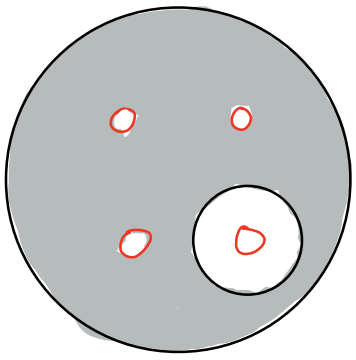
General microbiology

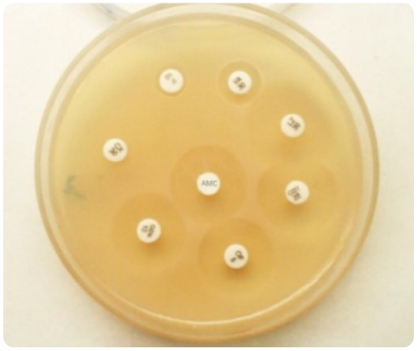
Identification of Ag

- **Microscopy**
  - 1) Gram stain
  - 2) culture & 3) Identification (Biochemical reactions) } Gold std
- **Molecular (Gene)**
  - PCR
- **Immunological Ag / Ab**
  - ELISA, IFA
  - Rapid tests

can perform antibiotic sensitivity testing (AST) on Mueller Hinton Agar (MHA)



Lawn culture + Antibiotic disc



SCIENTISTS

1. Antonie Van Leeuwenhoek - Father of microscopy

- invented simple microscope

2. Louis Pasteur - Father of Microbiology

i) Vaccination ↘ coined the term "vaccine"
↘ CAR - Cholera
Anthrax vaccines
Rabies

ii) Sterilization ↘ discovered autoclave, hot air oven
↘ pasteurization named after him

Flash method	72°C	for 15-20 sec	} followed by cooling to 13°C or lower
Holder method	63°C	for 30 mins	

(All non sporing killed except Coxiella burnetti)

iii) fermentation - utilization of carbohydrate (sugar) in absence of O_2

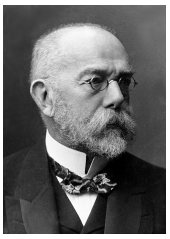
Ex: Lactose ↘ lactose fermenters ↘ acid - pH indicator
gas - Durham tube
↘ non lactose fermentors

maltose ↘ fermented - meningococci
↘ not fermented - Gonococci

iv) Germ theory of disease

v) liquid culture media

3. Robert Koch - Father of bacteriology
Father of modern microbiology



i) discovered solid culture media

ii) Isolation of organisms CAT - Cholera
- Anthrax
- TB bacilli (MTB)

iii) Hanging drop – motility

iv) Staining technique - basic dyes (Aniline dyes)

Note:

Dyes

Acidic (Acridini dye) fluorescent

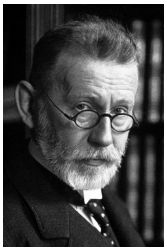
Basic (Aniline dye)

v) Koch postulates - establish causative relationship b/w microbe & disease

Organisms which do not obey Koch postulates

- Non cultivable bacteria (Treponema, M. leprae, Chlamydia, Rickettsia)
- Viruses

4. Paul Ehrlich - father of chemotherapy

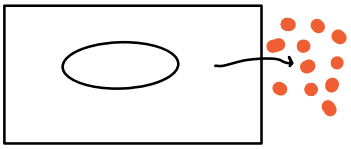
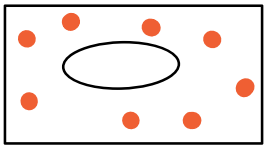


i) Salvarsan — to treat Syphilis
(1st Antibiotic)

ii) Acid fast staining

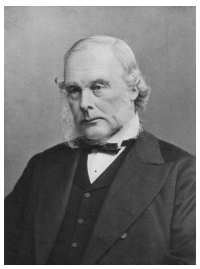
iii) standardized toxin & antitoxin

Toxin

Exotoxin	Endotoxin
- Gram +ve & Gram -ve	- Gram -ve except: Listeria (gram +ve producing endo)
	
