Methods of Bacterial Identification

1-Microbial Causes of Infection

Infectious diseases are caused by different types of microorganisms, including:

- **Bacteria**
- **Viruses**
- **Fungi**
- **Parasites**

2- The source of the pathogen may be:

- **Exogenous**: acquired from the environment, animals, food, water, or other people.
- **Endogenous**: originating from the body's normal flora when host defenses are compromised

3- Specimen Selection, Collection, and Processing

Accurate laboratory diagnosis depends on proper specimen handling:

- The **quantity of the specimen must be adequate** and representative of the disease process.
- Specimens are selected based on **clinical symptoms**.
- **Contamination must be avoided** by using sterile equipment and aseptic techniques.
- Specimens should be transported to the laboratory **as quickly as possible**.
- **Transport media** may be required to preserve organisms.
- Specimens should be collected **before antimicrobial therapy** to ensure reliable results.

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4- Laboratory Specimens

Specimen	Associated Infection
Blood	Septicemia
Urine	Urinary tract infections
Stool	Gastrointestinal infections
Sputum	Respiratory infections
Vaginal swabs	Vaginal infections
Nose & ear swabs	ENT infections
Cerebrospinal fluid (CSF)	Central nervous system infections
Food & vomit	Food poisoning
Pus	Acne, burns, wound infections
Seminal fluid	Urethral discharge

5. Microscopic Examination

Gram Staining

Gram staining is a **differential staining technique** used to classify bacteria based on their ability to retain crystal violet dye.

- **Gram-positive bacteria**: appear **purple:** *Staphylococcus*, *Streptococcus*
- **Gram-negative bacteria**: appear **pink or red:** *Escherichia coli*, *Klebsiella*

Gram staining also reveals:

Cell shape (cocci or bacilli) Arrange

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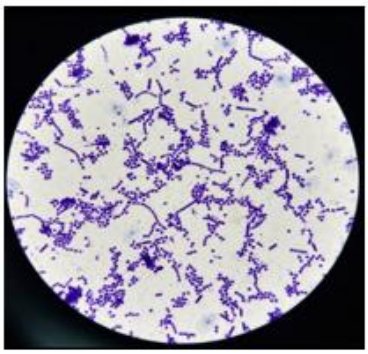
What Is a Gram Stain?

A Gram stain is a laboratory test used to detect bacteria in clinical specimens or body fluids. It provides rapid preliminary information that helps healthcare providers select appropriate therapy.

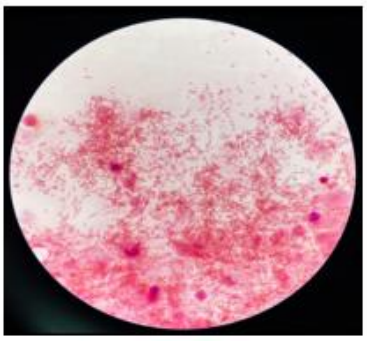
Although Gram staining does not usually provide a definitive diagnosis, it is a **fundamental technique** in microbiology and an essential first step in bacterial identification. The method was developed by **Hans Christian Gram in 1882**.

- **It is one of the most important first tests in the lab.**
- **The test depends on the thickness of the cell wall.**
- **Gram-positive bacteria keep the purple color because they have a thick wall.**
- **Gram-negative bacteria lose the purple color and take on a pink color instead.**

Gram-positive (Purple)



Gram-negative (Pink)



Gram-positive organisms

Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan, a substance that forms the cell walls of many bacteria. The peptidoglycan forms about 90% of the cell wall in gram-positive bacteria. This causes them to appear blue to purple under a Gram stain.

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Gram-positive organisms include:

- ***Staphylococcus* species.**
- ***Streptococcus* species.**
- ***Corynebacterium* species.**
- ***Clostridium* species.**
- ***Listeria* species.**

Gram-negative organisms

Gram-negative bacteria have cell walls with thin layers of peptidoglycan (10% of the cell wall) and high lipid (fatty acid) content. This causes them to appear red to pink under a Gram stain.

