MICROBIOLOGY

1 GENERAL MICROBIOLOGY

Scientists

00:01:08

Louis Pasteur (Mnemonic: Louis Pasteur, FATHER CAR)


Louis Pasteur

- Pasteur Institute, Paris
- Pasteurization (milk)
- Father of modern microbiology
- Given:
  - Liquid media
  - Fermentation Principle
  - Autoclave
  - Disapproved Theory of abiogenesis
  - Germ cell Theory
  - **Vaccines for**

  Mnemonic: CAR

  → Cholera

  → Anthrax

  → Rabies

Robert Koch (Mnemonic: KOCH)


Robert Koch

- Father of modern microbiology
- Koch postulates
  - Koch Bacilli (Tuberculosis)
  - Cholera organism
  - Solid culture media
  - Aniline dye color
  - Hanging drop motility - motility test
- **Koch postulates Mnemonic: (4+1)**
  - **Old (4)**

    → Constant association of causative organisms with disease.

    → Isolation in culture media possible

    → Culture growth inoculated in animals should produce the same lesion.

    → Re-isolation from the experimental animals is possible.
  - **New (1)**

    → Whenever there is an infection (antigen), humans should be able to produce antibodies in serum.
- **Exception from postulates:**

  Mnemonic- LPG
  - L-Mycobacterium leprae (No culture media, grown in armadillo animal)
  - P-Treponema Pallidum
  - G-N. Gonorrhea

Paul Ehrlich (Mnemonic: Paul Ehrlich)


Paul Ehrlich

He gave:

- Ehrlichia organism
- Father of chemotherapy
- Toxin-antitoxin standardization (Nobel prize)
- Acid-fast stain/ Ziehl Neelsen stain (modified of ZN stain)

Other Scientists

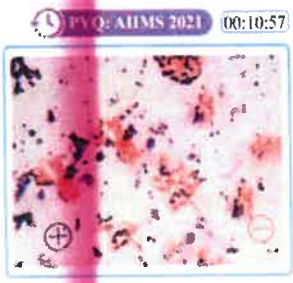
- **Joseph Lister (Mnemonic: JOSEPH):** Father of Antiseptic surgery (carbolic acid)
- **Anton Von Leeuwenhoek:** Father of the light microscope (Unilocular)
  - First saw little animal cules
- **Ernst Ruska:** Electron microscope
  - Mnemonic: Light microscope: Leeuwenhoek (L-L)
  - Electron microscope: Ernst (E-E)
- **Edward Jenner:** First vaccine- smallpox: 1980 eradicated
- **Karry B Mullis:** PCR
- **H C Gram:** Gram staining
- **Kleinberger:** L forms (cell wall deficient form)
- **Alexander Fleming:** Penicillin
- **Barbara Mcclintock:** Transposons (Jumping genes)

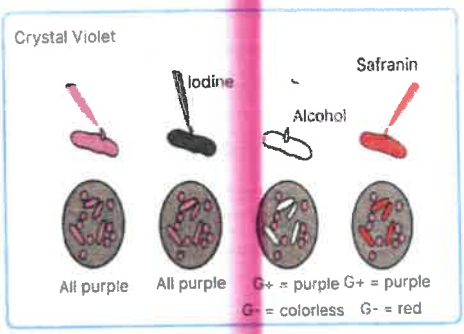
Nobel Prizes During Covid Era

- **HCV mechanism-** Michael Hougton, Harvey J. Alter, Charles M. Rice
- **CRISPR Cas9 -** Emmanuelle Charpentier and Jennifer A. Doudna.