- neutralize ⊂ antitoxin - can be toxoided (vaccine) - different for diff bacteria	- cannot be neutralized X - same for all i.e Lipopolysaccharide

5. Joseph Lister – Father of aseptic Surgery

i) Phenol (5% carbolic acid) - Surface disinfectant
(∵ irritates skin)

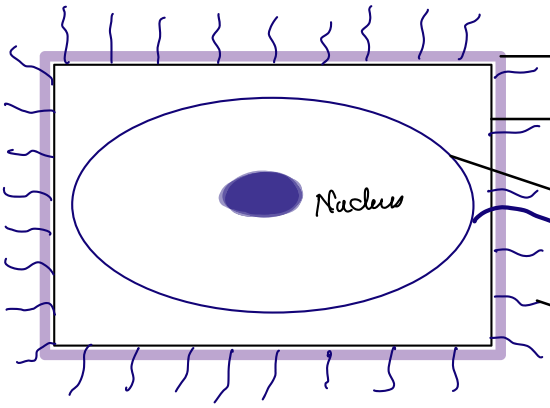


Disinfectant → skin
→ surface

6. Ignaz Semmelweis

introduced 'Surgical handwashing'

MORPHOLOGY & GROWTH OF BACTERIA



Capsule → K-Ag - Antiphagocytic
Cell wall → O Ag - protection
Cell membrane
Flagella → H Ag - locomotion
Fimbria → F Ag - Adhesion

Capsule

Intro : Glycocalyx

- loosely arranged → slime layer (sticky) → ∴ coalesce to form sheath → BIOFILMS
  - protects against immune system
  - antibiotics
  - attach to plastic/rubber
  - leading to implant infection
- compactly arranged → capsule

Capsulated bacteria

- Pneumococci
- Meningococci
- H. influenza
- Klebsiella
- Bacillus
- Clostridium

Function: antiphagocytic

Note: Rapid test for all capsulated organisms - Latex agglutination test

q

Mechanisms of biofilm-mediated resistance to antibiotics:

- **Mechanical barrier**: The lipopolysaccharide biofilms prevent entry of water-soluble drugs.
- **Efflux pumps**: These pumps on the biofilms actively excrete drug out.
- **Slow metabolism**: Decreased oxygen and nutrients passage through the biofilms slows down the metabolism of bacteria.
- **Anaerobic pathway**: Because of the above reason bacteria follow anaerobic metabolism.
- **Persisters:** Some bacteria undergo phenotypic modification into dormant, spore-like structure and survive antibiotic insult. These are called as persisters which are activated later.
- **Enzymatic inactivation** of drugs by biofilms
- **Genetic adaptation** to antibiotic insults
- **Quorum sensing**: It is cell to cell signaling among the bacteria to coordinate among themselves for contribution in biofilm synthesis.

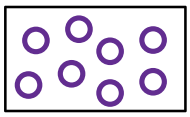
Not adherence

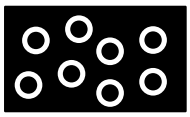
Note: Meningitis

Neonate	Early childhood	Adult	Elderly
- E. coli	H. influenza (mc)	Pneumococci	Pneumococci
- Gr. B streptococci		Meningococci	
- Listeria		H. influenza	
		Klebsiella	
		capsulated	

Demonstration:

- Staining
  - positive
    - His staining (crystal violet)
  - Negative
    - India Ink (mc)
    - Nigrosin (best)
- Immunological
  - Quellung's (Methylene blue) → polysaccharide capsule → most bacteria except Bacillus anthracis
  - McFayden's (Methylene blue) → polypeptide capsule





