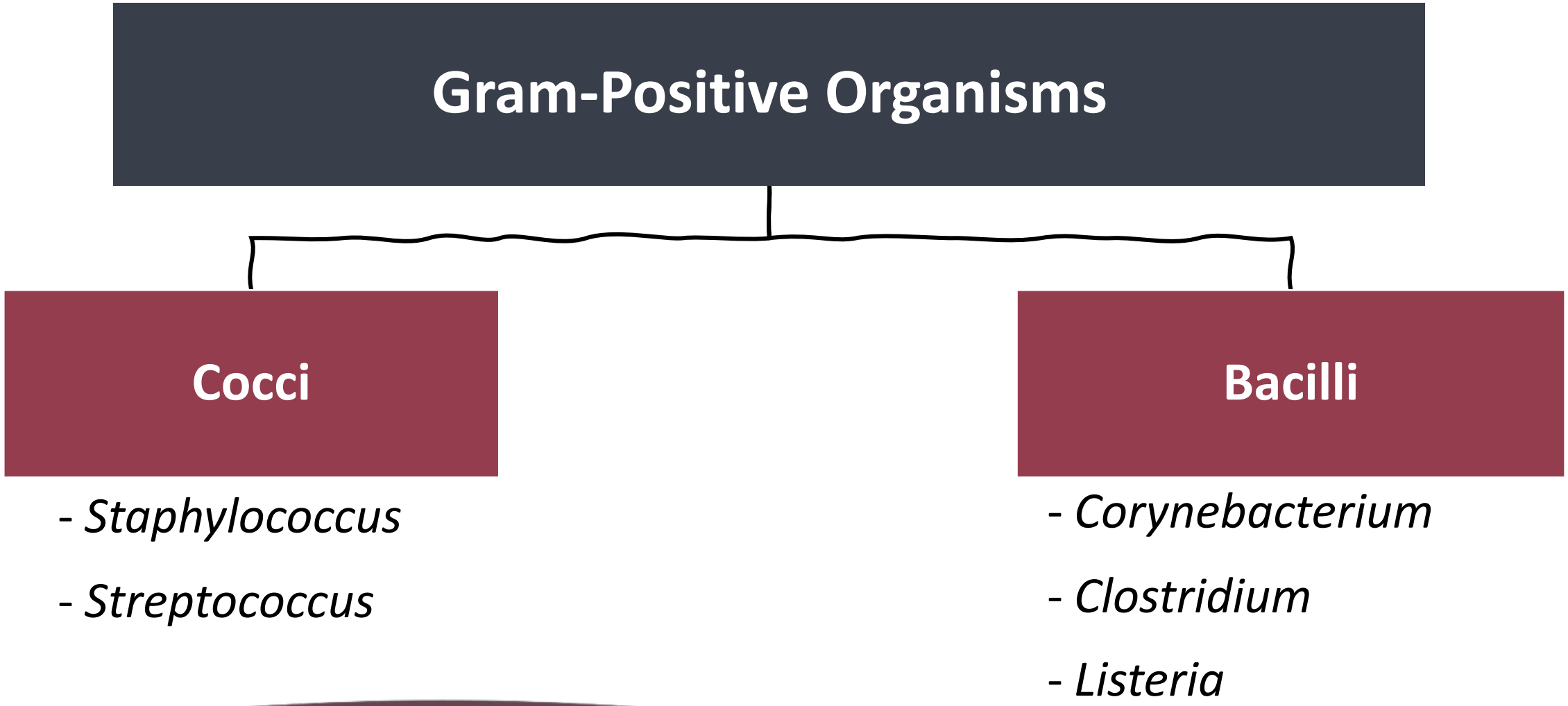
- **Gram stain helps in:**

Early identification of bacteria

Preliminary diagnosis

Guiding initial antibiotic therapy

Gram staining aids in the diagnosis of a disease or a pathologic condition, for example:



