

Microbiology

Marrow Edition 8

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Instructions

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MICROSCOPES

----- Active space -----

Types and Features

00:00:15

- Resolution
- Naked eye : 0.2mm
 - Light microscope : 0.2 μ m
 - Electron microscope : 0.2 nm

LIGHT/BRIGHT FIELD MICROSCOPE

General features :

AKA : Compound microscope.

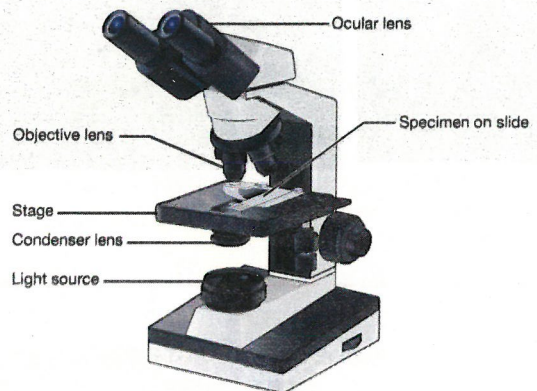
Components :

- Light source : Visible light (Wavelength : 550nm).
 - Objective lens.
 - Ocular lens.
- } magnification.

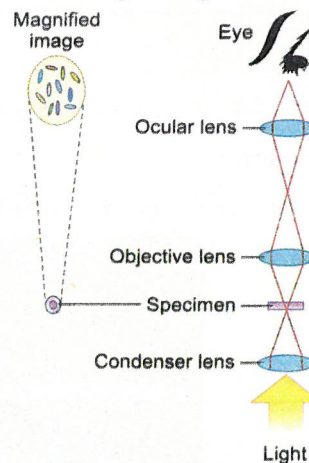
Resolution of light microscope :

- Resolution : 0.2 μ m.
- Depends on
 - Wavelength of light.
 - Numerical aperture of objective lens.

magnification of light microscope :



Light/bright field microscope



Ray diagram of light microscope

Lens	magnification of objective lens	magnification of ocular lens	Final magnification
Scanner lens	4x	10x	40x
Low dry lens	10x		100x
High dry lens	40x		400x
Oil immersion lens	100x		1000x

DARK FIELD MICROSCOPE

General features :

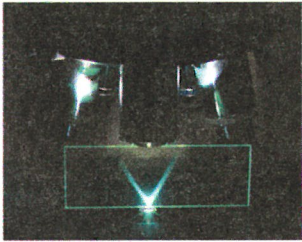
Advantages over light microscope :

1. unstained/transparent specimen : **Live organism.**
2. Slender bacteria visible.

----- Active space ----- modification :

Condenser lens has **central opaque light stop** :

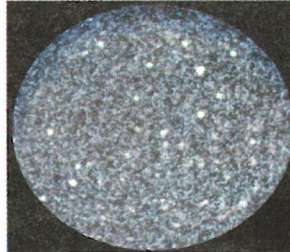
- Illuminates specimen with a hollow cone of light.
- Organism brightly lit against bright background.



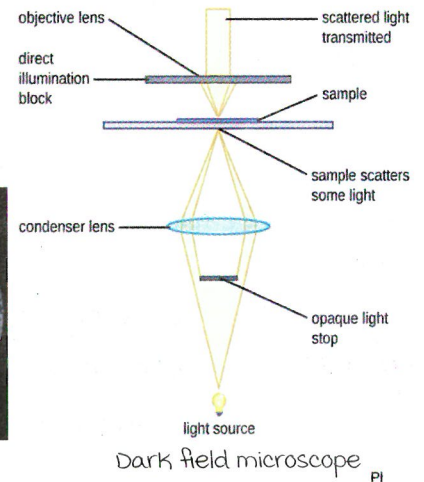
Hollow cone of light



T. pallidum under dark field microscope



Leptospira



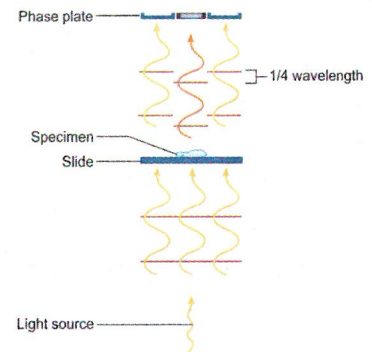
PHASE CONTRAST MICROSCOPE

General features :

Advantages over light microscope :

1. unstained/transparent specimen : **Live organisms.**
2. 3D effect and better contrast of internal structures.