Gram-negative organisms include:

- ***Neisseria gonorrheae and Neisseria meningitides.***
- ***Moraxella* species.**
- ***Escherichia coli* (E. coli).**
- ***Pseudomonas* species.**
- ***Proteus* species.**
- ***Klebsiella* species.**

Gram staining procedure

At the lab, a medical laboratory scientist smears or spreads the sample on glass microscope slides. These slides are known as smears. They then apply a series of stains to the smear to perform a Gram stain.

The Gram staining process includes four basic steps, including:

1. Applying a primary stain (crystal violet).1min
2. Adding a mordant (Gram's iodine).1min
3. Rapid decolorization with ethanol, acetone or a mixture of both.20 sec
4. Counterstaining with Safranin.0.5 min

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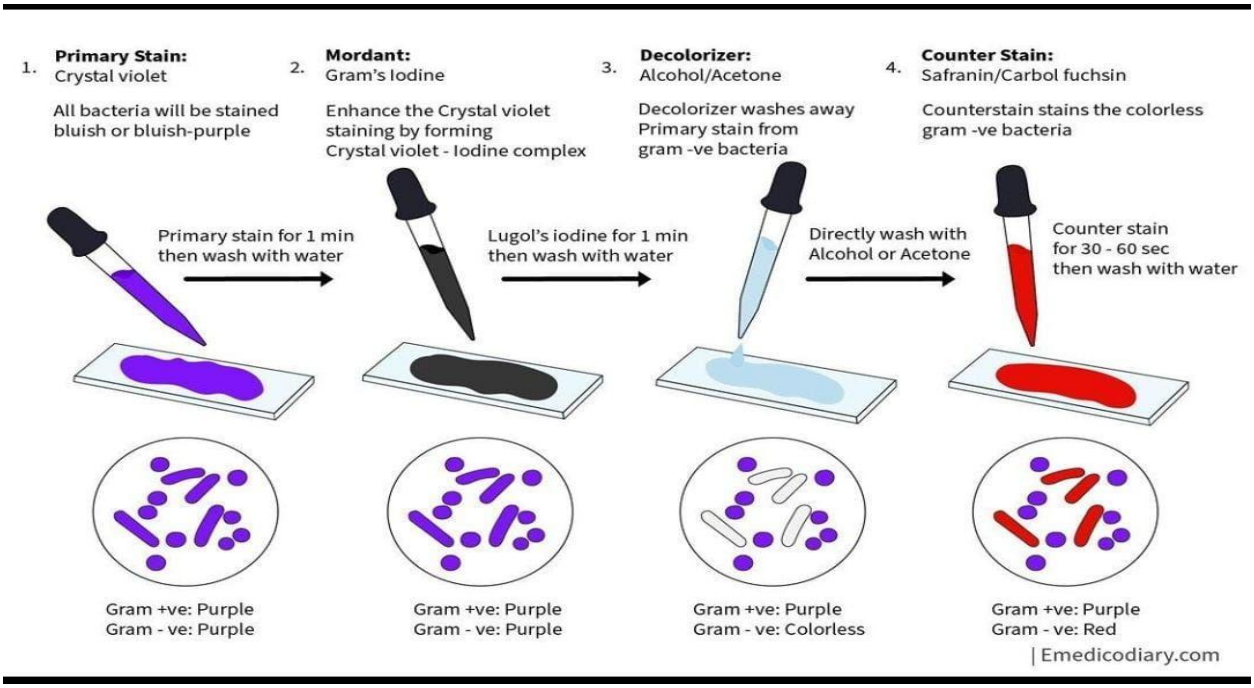
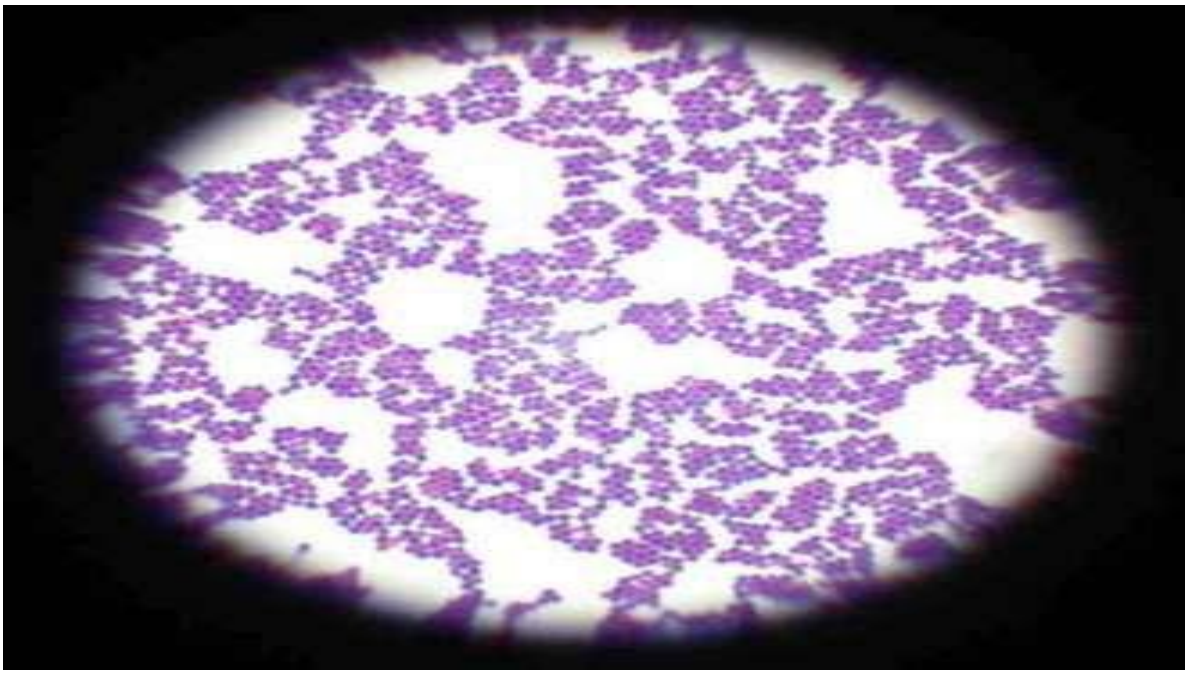
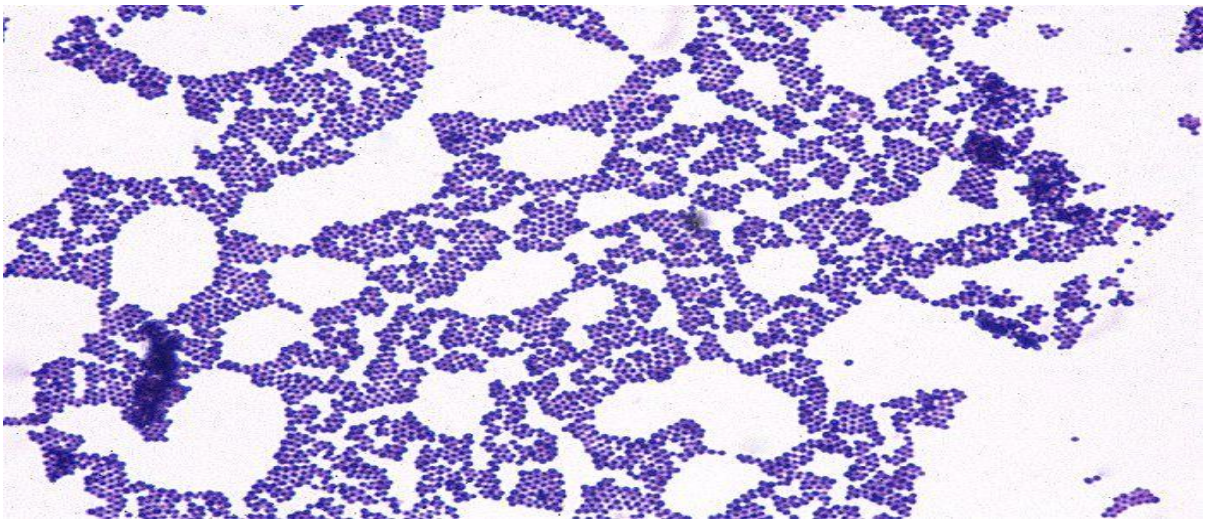