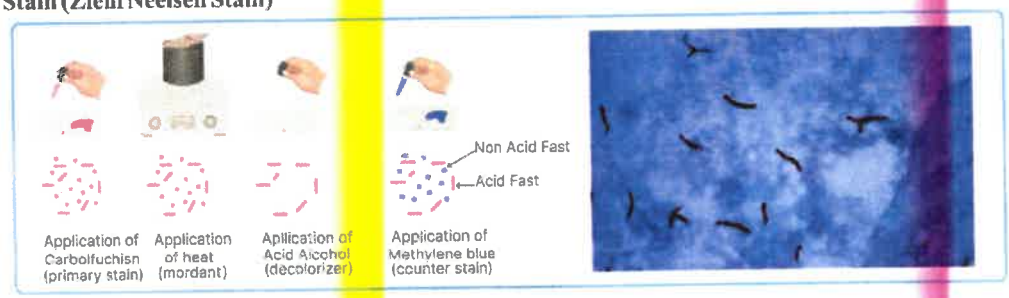
Differential Stains

1. **Gram stain (+/-)**
   - Mnemonic: Come In And Stain
     - Crystal Violet (primary stain)
     - Iodine (mordant)
     - Alcohol/ Acetone (decolorizer) - Important step
     - Safranin (secondary stain)
   - **Results**
     - **Gram +ve:** Purple
     - **Gram -ve:** Pinkish reddish color (decolorized)
   - **Poorly gram staining**
     Mnemonic- MRCS
     - Mycoplasma
     - Rickettsia
     - Chlamydia
     - Spirochetes





2. **Acid-Fast Stain (Ziehl Neelsen Stain)**



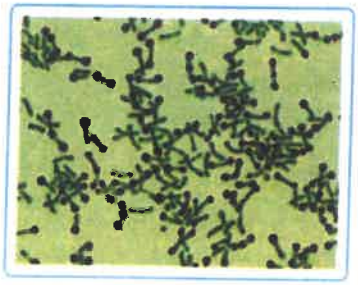
- Mnemonic: Class Has Asked Mnemonic
  - Carbol Fuschin (red) - Organism colors
  - Heating (mordant)
  - Acid/ Acid-alcohol - Sulphuric acid (decolorized)
  - Methylene Blue - Background color

- **How Much H_2SO_4**
  - Mycobacterium **(Mnemonic: T20/25)**
    - → TB - 20-25% H_2SO_4
    - → Lepra - 5% H_2SO_4 (Fite foracco stain)
  - Nocardia, Legionella - 1% H_2SO_4 (Modified ZN stain)
  - Spores, Head of sperm: 0.25 -0.5% H_2SO_4
- **Acid Fast Organisms (Mnemonic-My Nose IS Cold Hot)**
  - **My** - Mycobacteria
  - **Nose** - Nocardia, Legionella
  - **IS** - Isospora, Sperm
  - Cold - Cyclospora Cryptosporidium } (Coccidian parasites)
  - **Hot** - Hooklet of hydatid, Taenia saginata egg

To Remember

- If there is no heating, it is a cold Ziehl Neelsen Stain (Kinyoun stain/Gabbet stain). It is used for Coccidian parasites
- Mordant: increased concentration of phenol

3. Albert Stain



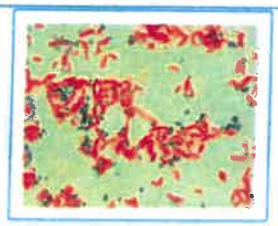
- Used for C diphtheria: Mnemonic: TIM
- Used for Volutin / Babes Ernst/ Bipolar/Metachromatic Granules
- **Albert solution 1:**
  - Malachite green - Organism
  - Toluidine blue - Volutin granules
  - Glacial Acetic acid
- **Albert solution 2: only has** Iodine

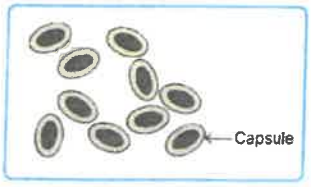
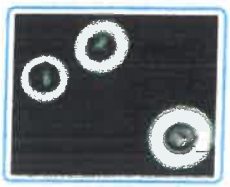
Stains for Volutin Granules

- **Mnemonic:** PLAN
  - Ponder's stain
  - Loeffler methylene blue (best stain)
  - Albert stain (TIM)
  - Neisser stain

4. Miscellaneous Stains

Stain	Usage

• Spore stains (mnemonic: MS SPORE)
• Spore - Resting/ dormant form of bacteria
• ZN stain 0.25 -0.5% H_2SO_4
• Schaeffer and Fulton stain/ Modified Ashby stain
  ○ Malachite green - Spore
  ○ Safranin Red- organism 	

• Negative stain (staining the background, so the sample is highlighted)
  ○ India Ink
  ○ Nigrosin  
	Cryptococcus (meningitis)