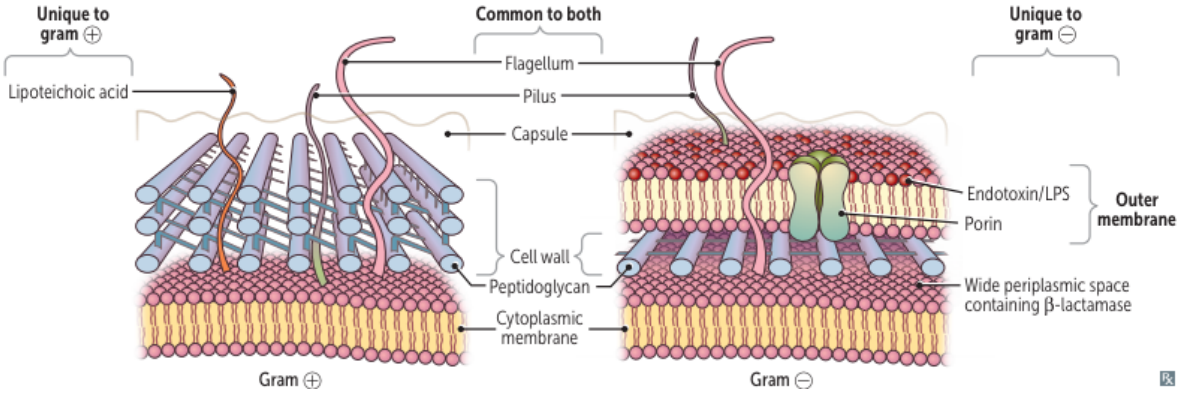
Cell wall

Intro :

Cell wall

		'O' Antigen
Gram positive	peptidoglycan + (100 layers)	teichoic acid — most antigenic
Gram negative	peptidoglycan + (10 layers)	lipopolysaccharide
Mycobacteria	peptidoglycan + mycolic acid + (acid fastness)	lipoarabinomannan
Fungal		- chitin

Cell envelope



Cell wall deficient forms

Natural

- permanent

Mycoplasma

Derived

due to stress factor (cellwall inhibitor)

- temporary

aka Involution forms / L-forms

Protoplast

Gram +ve

Spheroplast

Gram -ve

Function : protection

Demonstration
- Electron microscopy
- plasmolysis
- microdissection

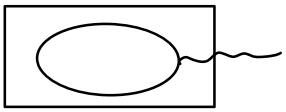
Flagella

Intro: made of protein - Flagellin
arises from cell membrane

all motile bacteria except → Spirochetes
- Treponema
- Borrelia
- Leptospira

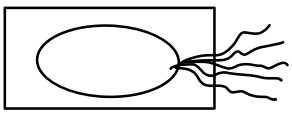
Arrangement:

- Monotrichous



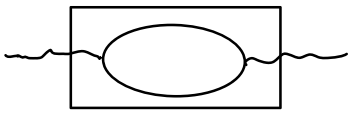
- Vibrio
- Pseudomonas
- Campylobacter

- Lophotrichous



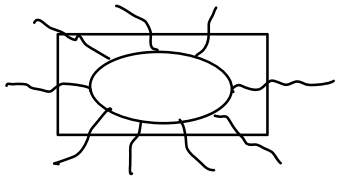
- Helicobacter

- Amphitrichous



- Alcaligenes fecalis

- Peritrichous



- E. coli

Function: locomotion

Demonstration

Direct (Flagella)	Indirect (motility)
- Electron microscope - Staining - Tannic acid stain	1) Wet mount 2) Hanging drop 3) phase contrast microscope 4) Dark field microscope 5) Semisolid agar 6) Antiflagellar antibody

Fimbria (Pili)

made of protein - Pilin

Function : Attachment

Demonstrate :
- Electron microscope
- Haem agglutination

Bacterial growth ↑se in number

Vegetative bacteria → favourable conditions for growth
- Nutrients } culture media
- pH } culture media
- Temp - incubator
- O_2 conc - anaerobic methods