Staphylococcus spp = Cocci , Grape like clusters and Purple



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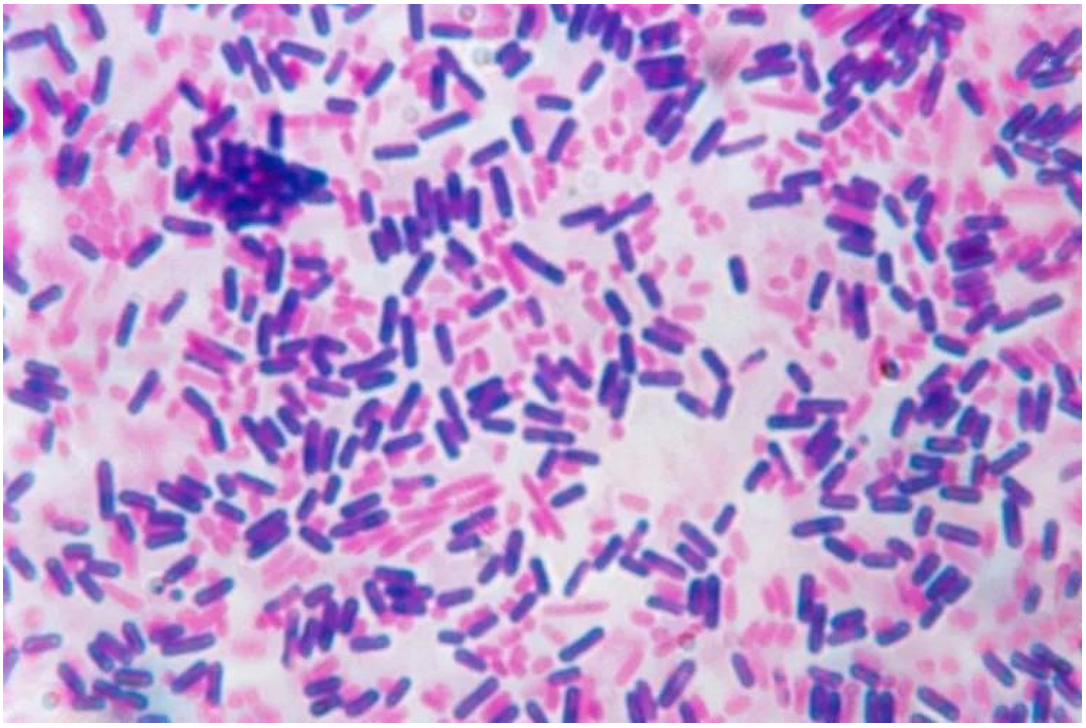


Red: Gram negative ……. Purple: Gram positive

6. Microbiological Culture Examination (Media Selection)

Culture examination allows isolation and identification of bacteria:

- Media selection depends on the **source of the specimen**.
- Appropriate **temperature and incubation conditions** (aerobic or anaerobic) are required.

Types of Media

- **Non-selective media**: support the growth of many microorganisms.
- **Selective media**: contain inhibitory substances that allow growth of specific bacteria.

Colony Characteristics

- Size
- Color
- Odor
- Hemolysis on blood agar

7. Biochemical Identification

Biochemical tests identify bacteria based on their metabolic capabilities:

- Sugar fermentation
- Amino acid utilization
- Enzyme production

These tests may be:

- **Single tests** (e.g., coagulase test for *Staphylococcus aureus*)
- **Multiple test systems** for Gram-negative bacilli ent (clusters, chains, pairs)

Commercial biochemical identification kits are widely used in diagnostic laboratories.