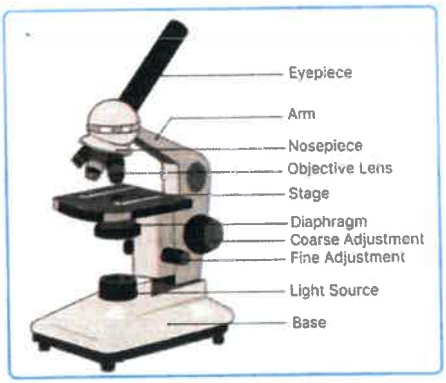
• Silver stains- black color • Fontana stain • Levaditi's stain 	Flagella, Spirochetes
Leifson and ryu's stain (Mordant tannic acid)	Flagellar stain

Stains	Mordant
Gram Stain	I_2
ZN Stain	Heat
Kinyoun Stain	Phenol
Leifson & Ryu Stain	Tannic Acid

PYQ: AIIMS 2018 00:28:48

Microscopes

1. **Light Microscope**
   - Light source - Transmitted light for microscopes
   - **Two lenses**
     - Eyepiece lens - 10X
     - Objective lens
       - → Scanner - 4X
       - → Low Power - 10X
       - → High Power - 40X
       - → Oil Immersion - 100X
   - **Total magnification = Eyepiece x objective**



To Remember

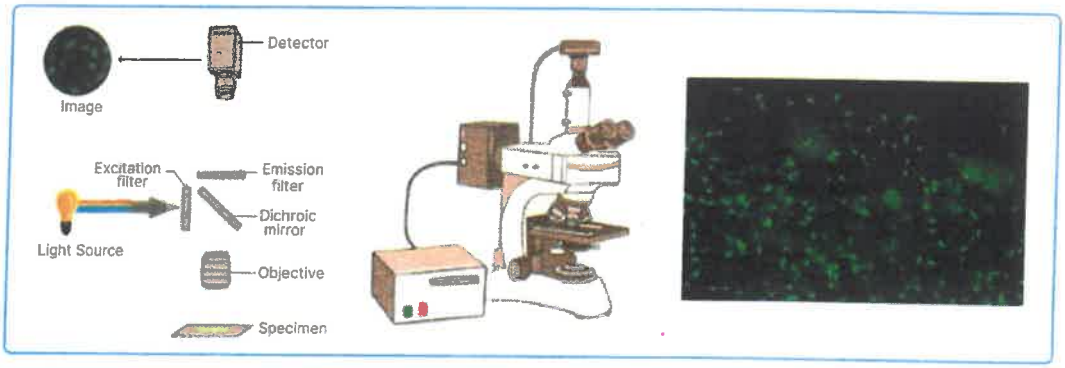
- Max magnification offered by light microscope is 1000X

PYQ: NEET PG 2021
PYQ: FMGE 2021

2. **Dark Field Microscope**
   - Light source : reflected light
   - Used for thin structures
     - Flagella
     - Spirochetes - Spiral structures

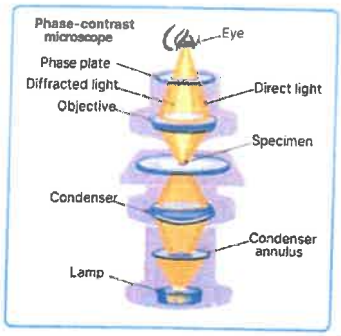
3. **Interference Contrast Microscope**
   - Reveals cells organelles
   - Measurements of chemical constituents of cells, such as:
     - Lipids
     - Protein
     - Nucleic acid

4. **Fluorescence Microscope**



- **Source of light:** Ultraviolet light
- **Mirror:** Dichroic mirror
- **Principle**
  - Light of a shorter wavelength will be converted to light of a longer wavelength light.
- **Different dyes**
  - **TB** - Auramine and Rhodamine
  - **Malaria** - Acridine Orange
  - **Fungus** - Calcofluor White
  - **Flow Cytometry** - FITC (Fluoro isothiocyanate)
- **Autofluorescence micro-organisms** (shine on their own)
  - Cyclospora
  - Isospora
  - Formalin
  - NADPH

5. **Phase Contrast Microscope**
   - **Use:** Internal details of cells
   - Principle
     - Differences in refractive indices will be shown in different phases
   - **Parts**
     - Annular diaphragm in front of the condenser - Bottom
     - Annular phase plate - Top



PYQ: AIIMS 2022

Note: Hairy cells in hairy cell leukemia is seen on phase contrast microscope.