Spore

vegetative bacteria ⇌ (No nutrition / Nutrition) ⇌ Spore — Exosporium

Function : Survival of bacteria ∈ available nutrition

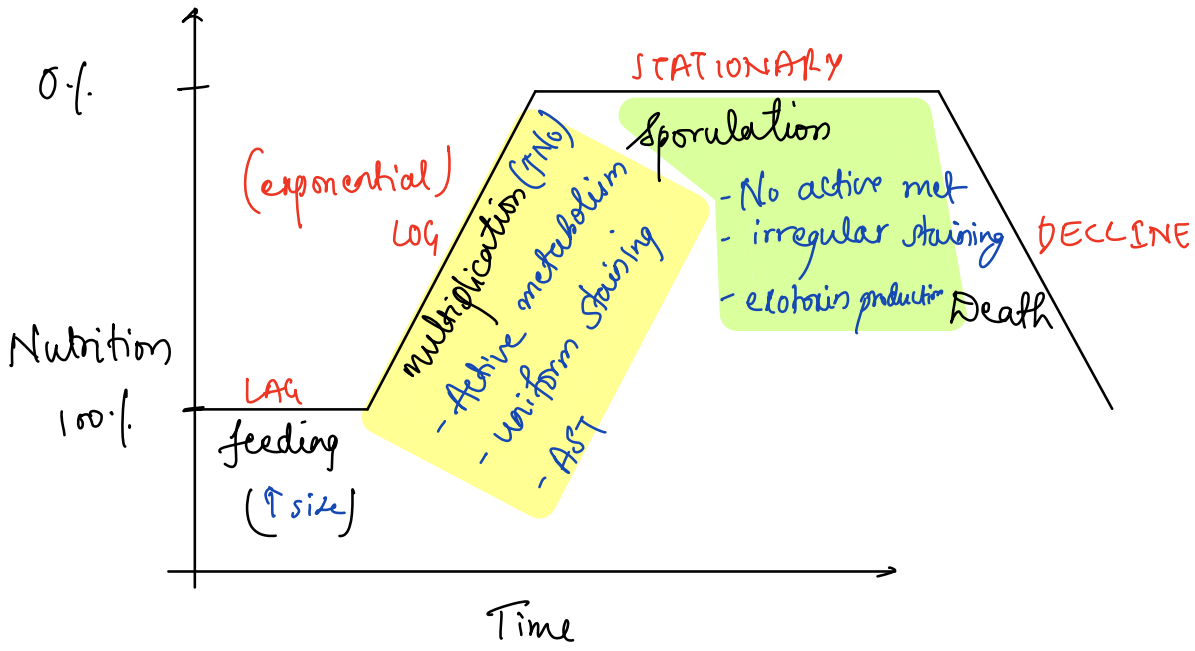
Demonstration :
- F - Fluton's method
- A - Ashby's method
- A - Acid fast method
- R - Ryu's method

Note:

- Bacillus — non bulging spore
- Clostridium — bulging spore

} have Spore in the vegetative state itself

Bacterial growth curve



STERILIZATION & DISINFECTION

Bacteria < pathogenic
non-pathogenic (Commensals)

Bacteria < vegetative
spore

Sterilization - All forms destroyed

Disinfection - except spores

Note: Rideal Walker co-efficient → helps in determining phenol co-efficient (indicates power of disinfectant)

> 1 - more effective than phenol

Other tests :- Chick Martin test
- Kelsey Skyes test (Capacity test)
- In use test

Physical methods

Heat

Dry

Hot air oven

Mechanism

- Denaturation of proteins
- Electrolyte imbalance
- Oxidative damage

Moist

Autoclave

- Denaturation of proteins
- Coagulation of proteins

Filtration

Depth filter

Asbestos (Seitz)

Membrane filter

membrane filter

Radiations

Ionizing

X/γ rays

Non Ionizing

IR rays

UV rays

Mechanism: Nucleic acid damage

Hot air Oven

Scientist : Louis pasteur

temp :
- 160° – 2 hrs
- 170° - 1 hr
- 180° - 30mins

Mechanism: D - Denaturation of proteins
E - Electrolyte imbalance
O - Oxidative damage