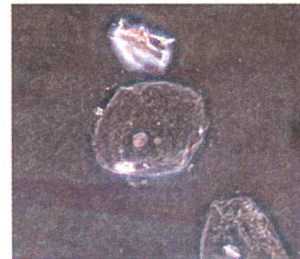
Principle : **Difference in amplitude of light** → **Differing light intensities.**



Ray diagram



Light microscope



Phase contrast microscope

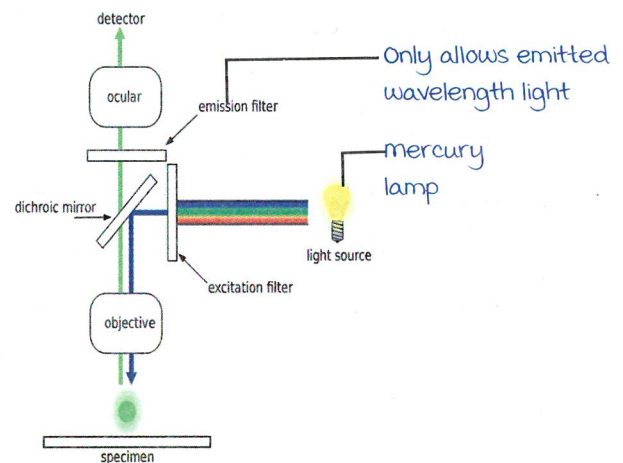
FLUORESCENT MICROSCOPES

Fluorophores :

- Excitation wavelength : Short wavelength (uv rays).
- Emitted wavelength : Longer wavelength (visible light).

Examples :

- Calcofluor white : Binds to chitin (Fungal cell wall).
- Acridine orange : Binds to DNA.



Ray diagram of fluorescent microscope

- Auramine O
 - Rhodamine B
 - Fluoresceine isothiocyanate
 - Lissamine rhodamine
- Active space -----

Binds mycolic acid (mycobacterial cell wall)

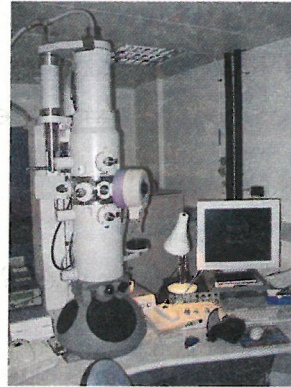
Binds antibodies (F)

ELECTRON MICROSCOPE

Introduced by Knoll and Ruska

Components :

- Beam of electrons used (Wavelength : 0.005 nm).
 - Only dead organisms visualised.
 - Condensed lens
 - Objective lens
 - Projector lens
- magnetic lens



Electron microscope

Types :

	Scanning electron microscope	Transmission electron microscope
Stain	vapourised gold/palladium → secondary electrons released	uranyl acetyl/Osmium tetroxide
mechanism	Secondary electrons create image	Variation in energy of transmitted electrons create image
uses	Study of topography of microorganisms	Study internal structures of organisms
microscope		
Image seen		

----- Active space -----

BACTERIAL STAINS

Reason for staining bacteria :

- Bacteria are colourless.
- Too small to be visualized.
- To provide contrast for better visualization.

Types of stains :

- Simple stains :
 - Single dye is used for staining → Every structure is stained uniformly.
 - Examples :
 - a. methylene blue : Structures appear blue.
 - b. Basic fuchsin : Structures appear pink.
- Differential stains.
- Negative stains.
- Impregnation stains.
- Special stains.

Differential Stains

00:01:40

1. GRAM STAIN

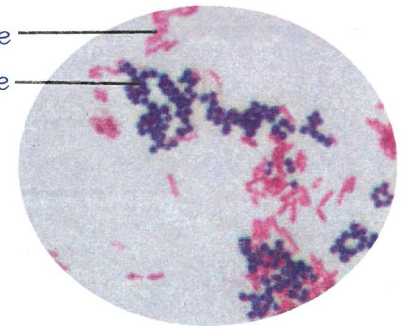
- m/c used stain in bacteriology.
- Introduced by : Hans Christian Gram.

Gram negative

Gram positive

Steps :

1. Primary stain : Crystal violet/methyl violet/gentian violet.
2. Mordant : Iodine.
 - Fixes primary stain to the structures.
3. Decolouriser : Alcohol/acetone/alcohol-acetone mixture.
 - Decolourises certain bacteria which take up primary stain.
4. Counterstain : Neutral red/carbol fuchsin/safranin (m/c used).
 - Counterstains bacteria that had undergone decolourisation.



Gram staining

Effects :

	Gram positive	Gram negative
Primary stain	Purple	Purple
Mordant	Purple	Purple
Decolouriser	Purple	Colourless
Counterstain	Purple	Pink

Note : Smear prepared is fixed by heating (kill all bacteria).