6. **Electron Microscope**
   - Invented by Ernst Ruska
   - No light is used
   - **Source:** A beam of an electron is used (electron is enclosed)
   - **Medium:** Vacuum

Differences between Electron light Microscope

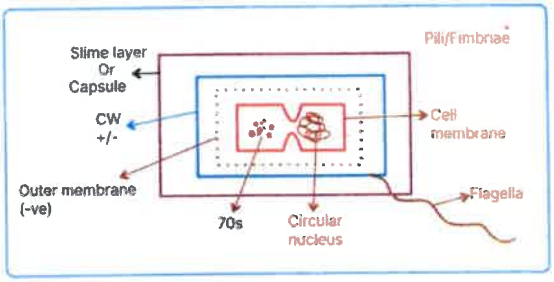
Electron Microscope	Features	Light Microscope
2-2.5% Glutaraldehyde	**Fixation**	10% Neutral Buffered Formalin(NBF)
Resin	**Embedding**	Paraffin wax
Copper metal slides	**Slide**	Glass slides
Electron	**Source**	Transmitted light
Vacuum	**Medium**	Air
Higher	**Resolution**	Lower

Types of Electron Microscope

Scanning Electron Microscope (SEM)	Features	Transmission Electron Microscope (TEM)
Scattered electrons	**Principle**	Transmitted electrons
3D	**Dimensional**	2D
More samples viewed in less time	**View and Time**	Less samples viewed in the same time
Surface details seen	**Details**	Internal Details

Bacterial Anatomy

00:43:00



1. Capsule

- Capsule – tough and demarcated
- Slime layer - loose

To Remember

- Streptococcus salivaris has both a slime layer and a capsule.

Capsulated organisms

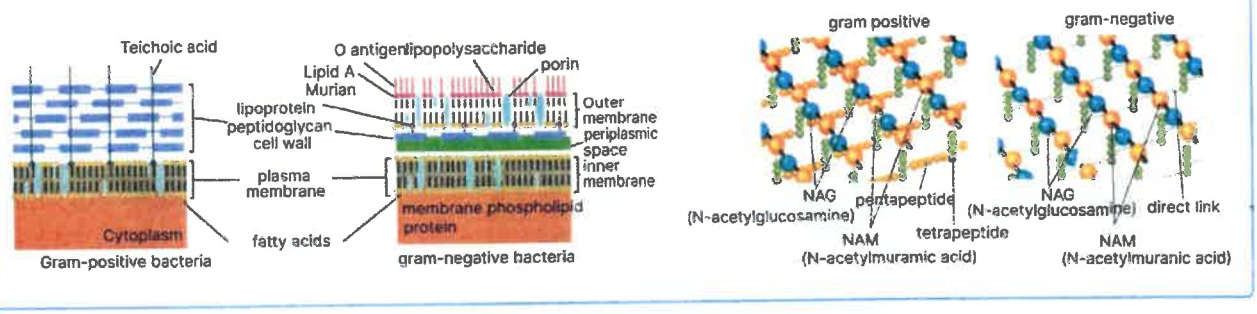
(Mnemonic: Pretty NIce Capsule)

- Streptococcus Pneumonia
- Klebsiella Pneumoniae
- Bordetella Pertussis
- Vibrio Parahaemolyticus
- Clostridium Perfringens
- Yersinia Pestis
- Neisseria meningococcus
- Hemophilus Influenza
- Cryptococcus (fungus)
- Staphylococcus Aureus
- Bacillus Anthracis

To Remember

- All capsules are made of polysaccharides.
- Except
  - Yersinia pestis - F1 peptide
  - Bacillus anthracis - polypeptide
- S. aureus has microcapsule