Control : Biological - Bacillus subtilis / Bacillus atrophaeus
(purple → green)

Material : 1) Glass : glass syringe, flask petri dish

2) Sharp surgical instruments : blade, scalpel scissors

3) oily & powdery materials : glove dust powder liq. paraffin

All materials can be autoclaved

Autoclave

Scientist : Louis Pasteur

temp : 121° 30 mins 15 lbs

Mechanism: Denaturation of proteins
Coagulation of proteins

Control : Biological : Bacillus stearothermophilus
(Geobacillus stearothermophilus)

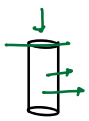
Material : 1) Culture media except
- Sugar containing : Tyndallization 100°C 20 mins
- Serum containing : Inspissation 80-85°C 30 mins
  - done for 3 days
  - Intermittent sterilization

2) Surgical instruments incl. sutures except catgut
↓
ionizing radiation

3) plastic & rubber - Gloves, syringe, Foley's catheter

Filtration

Depth filters (Asbestos)



filtration at surface & depth

Materials sterilized:
1) Serum
2) Vaccines
3) Antibodies (Eg)

Now membrane filter used.

Membrane filters

filtration only on surface (better)

pore size: 0.22 – 0.44 µ

1) Bacterial isolation
2) Masks
3) HEPA / ULPA filters used in biosafety cabinets

Nowadays Depth filters not used.
∵ - membrane filters better
- asbestos is carcinogenic

Radiation

Ionizing	Non ionizing
aka cold sterilization	
X rays γ rays	UV rays IR rays
high penetrating power	low penetrating power
very effective	less effective
heat labile materials	not used commonly
1) disposable (plastic & rubber) 2) catgut sutures 3) cotton swab	- biosafety cabinet

Chemical methods

High level
Sporicidal

- ETO - alkylating agent
- Plasma - oxidising agent
- Aldehyde alkylating agent

Intermediate level
(Sporicidal under special conditions)

- Halogenic compounds - oxidising agent
  (Med temp)
- Phenolic compounds - cell membrane damage
  (Med contact time)

low level
non-sporicidal

- Alcohol cell membrane damage
- Dyes
- Surface active agents
  (Quaternary compounds)
  - Cetrimide
  - Benzalkonium chloride
- Heavy metal salts

Ethylene oxide

Gas
- ETO
- Formaldehyde
- BPL (β-propiono lactone)

Mechanism: alkylating agent

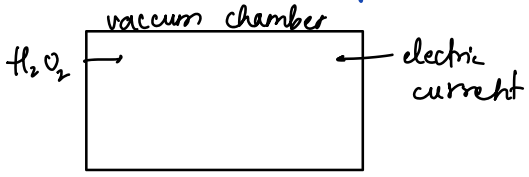
Control: Bacillus subtilis
Bacillus globigii

Materials: 1) Heart lung machine
2) ventilator
3) Dialysis machine
4) Dental equipments

- highly inflammable
- toxic
- carcinogenic

Plasma (ions)

Mechanism: oxidizing agent



Control: Bacillus stearothermophilus

Materials: 1) Heart lung machine
2) ventilator
3) Dialysis machine
4) Dental equipments
5) arthroscopes
6) Urethroscopes

Aldehyde	Alcohol
high level (Sporicidal)	low level (non sporicidal)
Mechanism: alkylating agent	Mechanism: cell membrane damage
1) Formaldehyde (Gas) (40%) OT sterilization (fumigation)	1) Ethyl alcohol (70%) Hand rub / sanitizer
2) Glutaraldehyde (2%) Cidex Endoscope (Nowaday, ortho Pthal aldehyde used)	2) Isopropyl alcohol (70%) clinical thermometer

Halogenic compound

Surface — Skin

Chlorine — Iodine (unstable)

1) free chlorine
↓ active form
hypochlorous acid
disinfect water

I_2 —alcohol→ Tincture iodine

I_2 —Povidine→ Povidine iodine (Betadine)