Principle :

1. Gram positive cytoplasm is more acidic → Better binding to primary basic stains → Slower decolourisation.
2. Gram positive cell wall is thicker → Better retaining of primary stain → Slower decolourisation.
3. Gram negative cell wall : Lipid composition is more → more pore formation while adding acetone/alcohol → Rapid decolourisation.

----- Active space -----

Exceptions :

Bacteria	Reason for not taking Gram's stain
mycoplasma	Small in size
Chlamydia Rickettsia	Small in size & obligate intracellular organism
mycobacteria	Lipid rich cell wall (Reagents cannot penetrate)
Spirochetes	Too slender

Note : Cytoplasm is stained (Not the cell wall).

2. ACID FAST STAINS

ZN staining :

- Introduced by : **Paul Ehrlich** (Father of chemotherapy).
- modified by : **Ziehl and Neelson**.
- used to demonstrate mycobacterium.

Steps :

1. Primary stain : Carbol fuchsin (Basic fuchsin dissolved in phenol).

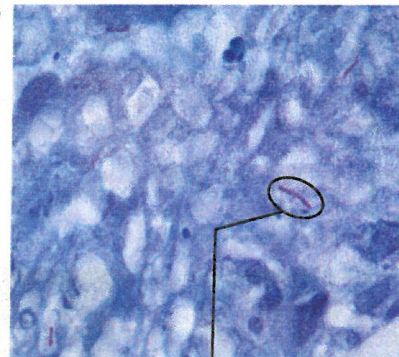
- Heat for 3-5 minutes : Helps carbol fuchsin to enter through cell wall to stain cytoplasm.

2. Decolouriser : 20% H_2SO_4 .

- Retain primary stain : Acid fast.
- Do not retain primary stain : Non acid fast.

3. Counterstain : methylene blue/malachite green/picric acid.

- To demonstrate non acid fast structure.



Acid fast bacilli
(mycobacterium tuberculosis)

----- Active space ----- Effects :

	Acid fast	Non acid fast
Primary stain	Pink	Pink
Decolouriser	Pink	Colourless
Counterstain	Pink	Blue/green/yellow (Based on counterstain)

3. Kinyoun stain :

- AKA cold stain/Gabbet stain.
 - modification of Ziehl Neelson stain: No heating post primary stain.
 - ↑ concentration of phenol in carbol fuchsin
 - ↑ time of exposure to carbol fuchsin
- } Counter omission of heating.

Acid fast v/s partially acid fast :

	Acid fast	Partially acid fast
Features	Acid fast with 20% H_2SO_4	Retain primary stain with lower concentration of H_2SO_4
Example	• mycobacterium tuberculosis complex : - mycobacterium tuberculosis - mycobacterium bovis - mycobacterium africanum - mycobacterium caprae • Atypical mycobacteria	5% H_2SO_4 : • mycobacterium leprae • Oocyst of cystoisospora, cyclospora & cryptosporidium (Protozoans causing diarrhoea in HIV positive pts.) 0.5-1% H_2SO_4 : • Nocardia • Head of sperm • Rhodococcus • Legionella micdadei 0.25%-0.5% H_2SO_4 : • Bacterial spores

Negative Stain

00:18:15

Outlining the structures against the dark background of India ink/nigrosine.

Uses :

- Demonstrate capsules of organism.
- Demonstrate very slender bacteria : Spirochetes.



Negative staining of T. pallidum.



Cryptococcus neoformans

Yeast budding from one side

Impregnation Staining

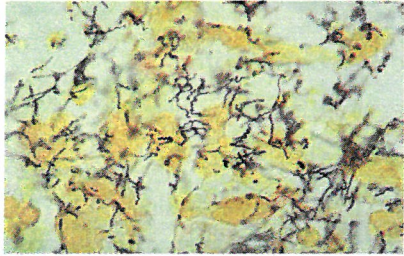
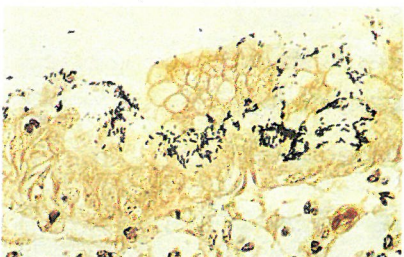
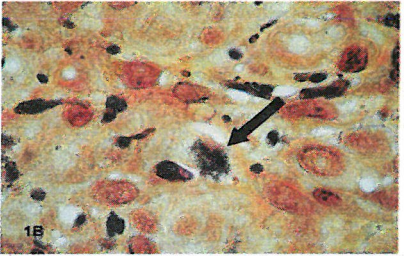
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----- Active space -----

Principle : Impregnating organism → Organism become thick enough to be visualized by light microscope.

