

Microbiology

Edition 8.5

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Contents

General Microbiology

Introduction to General Microbiology	1
Diagnostic methods • Antimicrobial susceptibility testing (AST)	
Pioneers in Microbiology	5
Leeuwenhoek, Pasteur & Koch • Ehrlich, Lister & Semmelweis	
Morphology & Growth	7
Capsule • Cell wall • Fimbria & flagella • Growth	
Microscopy & Staining	11
Bright field/light microscope • Darkfield & phase contrast microscope • Fluorescent & electron microscope • Staining • Gram stain • Modified acid fast/ZN stain • Special stains	
Bacterial Culture Media & Biochemical Tests	16
Culture media : based on constituents • Culture media: based on O ₂ requirement & consistency • Biochemical tests	
Genetics	22
PCR (polymerase chain reaction) • Mutation • Gene transfer	
General Bacteriology	26
Classification based on morphology & staining • Lab diagnosis • Other classifications	
General Mycology	30
Morphological classification • Fungal disease classification • Lab diagnosis of fungal infections • Fungal spores and invasive markers	
General Virology	35
DNA/RNA virus classification • Morphology, receptors, capsid symmetry, nucleic acid • Lab diagnosis & culture identification • Direct identification & quantification of virus	
General Parasitology	42
Protozoa • Protozoa : lab diagnosis • Protozoa : life cycle & other classification • Helminths • Helminths : lab diagnosis • Helminths : life cycle & manifestations	

Hospital Infection Control

Hand Hygiene & Personal Protective Equipments	48
Hand hygiene • Personal protective equipments (PPE)	
Biomedical Waste Management	51
Management of biomedical waste	

Sterilisation & Disinfection

53

Sterilisation • Dry heat • Moist heat • Gas & plasma • Filtration & radiation •
Disinfection • Tests for disinfection • Spaulding's classification of medical devices

Immunology

Immunity

60

Acquired immunity

Antigen and Antibody

63

Antigen : properties & examples • Complete & incomplete antigens •
T-cell dependent & independent antigens • Super & heterophile antigens •
Antibody : structure • Enzyme action on antibody • Antigenic determinants of antibody •
Antibody formation • Antibody isotypes

Antigen-Antibody Reactions

69

Marrack's lattice hypothesis • Precipitation/flocculation reaction •
Agglutination reaction • Neutralization reactions • Immunoassays

Complement System

73

Complement pathways • Complement deficiency disorders (CDD)

Immunodeficiency Disorders

75

Cellular immunodeficiency disorders • Humoral immunodeficiency disorders •
Combined immunodeficiency disorders • Phagocytic immunodeficiency disorders •
Lab diagnosis & treatment

Hypersensitivity

79

Type I & II hypersensitivity • Type III & IV hypersensitivity

Transplant Immunology

82

Types of graft & graft rejection • Graft vs. Host disease (GVHD)

Central Nervous System

Introduction to Central Nervous System

84

Clinical presentation • Management: meningitis

Meningitis

86

Bacterial meningitis : classification &
etiopathogenesis • Pneumococcus • Meningococcus •
Hemophilus influenzae-b (HIB) • Listeria • Viral meningitis : Etiology •
Mumps virus • West Nile virus • Zika virus

Viral Encephalitis

95

Japanese encephalitis virus • Nipah virus • Rabies virus • Rabies : prophylaxis

Parasitic Encephalitis

100

Naegleria fowleri • Acanthamoeba • Balamuthia • Sappinia

Slow Viral & Prion Diseases

105

Subacute sclerosing panencephalitis (SSPE) •
Progressive multifocal leucoencephalopathy (PML) • Creutzfeldt-jakob disease (CJD)

Poliomyelitis	108
Clinical presentation • Management & prevention • VAPP vs. VDPV	
Neurotoxin Related Disorders	110
Botulism • Tetanus • Prophylaxis of tetanus	
Cardiovascular System	
Introduction to Cardiovascular System	114
Infective Endocarditis	115
Etiology & types • Clinical manifestations & diagnosis • Treatment	
Acute Rheumatic Fever	119
Pathogenesis & clinical manifestations • Diagnosis • Treatment & prophylaxis	
Bacterial Zoonotic Bloodstream Infections	122
Brucellosis • Leptospirosis	
Bacterial Vector-Borne Bloodstream Infections	125
Yersinia pestis • Rickettsia & orientia • Coxiella, ehrlichia & bartonella	
HIV Disease/AIDS	130
General characteristics of HIV • Clinical manifestations • Lab diagnosis • Treatment	
Viral Hemorrhagic Fever	134
Flaviviridae: kyasanur forest disease (KFD) virus • Flaviviridae: dengue virus • Flaviviridae : yellow fever virus • Arenaviridae, bunyaviricetes & filoviridae	
Blood & Tissue Flagellate Infections	138
Leishmaniasis • American trypanosomiasis (chagas disease) • African trypanosomiasis (sleeping sickness)	
Blood & Tissue Sporozoan Infections	143
Malaria • Babesiosis • Toxoplasmosis	
Schistosomiasis	151
Etiology • Clinical manifestation • Management	
Filariasis	153
Lymphatic filariasis • Management of lymphatic filariasis • Subcutaneous filariasis	
Respiratory System	
Introduction to Respiratory System	157
Pneumonia	
Bacterial Typical Pneumonia	160
Pseudomonas • Burkholderia • Acinetobacter	