2) 1% Na hypochlorite (disposables)
lab disinfectant
(also for blood spills)

Phenolic compounds

Surface — Skin

- Lysol household
- Cresol
  ↳ sharps

- Chloroxylenol (Dettol)
- Chlorhexidine (Savlon)
(also contain cetrimide)

mc contaminant of disinfectant → Pseudomonas (∵ cetrimide)

Note: Savlon - Cetavlon (Cetrimide) + Hibitane (Chlorhexidine)

Note: Sputum can be disinfected by autoclaving, boiling or usage of 5% cresol

The Spaulding Classification

Pearl #818 • Community Medicine

The Spaulding classification classifies medical devices according to the degree of risk of infection if they are contaminated.

Device	Description	Examples	Disinfection
Non-critical device	Lower risk of infection as the device comes in contact with intact or normal skin.	Stethoscope, walls, furniture, BP cuff, etc.	**Intermediate/low-level** disinfectant. Clean, dry and store for future use.
Semi-critical devices	Lower risk of infection as these devices come in contact with mucosa and do not penetrate into sterile areas of the body.	Fiber-optic endoscopes, laryngoscopes, bronchoscopes, cystoscopes, vaginal specula, etc.	**High-level** disinfectant to be used after proper cleaning or sterilization by heat.
Critical devices	High risks of infection as these devices come in contact with sterile areas of the body like blood.	Needles, cardiac catheters, implants, intrauterine devices, urinary catheters, etc.	**High-level** disinfectant is indicated with sterilization with heat, ethylene oxide, etc.

While collecting blood for samples, you have spilled some on the floor. What would be the immediate next step?

A. Call the infection control team of the hospital [4%]

B. Mop it [6%]

C. Cover it with 1% sodium hypochlorite [69%]

D. Clean with absorbable material [21%]

21% of the people got this right

5c5c5f01cef59144e429f9bb #AIIMS

The immediate next step after spilling blood sample on the floor is to clean it with absorbable material. Then discard the materials in appropriate labeled content. Following this sodium hypocholorite solution is used to decontaminate the surface.

A 1:10–1:100 dilution of 5.25%–6.15% **sodium hypochlorite (i.e., household bleach)** or an EPA-registered tuberculocidal disinfectant has been recommended for decontaminating blood spills.

- If sodium hypochlorite solutions are selected, use a 1:100 dilution to decontaminate non-porous surfaces after a small spill (e.g., <10 mL) of either blood or OPIM.
- If the spill involves large amounts (e.g., >10 mL) of blood or OPIM, or involves a culture spill in the laboratory, use a 1:10 dilution for the first application of hypochlorite solution before cleaning. This is done in order to reduce the risk of infection during the cleaning process in the event of a sharp injury.
- Follow this decontamination process with **terminal disinfection, using a 1:100 dilution of sodium hypochlorite.**

Microscopes

Source

Light beam

Visible light

- Bright field microscope 
- Dark field microscope 
- Phase contrast microscope 

Principle: Transmission of light
or
Scattering of light

UV light

- Fluorescent microscope

Principle: Fluorescence
absorption of low wavelength (UV light)
emit high wavelength (visible) light