2. Cell Wall

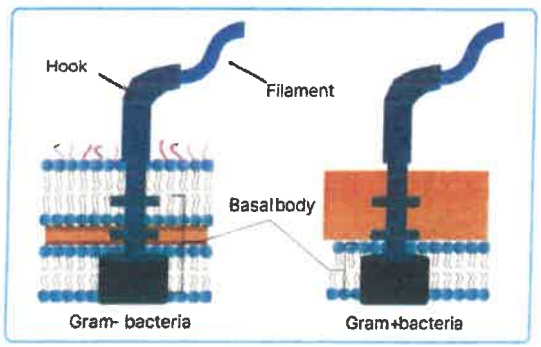


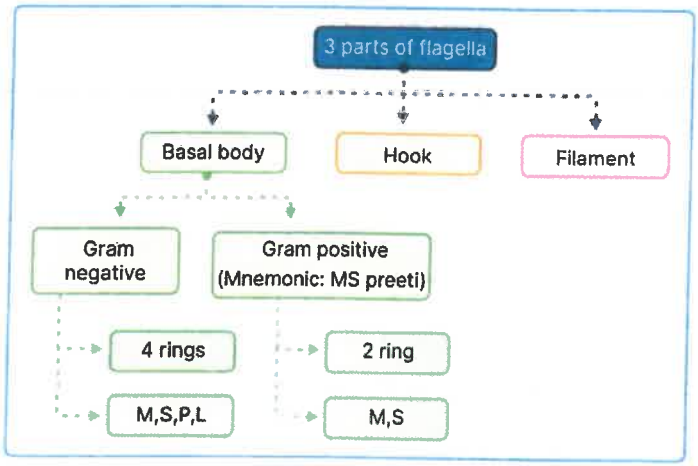
Gram positive Image A (gram +ve) Mnemonic: PPPT • P - Positive • P- Peptidoglycans • P - Pentapeptide cross bridge • T- Teichoic acid	**Features**	**Gram negative** Image B (gram -ve)
2-5 %	**Lipid content**	15-50%
Peptidoglycans++ Lipoproteins	**Composition**	Lipopolysaccharide ++ Mnemonic-OPA • O- O antigen • P - polysaccharide • A - lipid - A
Pentapeptide cross bridge present	**NAM NAG**	Pentapeptide bridge absent
+	**Teichoic acid**	-
-	**Periplasmic space, Porins, Outer membrane**	+

To Remember

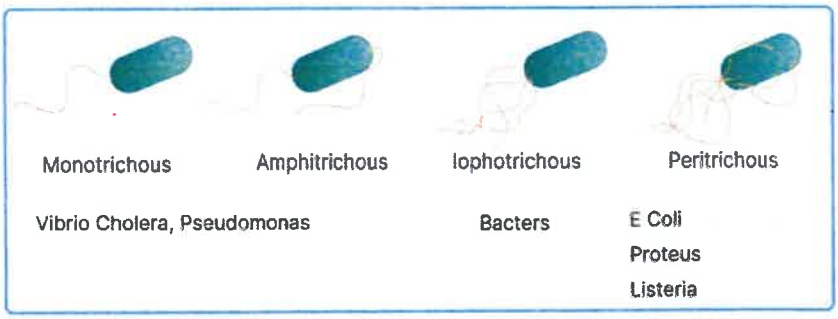
- Periplasmic space has endoflagella, which is a feature of spirochetes.

3. **Cell Membrane**
   - Invagination (Dip) in cell membrane - Mesosomes / Chondroids
     - Respiratory unit in bacteria
     - Seen in both gram-positive and gram-negative
     - Prominent in gram-positive
     - It is an invagination of cell membrane
4. **Ribosomes**
   - 70S
5. **Nucleus**
   - Single circular
   - Double stranded DNA
6. **Flagella**