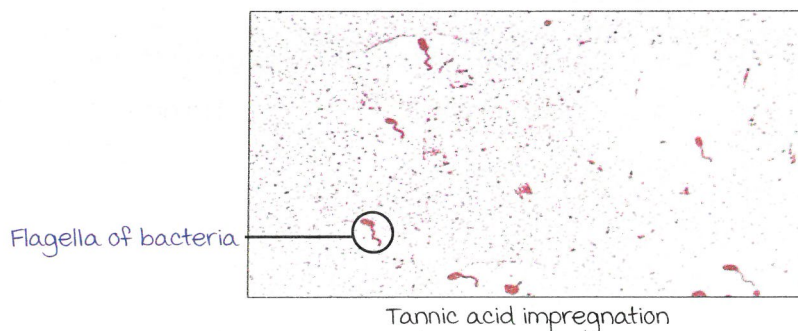
1. Silver stain :

Use :

Organism	Stains used	Image
Spirochetes	- Levaditi stain (Tissue) - Fontana stain (Fluid)	 Spirochetes
Helicobacter pylori	Warthin starry silver stain (Gastric biopsy)	 Helicobacter pylori
Bartonella	Warthin starry silver stain (Lymph node/vascular lesion biopsy)	 Bartonella

2. Tannic acid :

- Stain : Leifson and Ryu impregnation stain.
- use : Demonstrate flagella of bacteria.



Special Stains

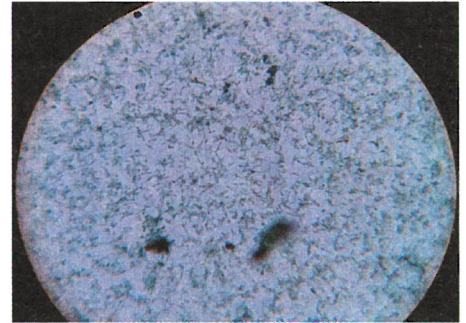
00:22:16

metachromatic granules :

- AKA volutin granules/Babes Ernst granules/polar bodies.
- Act as energy stores.
- metachromasia : Organisms take up colour other than colour of stain used.
 - For eg : Purple red colour when stained with toluidine blue.

Seen in :

- *Corynebacterium*.
- *Yersinia pestis*.
- *Bordetella pertussis*.
- *Gardnerella vaginalis*.
- Certain *Mycobacterium* species.



Albert's stain

Stains used :

- Albert's stain : Toluidine blue + malachite green + iodine.
- Neisser's stain.
- Ponder's stain.

Spores :

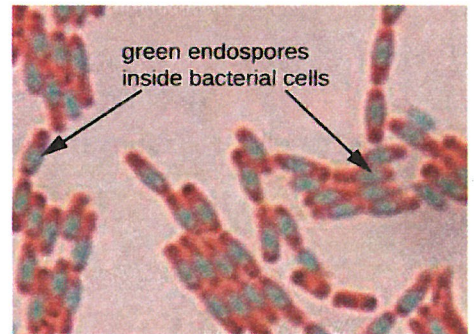
Do not take up gram stain (D/t thick cell wall) : Appear as empty spaces.

Seen in :

- *Bacillus*.
- *Clostridium*.

Stain used :

- Ashby staining : malachite green & safranin are dyes used.
- Schaeffer-Fulton stain (modification of Ashby stain).



Ashby staining

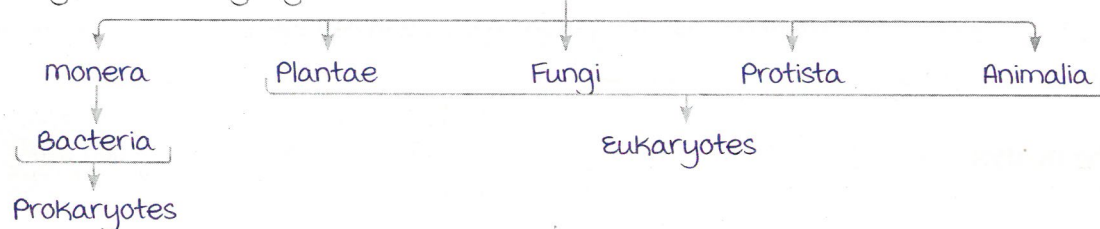
Lipid/polysaccharide granules :

	Lipid granule	Polysaccharide granule
Stain used	Sudan black B	Iodine

BACTERIAL ANATOMY AND TYPING

----- Active space -----

Kingdoms of living organisms :