Gram-Negative Organisms

Cocci

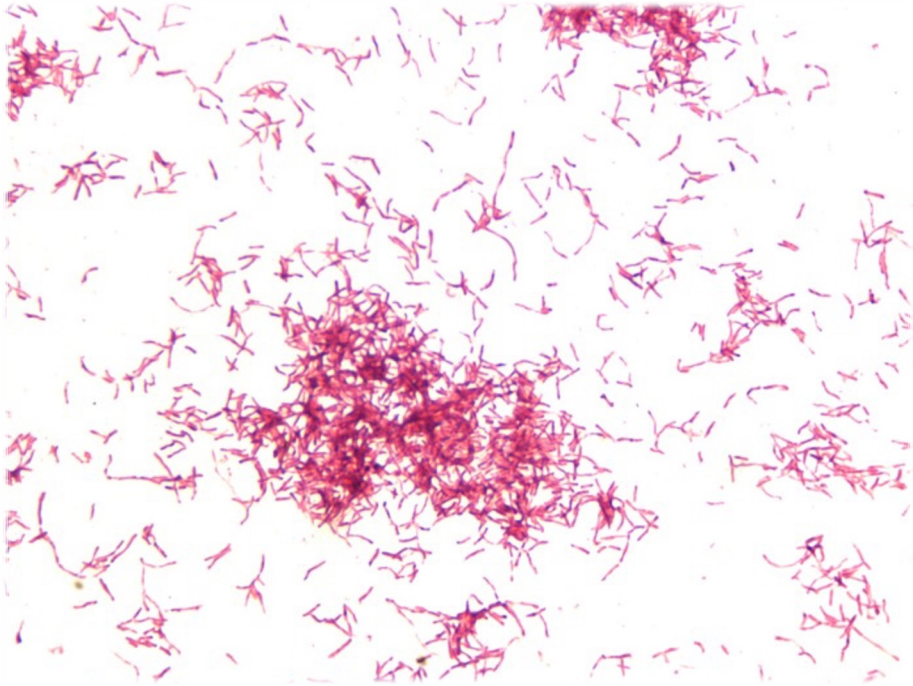
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Moraxella*

Bacilli

- *Escherichia coli*
- *Pseudomonas*
- *Proteus*
- *Klebsiella*

Gram-Variable Organisms

- *Actinomyces* species

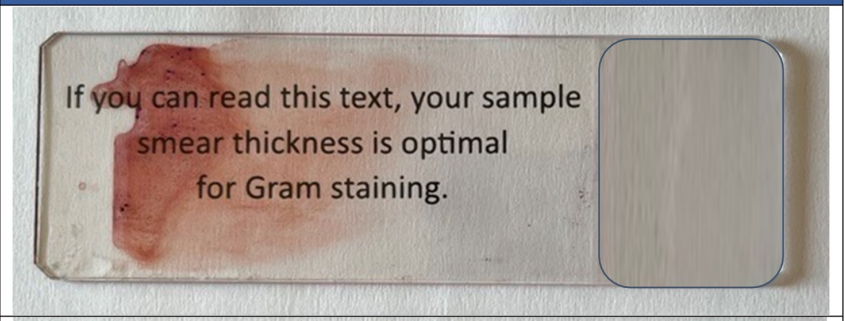


Interfering Factors

- If the specimen collection is **not sterile**, multiple organisms can contaminate the specimen
- **Improper specimen collection** and **prior use of antibiotics** can interfere with the growth of organisms.
- During the interpretation of the Gram stain, as described by the World Health Organization in 2003, **the following steps should be followed:**

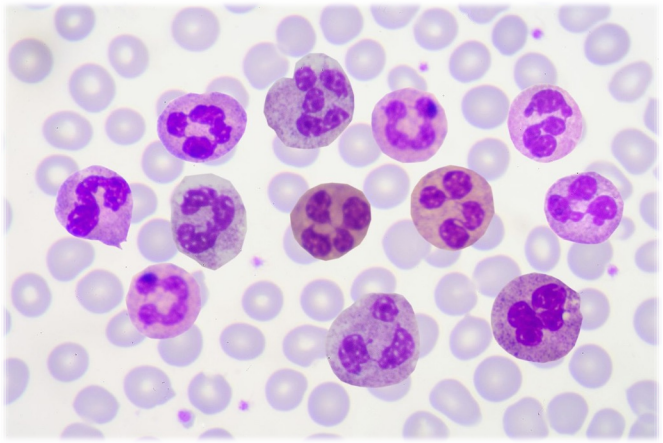
1. General nature of the smear requires analysis under low power magnification (10X)