8. Serologic Methods

Serologic tests detect specific antigens or antibodies:

- Known antigens are bound to latex particles.
- When mixed with corresponding antibodies, **visible agglutination** occurs.
- A positive reaction indicates the presence of the tested organism.

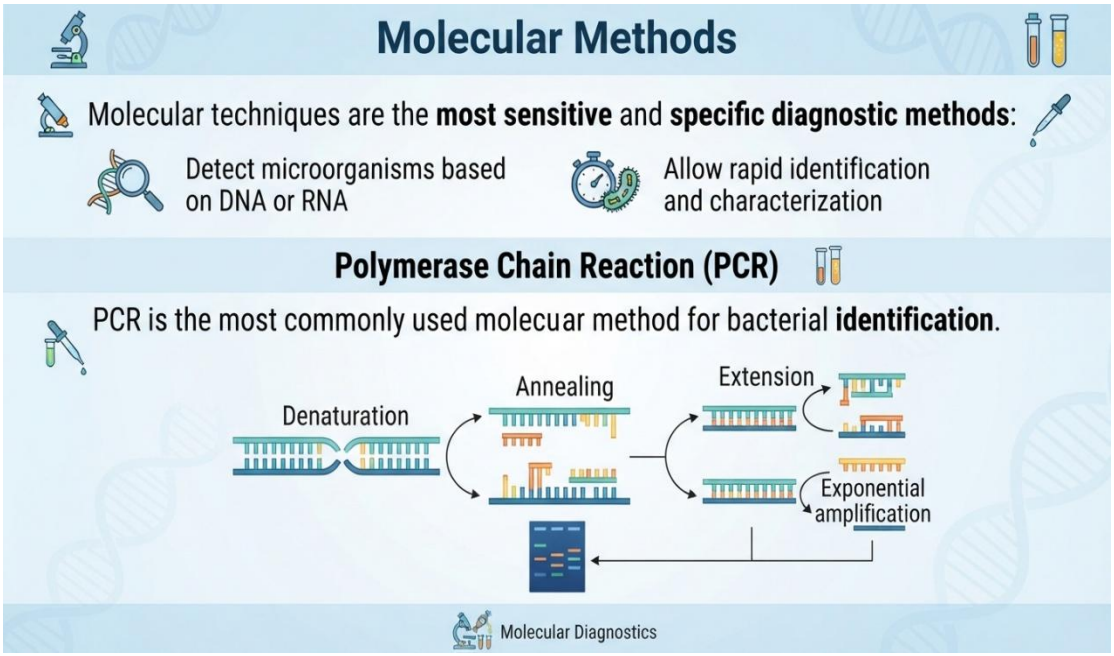
9. Molecular Methods

Molecular techniques are the **most sensitive and specific** diagnostic methods:

- Detect microorganisms based on **DNA or RNA**
- Allow rapid identification and characterization

Polymerase Chain Reaction (PCR)

PCR is the most commonly used molecular method for bacterial identification.



Comparison of Molecular Diagnostic Techniques

1. Conventional PCR:
   - Amplification of target DNA detected via Gel Electrophoresis at the end-point.
2. Real-Time PCR (qPCR):
   - Fluorescent detection monitors amplification in real-time during every cycle.
3. Multiplex PCR:
   - Multiple primer pairs amplify several targets simultaneously in a single reaction.
4. Sequencing (Sanger / NGS):
   - Determines the exact nucleotide sequence for precise identification and typing.

10. Antibiotic Susceptibility Testing

- Determines whether bacteria are **susceptible or resistant** to antimicrobial agents.
- Performed **in vitro** to guide appropriate treatment.
- Helps prevent misuse of antibiotics and reduces antimicrobial resistance.



Kirby Bauer disk diffusion antibiotic susceptibility testing