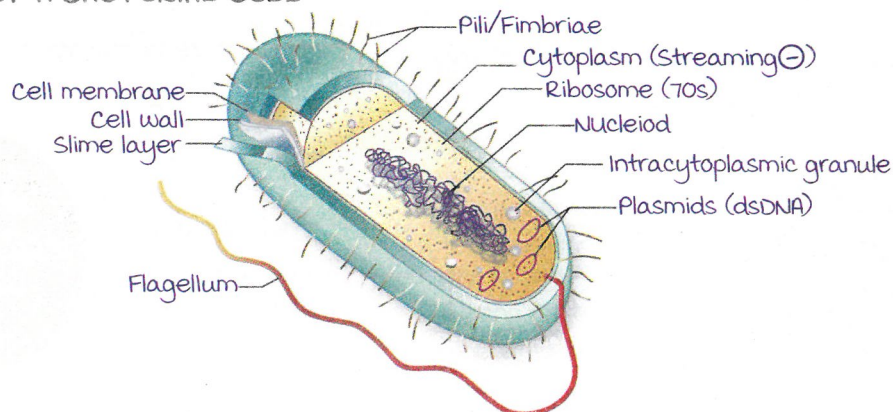
Prokaryotes

00:02:12

PROKARYOTES VS. EUKARYOTES

Parameter	Prokaryotes	Eukaryotes
1. Nuclear membrane, nucleolus and histone proteins	⊖	⊕
2. Cytoplasmic membrane-bound organelles (mitochondria, golgi body, lysosomes, endoplasmic reticulum).	⊖	⊕
3. Chromosome	Single, circular (dsDNA) contained within nucleoid.	multiple, linear.
4. Muramic acid in cell wall	⊕	⊖
5. Sterols in cell membranes	⊖	⊕
6. Ribosomes	70S 30S 50S	80S 40S 60S
7. Extrachromosomal DNA	Plasmids	mitochondria

STRUCTURE OF A BACTERIAL CELL



----- Active space -----

Exceptions :1. *Mycoplasma* :

- Only prokaryote → Lacking a cell wall.
- Containing sterols in cell membrane.

2. *Chlamydia* : Only prokaryote lacking muramic acid in cell wall.3. *Vibrio* : Contains 2 chromosomes.**Glycocalyx**

00:11:30

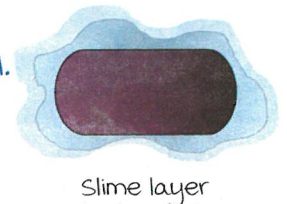
may be present/absent.

TYPES**a. Slime layer :**

Loose, ill-defined, polysaccharide layer all around the cell wall.

Significance : Biofilm formation :

- Antiphagocytic
- Adhesion
- ↓ Antibiotic activity (mechanical barrier).

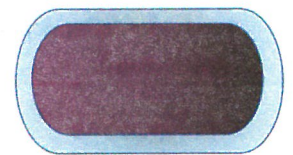


Examples :

- *P. aeruginosa*
- *Streptococcus mutans* (m/c cause of dental caries).
- *Staphylococcus epidermidis*.

b. Capsule :

- Well demarcated layer around cell wall.
- Composed of polysaccharides.
 - Exception : *B. anthracis* → polypeptide capsule (Polymer of D-glutamic acid).

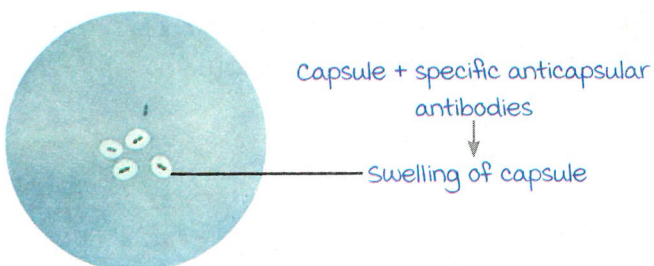


Significance :

- **Antiphagocytic** : most important virulence factor.
- Protection from phages (viruses that infect bacteria).
- Antigen : Induces antibody formation.

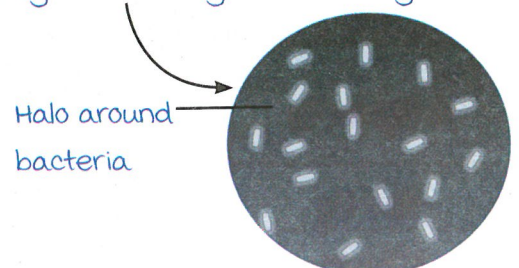
Gram stain : Not done (No net change).

Quellung/Neufeld reaction :



Capsule demonstration :

Negative staining (India ink/Nigrosine).



----- Active space -----

Capsulated organisms :

*Yersinia pestis**Streptococcus pneumoniae**Bacteroides fragilis*, *Bordetella pertussis*.*H. influenzae*.*Klebsiella*.*Bacillus anthracis*.*Meningococcus*.*Clostridium perfringens*.