- **Evaluate smear quality:**
  - Background should be clear or gram-negative
  - **WBCs** stain **gram-negative**
  - Smear should be one cell thick
  - Avoid confusion with crystal violet precipitate

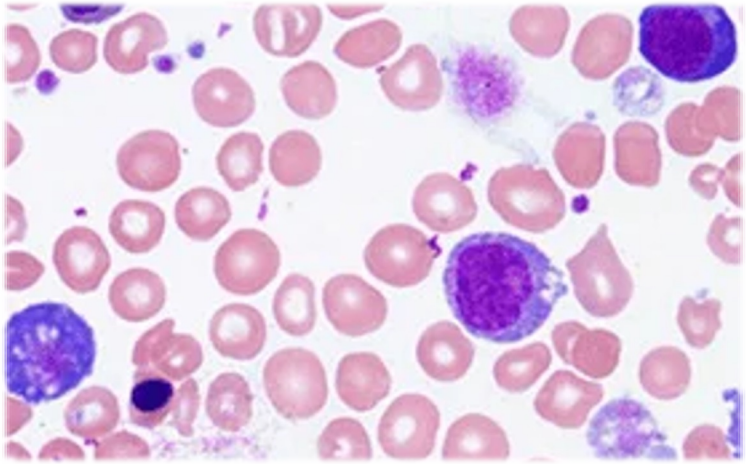
Smear characteristics	Blood culture sample after Gram-Hücker staining
Optimal thickness	
Non-optimal thickness	

2. Interpretation of low power magnification

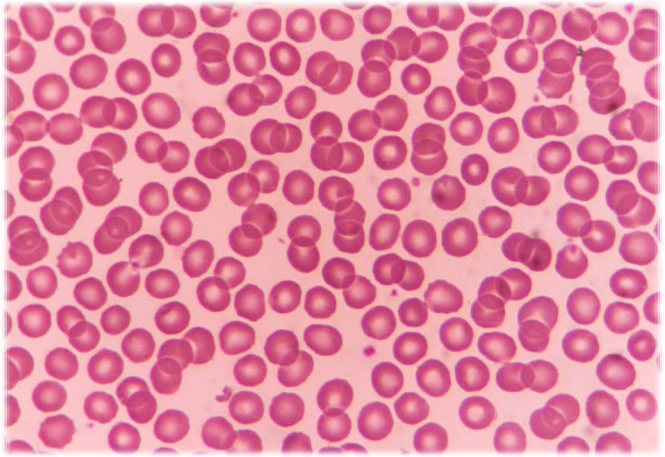
- **Should be utilized to note the following:**
  - Relative numbers of **polymorphonuclear neutrophils** (PMNs), **mononuclear** cells and **red blood** cells (RBCs)
  - Relative numbers of squamous **epithelial cells**, and **normal microbiota** bacteria
  - **Location, arrangement, and shape** of the organisms



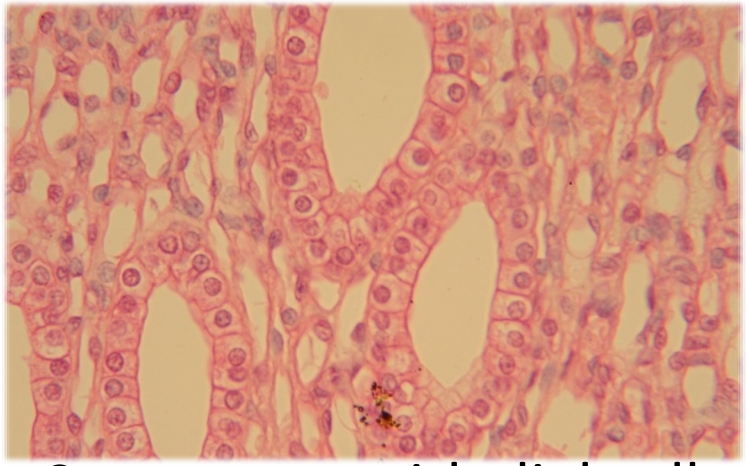
Polymorphonuclear neutrophils



Mononuclear cells



Red blood cells



Squamous epithelial cells

3. Interpretation of Oil immersion

- **Examination of multiple fields is necessary to note the following:**
  - Microorganisms' numbers and morphology
  - **Shape:** cocci, bacilli, coccobacilli, filaments, yeast-like
  - **Ends:** rounded, tapered, concave, clubbed, flattened
  - **Sides:** parallel, ovoid, irregular
  - **Axis:** straight, curved, spiral
  - Pleomorphism (variation in shape)
  - Branching or cellular extensions