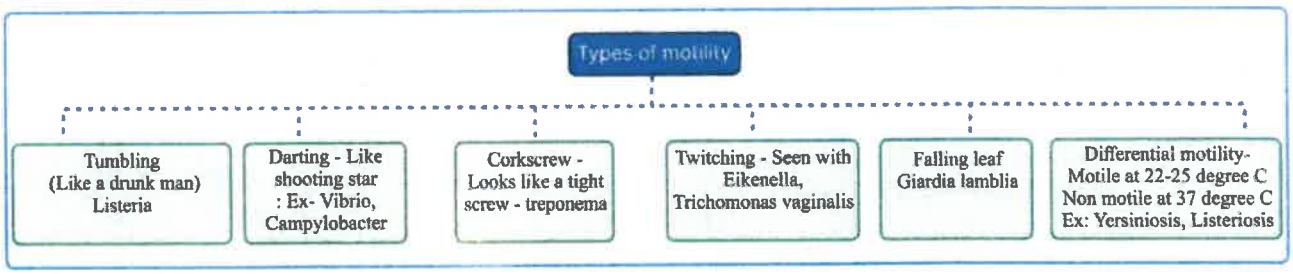




- **Use:** Locomotion
- **Flagella antigen** - H antigen
  - **Widal test for typhoid**
    - → O antigen - cell wall
    - → H antigen - flagella
- **Location (Types)**
  - Types of bacteria based on flagella location



Monotrichous	Amphitrichous	Lophotrichous	Peritrichous
• One flagella on side • **Ex:** Vibrio, Pseudomonas	• Flagella on either side	• Entire tuft of flagella on one side • **Ex:** Helicobacter	• Flagella all around the periphery • **Ex:** (Mnemonic: PERitrichous) o **PR**- Proteus o **E** - E.coli o Listeria



- Considered as growth pattern - in blood agar
- **Ex:**

PVCS-mnemonic:

- P - Proteus
- V - Vibrio parahaemolyticus, Vibrio alginolyticus
- C - Clostridium tetani, Bacillus Cereus
- S - Serratia

7. **Fimbriae/ Pili**
   - Hair like
   - **Use:** Adhesion
   - **Detection**
     - **Hemagglutination:** Gonococcus, E. coli, Klebsiella
     - **Surface pellicle test**

Important Information

- Type IV pili in Neisseria and pseudomonas are used for locomotion.

O_2- Oxygen

- Aerobic - Need O_2
- Anaerobic - Not needed O_2
- **Obligate aerobes** (Must Let Nagging Pets Breathe)
  - MTB
  - Legionella
  - Nocardia
  - Pseudomonas
  - Bacillus
  - Brucella
  - Bordetella
- **Obligate Anaerobes** (Choked By Air)
  - Clostridium
  - Bacteroides
  - Actinomyces
- **Facultative anaerobes**
  - Staphylococcus
  - Streptococcus
  - Enterobacteriaceae
  - Haemophilus
  - Vibrio
- **Microaerophilic** (5% of oxygen needed)
  - **Ex:** Helicobacter and campylobacter

CO_2- Carbon dioxide

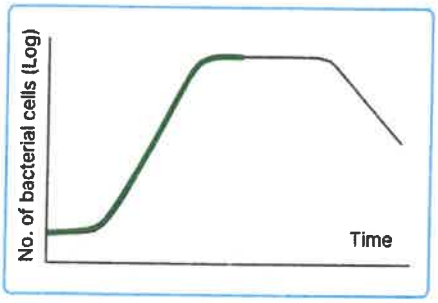
- Capnophilic
- **Ex:** Campylobacter
- **pH:** Usually 7.2 to 7.6
- **Except**
  - H.pylori - Need acidic pH
  - Proteus - Need alkaline pH

Temperature

Psychrophile	Mesophile	Thermophile
• <20°C • **Grow better at low temperatures**	20-40°C	• 55-80°C • **Ex:** Bacillus geothermophilus

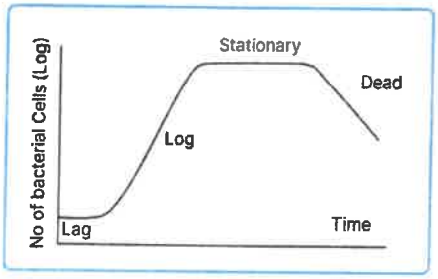
Bacterial Growth Curve

- **Phases**
  - Lag
  - Log
  - Stationary
  - Dead



Features of Phases

- Curve with phases
- **Lag Phase**
  - No increase in number of bacteria
  - Increase in size, enzymes and metabolite



To Remember

- Maximum size is seen at the end of the lag phase.
- **Log Phase**
  - Cell division
  - Smaller in size
  - Maximum metabolic activity
- **Stationary Phase**
  - Some remain viable and some are dead
  - Mnemonic-STAB
    - → Sporulation