Mnemonic :

Yes

Some

Bacteria

Have

Killer

And

mean

Capsules

Note :

1. Negative staining also used for the demonstration of *spirochetes*.2. Only capsulated fungus : *Cryptococcus*.

Cell Wall

00:19:24

Function : Provides shape and rigidity.

Composition : **Peptidoglycan**.

murein backbone (Carbohydrate) linked by peptides.

made up of 2 amino acids :

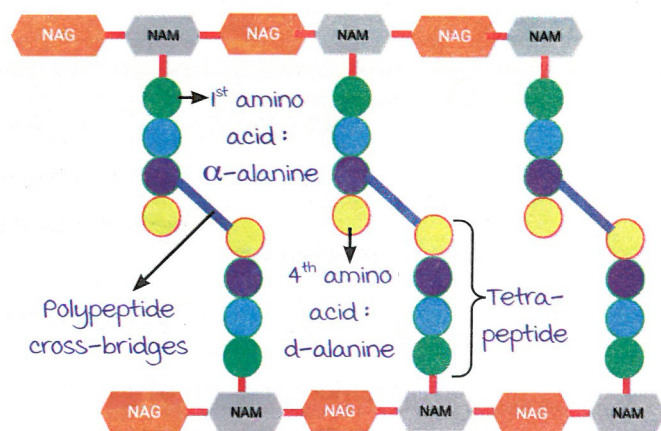
1. N-acetyl muramic acid (NAM)

2. N-acetyl glucosamine (NAG)

Cross linking of cell wall : mediated by

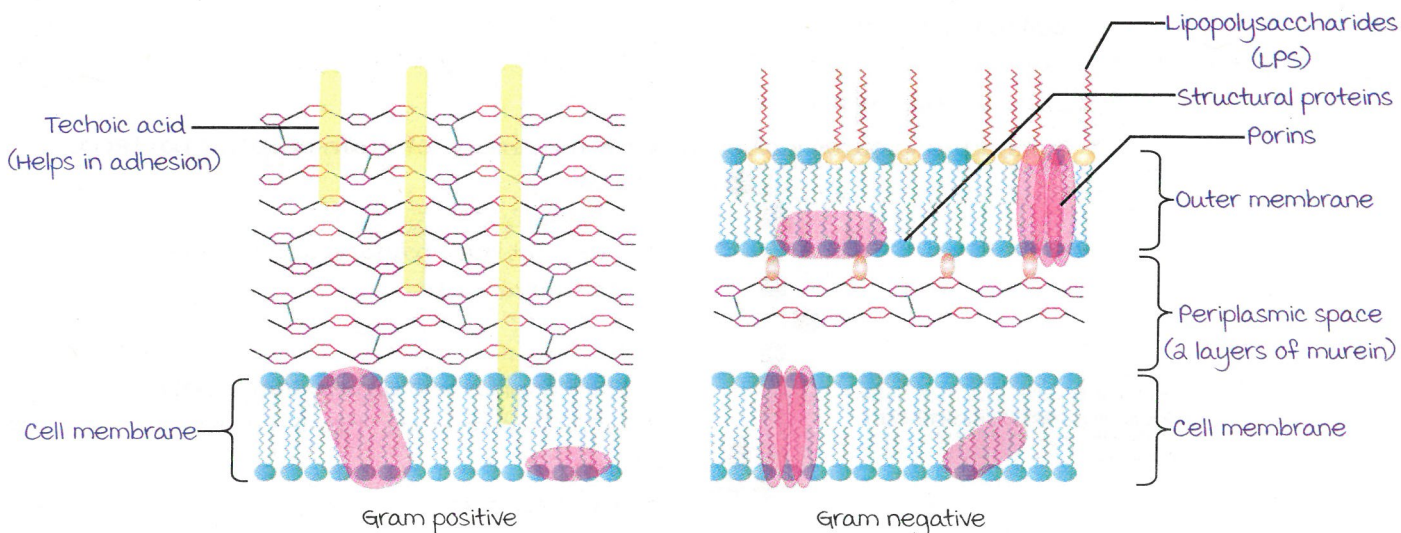
Transpeptidase

Transglycolase



Structure of a cell wall

GRAM POSITIVE VS. GRAM NEGATIVE



----- Active space -----

	Gram positive	Gram negative
1. Thickness	25-80nm	10nm
2. murein layers	50-100 layers, peptide cross linking $\oplus$	2 layers, peptide crosslinking $\oplus$
3. Amino acids (AA)	Aromatic & sulfur containing AA $\ominus$	All types of AA $\oplus$
4. Techoic acids (TA)	2 types $\oplus$: • Lipo TA • Cell wall TA	$\ominus$
5. Outer membrane	$\ominus$	$\oplus$
6. Porins		
7. Periplasmic space		
8. Endotoxins (LPS)		