Complications

- The interpretation of slides can be difficult if the **microscopic smear is thick and clumped.**
- **Decolorization time** should have very close monitoring to avoid **under-decolorization or over-decolorization** (Thicker smears require longer decolorizing time).
- Cultures should undergo evaluation while they **are still fresh**. **Old cultures** tend to **lose the peptidoglycan cell walls**, which predisposes gram-positive cells to be gram-negative or gram variable.

Before (Wrong Practice)

- Smear too thick, cells overlap

- Decolorizer applied too long or uneven

- Slide not properly air dried or heat fixed

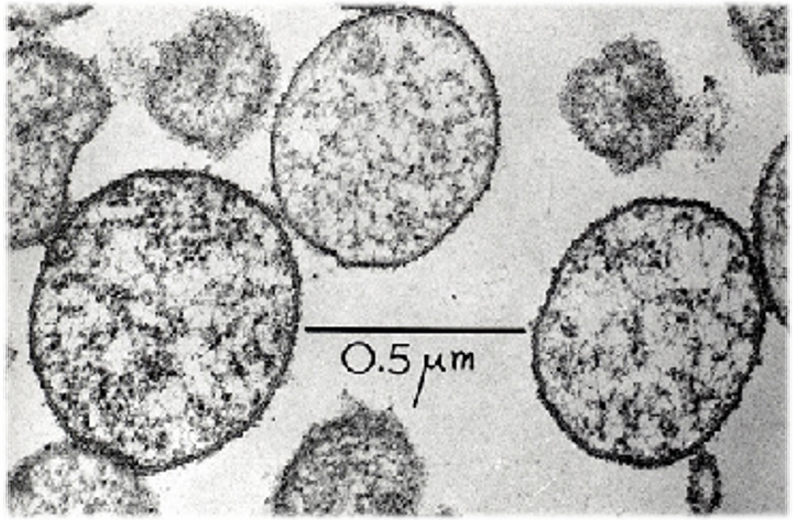
Correct Practice

- Thin, even smear prepared

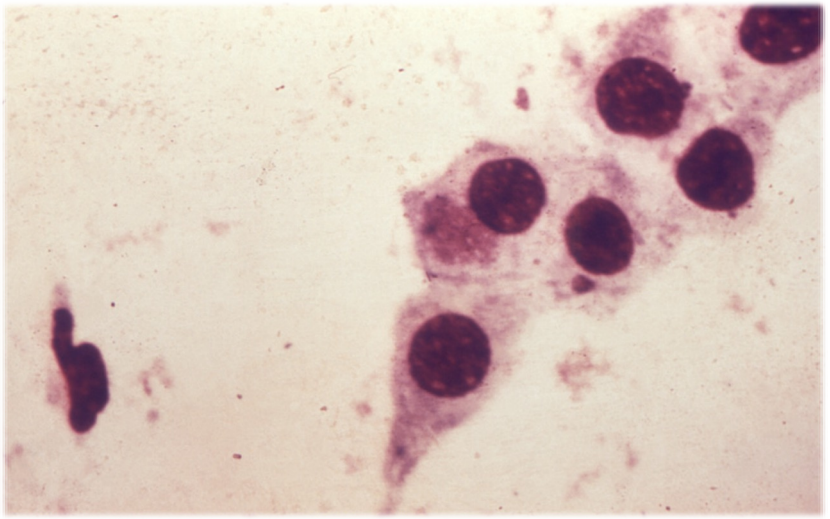
- Decolorization timed and controlled

- Slide fully air dried and correctly heat fixed

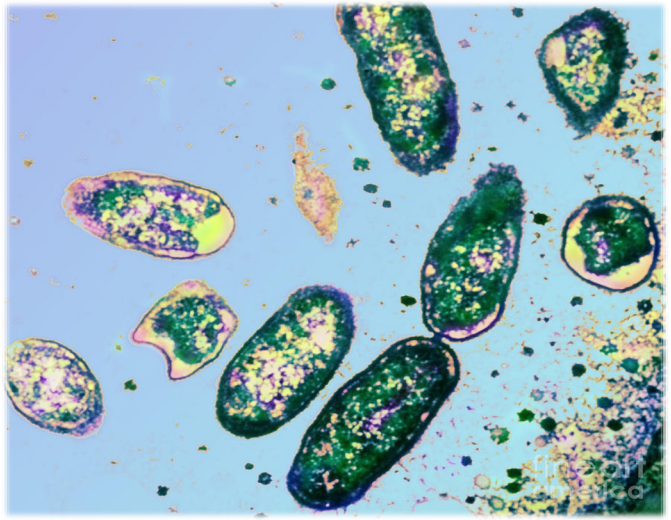