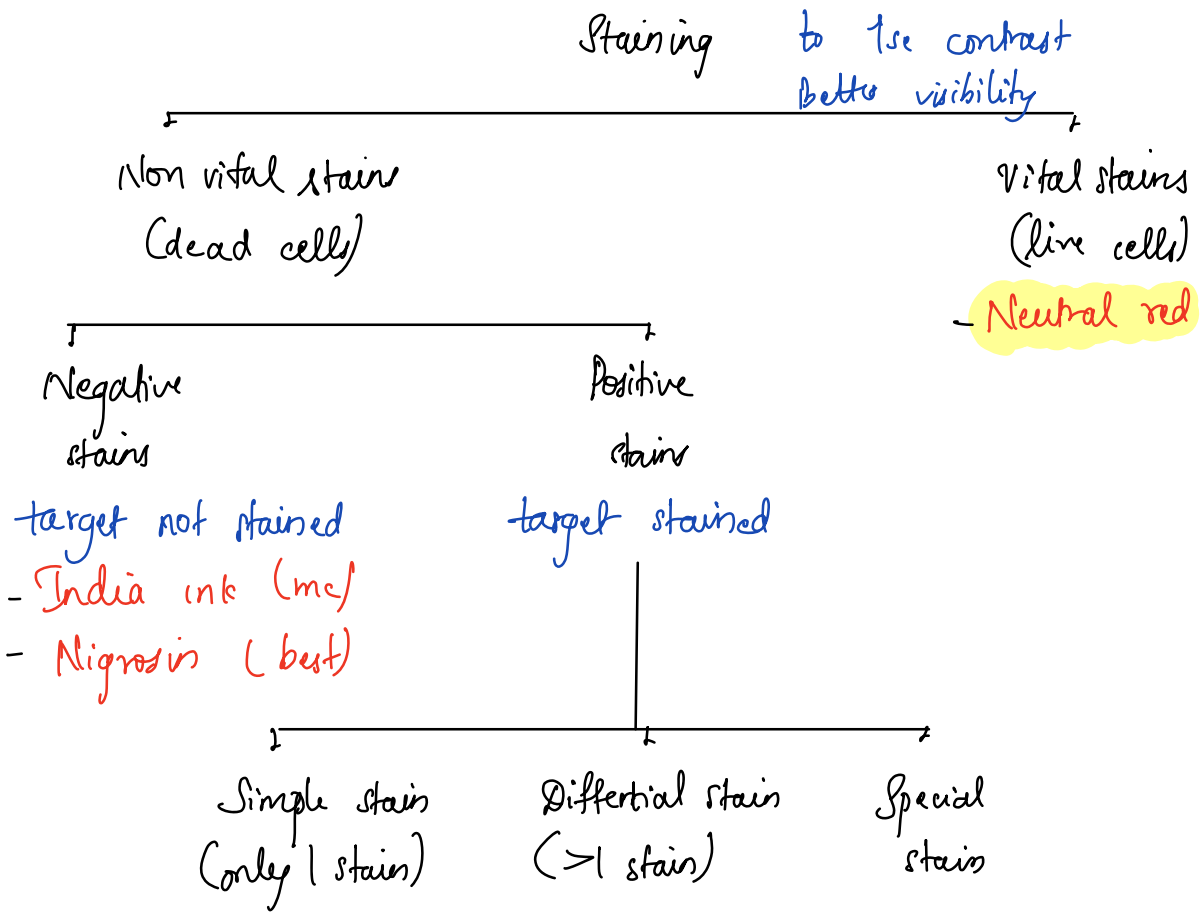
Electron beam

Electrons

- Electron microscope

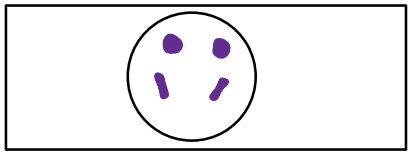
scattering of e^-
(shadow casting)

Microscopy


Staining
to use contrast
Better visibility
Non vital stain
(dead cells)
Vital stains
(live cells)
- Neutral red
Negative stains
Positive stains
target not stained
- India ink (mc)
- Nigrosin (best)
target stained
Simple stain
(only 1 stain)
Differtial stain
(>1 stain)
Special stain
- Methylene Blue
- Gram stain
- ZN stain
- Albert stain - metachromatic granules
- Bipolar stain
(Wayson stain)
- Silver impregnation method
(thin structures like fimbriae)

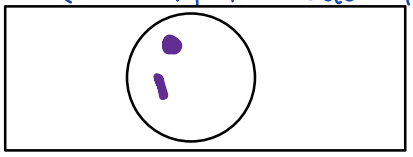
Gram stain

I : 1° stain – Crystal violet
(1 min) Methyl violet
Gentian violet



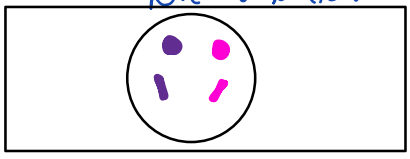
II : Mordant - Gram's Iodine
(penetration of stain inside)
(1 min)