Antibiotics acting on the cell wall :

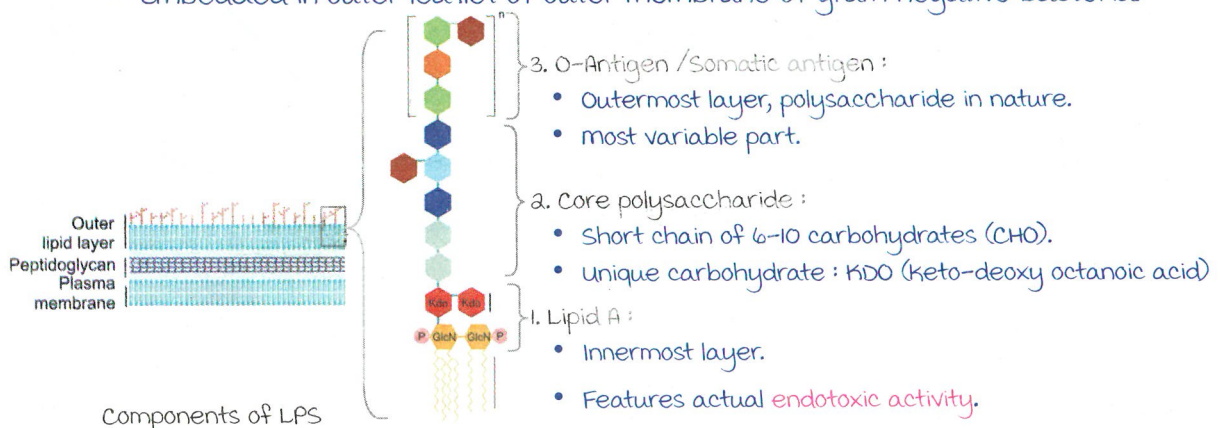
Antibiotic	β -lactams	vancomycin
mechanism	Bind to transpeptidase (AKA penicillin binding protein : PBP). ↓ Inhibit crosslinking $\rightarrow$ Lysis	Bind to D-ala D-ala moieties (cell wall precursor molecules). ↓ Disrupts action of transpeptidase

Demonstration of cell wall :

1. Electron microscope.
2. microdissection.
3. Plasmolysis : Bacteria placed in hypertonic solution $\rightarrow$ Water moves out, only cell wall remains.

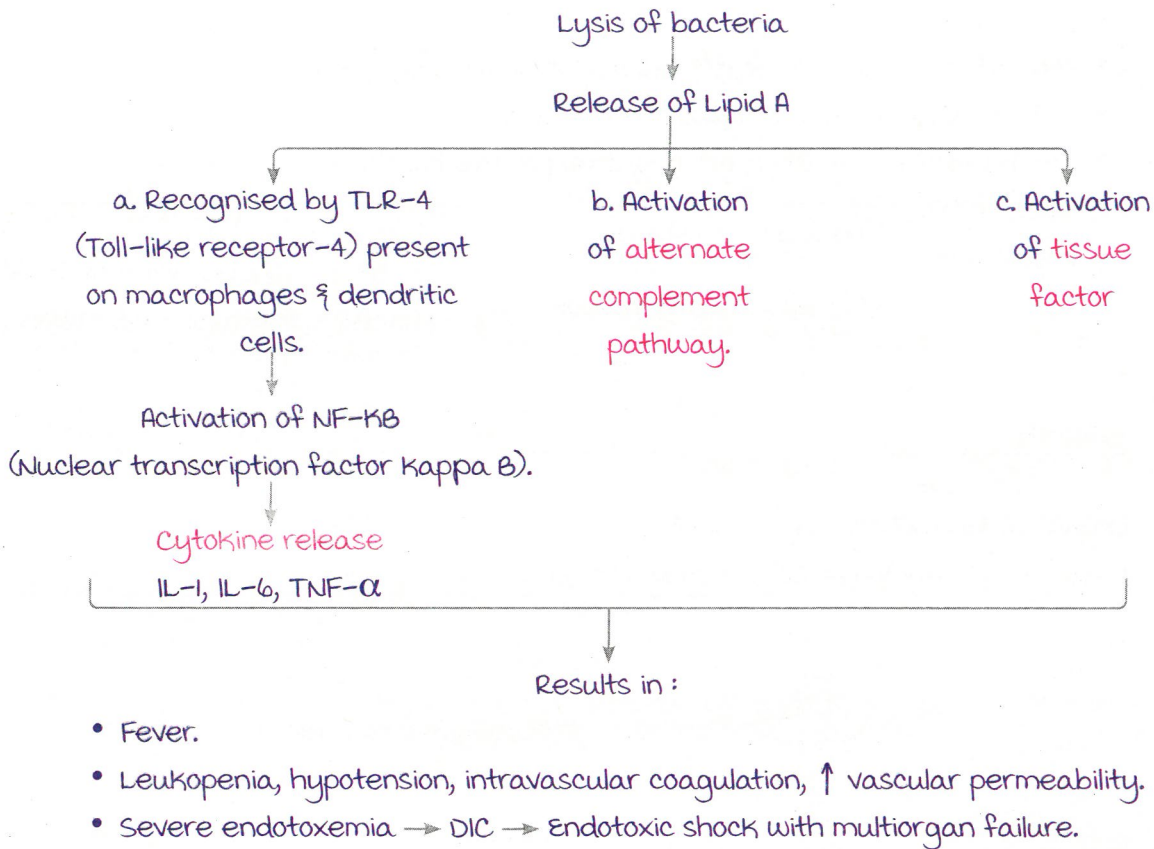
Endotoxin/Lipopolysaccharides (LPS) :