- **Gram stain is not useful** for organisms **without a cell wall** like ***Mycoplasma*** **species**, and for **smaller bacteria** like ***Chlamydia*** **and** ***Rickettsia*** **species**.



Mycoplasma



Chlamydia



Rickettsia

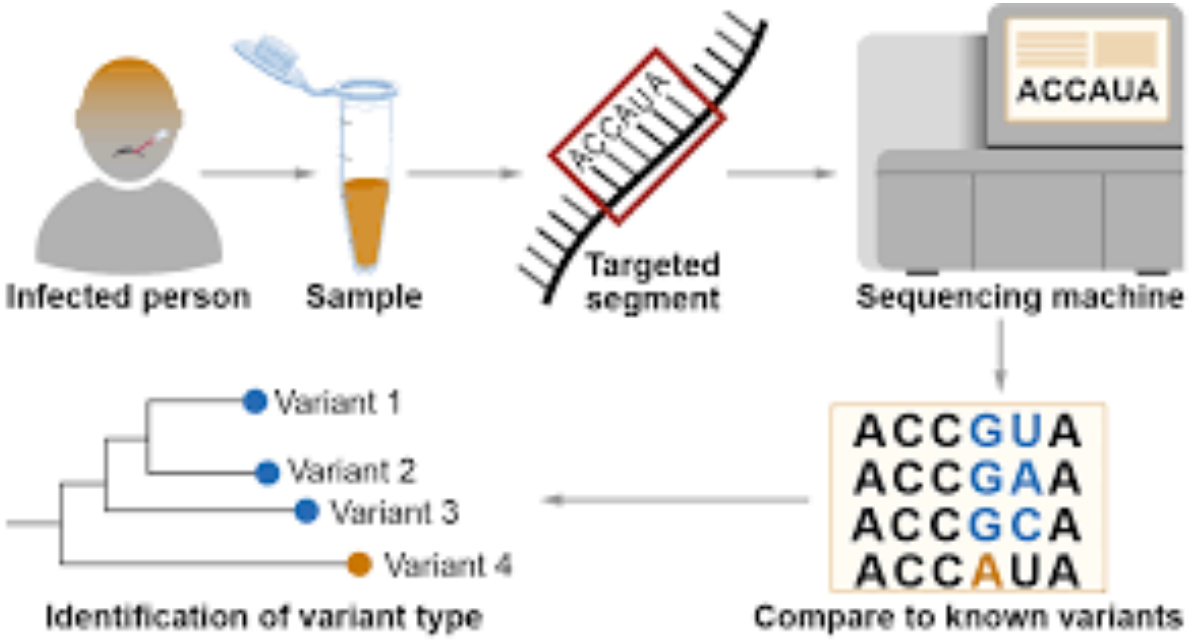
Causes of False Results

- Prior antibiotic use
- Smear too thick
- Fixing before drying
- Low crystal violet concentration
- Excessive heat fixation
- Excessive washing
- Insufficient iodine
- Prolonged decolorization
- Excessive counterstaining
- Inexperience in slide preparation

- Sometimes **results of Gram-stain may not match the results of cultures** and could potentially lead to **inappropriate use of antibiotics.**

Clinical Significance

- **Gram stain** is often the **initial diagnostic test** for the evaluation of infections.
- The use of Gram stain facilitates the **rapid use of appropriate antibiotics.**
- **Genetic sequences** and **molecular techniques** are **more specific** than classic gram stain.



"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 🤓