III : Decolorizer - Acetone (2-3 sec)
(10 sec) Acetone alcohol



Gram +ve thick cell wall
∴ not decolorized

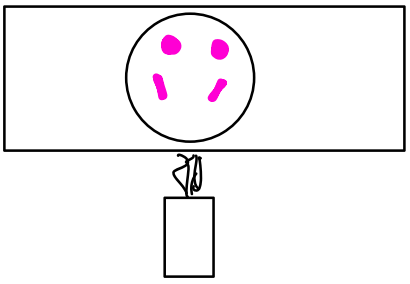
IV : 2° stain - Safranin
(1 min) Dil carbolfuschin



Gram +ve Gram -ve

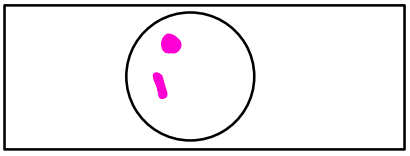
ZN stain

I : 1° stain : Conc. Carbolfuschsin
(5 mins)



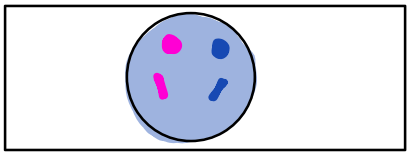
Mordant : Heating
till fumes arises

II : Decolorizer - H_2SO_4
(1 min)



Acid fast - mycolic acid
∴ not decolorized

III : 2° stain - Methylene Blue
(1 min)



background Blue
∴ only acid fast seen

Modified ZN stain

- Absolute methanol (fixation)
- Kinyoun's carbol fuchsin (1° stain)
- Acid alcohol (decolorizing agent)
- Methylene blue / Malachite green (counter stain)

Stains

Gram stain	First-line lab test in bacterial identification. Bacteria with thick peptidoglycan layer retain crystal violet dye (gram ⊕); bacteria with thin peptidoglycan layer turn red or pink (gram ⊖) with counterstain. These bugs do not Gram stain well (These Little Microbes May Unfortunately Lack Real Color But Are Everywhere):	
	Treponema, *Leptospira*	Too thin to be visualized
	Mycobacteria	Cell wall has high lipid content
	Mycoplasma, *Ureaplasma*	No cell wall
	Legionella, *Rickettsia*, *Chlamydia*, *Bartonella*, *Anaplasma*, *Ehrlichia*	Primarily intracellular; also, *Chlamydia* lack classic peptidoglycan because of ↓ muramic acid
Giemsa stain	*Chlamydia*, *Rickettsia*, Trypanosomes A, *Borrelia*, *Helicobacter pylori*, *Plasmodium*	Clumsy Rick Tripped on a Borrowed Helicopter Plastered in Gems
Periodic acid–Schiff stain	Stains glycogen, mucopolysaccharides; used to diagnose Whipple disease (*Tropheryma whipplei* B)	PaSs the sugar
Ziehl-Neelsen stain (carbol fuchsin)	Acid-fast bacteria (eg, *Mycobacteria* C, *Nocardia*; stains mycolic acid in cell wall); protozoa (eg, *Cryptosporidium* oocysts)	Auramine-rhodamine stain is more often used for screening (inexpensive, more sensitive)
India ink stain	*Cryptococcus neoformans* D; mucicarmine can also be used to stain thick polysaccharide capsule red	
Silver stain	Fungi (eg, *Coccidioides* E, *Pneumocystis jirovecii*), *Legionella*, *Helicobacter pylori*	
Fluorescent antibody stain	Used to identify many bacteria, viruses, *Pneumocystis jirovecii*, *Giardia*, and *Cryptosporidium*	Example is FTA-ABS for syphilis