- Embedded in outer leaflet of outer membrane of gram negative bacteria.



mechanism of action :

----- Active space -----



Detection of endotoxin :

1. Rabbit pyrogenicity.
2. Limulus amoebocyte lysate (LAL) assay.

Note :

- | | |
|---------------|---|
| H. influenzae | } • Lipo-oligosaccharide ⊕
• No LPS. |
| Neisseria | |

Endotoxin Vs. Exotoxin :

	Endotoxin	Exotoxin
Nature	LPS	Protein.
Site of release	Present in cell wall of gram negative bacteria.	Secreted by either gram positive/negative.
Mechanism of release	Released only on lysis.	Actively secreted. Exception : Botulinum toxin → released on lysis.
Effect of heat	Heat stable.	Heat labile. Exception : S. aureus enterotoxin.
Antigenicity	Low antigenicity	Highly antigenic (Protein). Can be used for synthesis of toxoid.
Toxicity	Low toxicity	Highly toxic.
Effects	Constant effects.	Variable actions.

----- Active space -----

L-forms :

- Cell wall deficient bacteria.
- Formed spontaneously or d/t cell wall inhibitors (Eg : penicillin).
- 1st demonstrated on streptobacillus moniliformis.
- Can be formed by any gram negative/positive bacteria.
- Pleomorphic.
- Importance :
 - Antibiotic resistance
 - Persistence of infections.

Note :

Listeria → Only gram positive bacteria containing endotoxin.

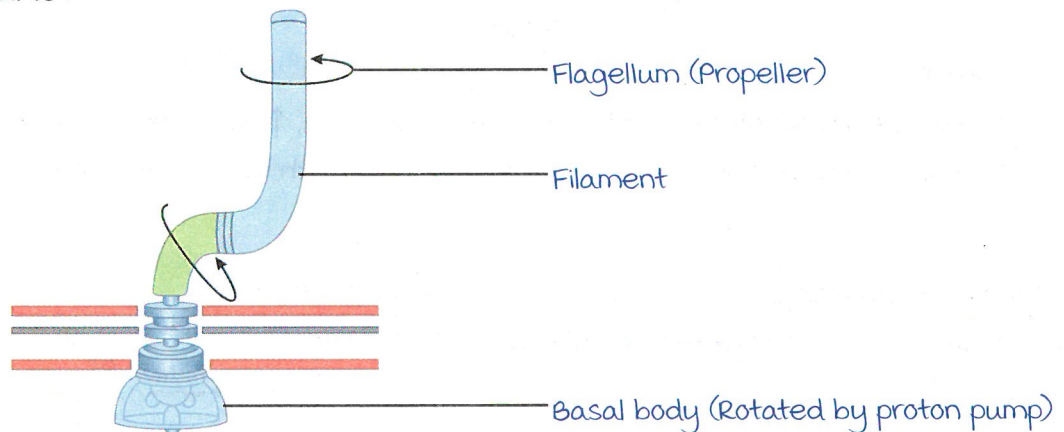
Flagella

00:48:11

Organs of locomotion, help in motility.

made up of flagellin protein : Highly antigenic → H. antigens.

Parts :



Distribution :

monotrichous	Lophotrichous	Amphitrichous	Peritrichous
Vibrio P. aeruginosa	Helicobacter	Campylobacter Spirillum	Enterobacteriaceae Clostridium Bacillus

Note :

- WIDAL test : Detection of 'H' and 'O' antibodies.
- All pathogenic cocci are atrichous.