Bacterial Atypical Pneumonia	163
Chlamydia • Mycoplasma • Legionella	
Viral Pneumonia	167
Influenza virus • Corona virus • Adenovirus	
Systemic Fungal Pneumonia	172
Histoplasmosis • Blastomycosis • Coccidiomycosis • Paracoccidiomycosis • Talaromycosis	
Opportunistic Fungal Pneumonia	177
Candidiasis • Cryptococcosis • Aspergillosis • Mucormycosis/zygomycosis • Pneumocystosis	
Parasitic Pneumonia	185
Tuberculosis	186
Etiology & manifestations • Diagnosis • Treatment • Prophylaxis	
Gastrointestinal System	
Atypical Mycobacterial Infections	190
Runyon's classification • Presentation & management	
Diphtheria and Pertussis	192
Diphtheria : etiology & clinical features • Diphtheria : diagnosis • Diphtheria : treatment & prophylaxis • Pertussis / whooping cough	
Bacterial Food Poisoning	196
Etiological agents • Clinical presentation & management	
Pseudomembranous Colitis	198
Pathogenesis & clinical manifestations • Diagnosis & treatment	
Escherichia Coli Diarrhoeal Disease	200
Etiology • Clinical manifestations • Management	
Enteric/Typhoid Fever	202
Clinical presentation • Diagnosis • Management	
Shigellosis/Bacillary Dysentery	205
Etiology & clinical features • Management	
Cholera	207
Vibrio • V. Cholerae O1 • V. Cholerae O139 & halophilic vibrio	
Campylobacteriosis	211
Peptic Ulcer Disease	212
Management	

Viral Gastroenteritis	214
Etiological agents • Rotavirus • Clinical manifestations & management	
Non-Acid Fast Protozoal Gastroenteritis	216
Balantidiasis • Amoebiasis • Giardiasis	
Acid-Fast Protozoal Gastroenteritis	221
Intestinal Cestodiasis	222
Taeniasis • Hymenolepiasis • Diphyllbothriasis	
Intestinal Trematodiasis	229
Etiological agent • Manifestations & management	
Intestinal Nematodiasis	231
Strongyloidiasis • Hookworm infection • Ascariasis • Enterobiasis • Trichuriasis	
Fungal Food Poisoning	240
Mycotoxicosis • Mycetism	
Hepatobiliary System	
Viral Hepatitis	241
Hepatitis A • Hepatitis B : HBV & clinical manifestations • Hepatitis B : Diagnosis, treatment & prophylaxis • Hepatitis C, D, E	
Hydatid Cystic Disease	247
Cystic echinococcosis: Etiology & manifestations • Cystic echinococcosis : Management • Alveolar echinococcosis	
Fascioliasis	249
General features • Clinical manifestations • Diagnosis & treatment	
Genitourinary System	
Genital Ulcerative Disease	251
Granuloma inguinale/donovanosis • Chancroid • Syphilis • Lymphogranuloma venereum • Herpes genital ulcerative disease	
Vaginitis	255
Bacterial vaginosis • Trichomoniasis • Vaginal candidiasis	
Urethritis	258
Urinary Tract Infection	260
Uropathogenic E. coli (UPEC) • Klebsiella pneumoniae • Proteus mirabilis • Citrobacter freundii • Enterococcus	

Skin, Subcutaneous and Musculoskeletal System

Staphylococcal Infections	264
Staphylococcus aureus • Lab diagnosis : Staph. Aureus • Treatment : Staph. Aureus • Coagulase negative staphylococcus	
Streptococcal Infections	269
Virulence factors • Clinical manifestations • Diagnosis & treatment	
Anthrax	273
Clinical manifestations • Management	
Gas Gangrene	276
Etiological agent • Clinical manifestations • Lab diagnosis • Treatment	
Leprosy	279
Clinical manifestations • Lab diagnosis & treatment	
Actinomycosis & Actinomycetoma	282
Actinomycosis : etiology & clinical manifestations • Management • Actinomycetoma : etiology & clinical features • Management	
Borreliosis	286
Etiology & presentation • Management	
DNA Viral Skin Infections	288
Human herpes virus (HHV) • HHV : affecting skin • HHV : affecting respiratory system • Pox virus • Human papilloma virus • Parvovirus	
RNA Viral Skin Infections	295
Measles virus • Rubella virus • Coxsackie virus	
Superficial Fungal Infections	297
Tinea versicolor • Piedra • Dermatophytosis/tinea • Management of dermatophytosis	
Subcutaneous Fungal Infection	302
Eumycetoma • Sporotrichosis • Chromoblastomycosis • Pheohyphomycosis	
Tissue Parasitic Infections	305
Trichinellosis • Dracunculosis	
Miscellaneous	
Congenital Infections	308
Manifestations of congenital infections • Management	
Bioterrorism Agents & Biosafety Levels	310
Bioterrorism agents • Biosafety levels	

INTRODUCTION TO GENERAL MICROBIOLOGY

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

Objective : To **diagnose & treat** infectious diseases.

Diagnostic Methods

00:02:45

Objective :

Detect infecting **organism/antibody** in patient → Diagnosis.

methods :

1. microscopic method.
2. Culture method.
3. molecular method.
4. Immunological method :
Only method that detects **antibodies**.



Light microscope

microscopy :

- Identifies **morphology & motility** of organism.
- E.g. : Comma-shaped bacteria with darting motility → *Vibrio cholerae*.



Vibrio cholerae :

Fish in stream appearance

Culture method :

- Culture : media with nutrients supporting growth.
- microorganisms grow & reproduce → Form colonies.

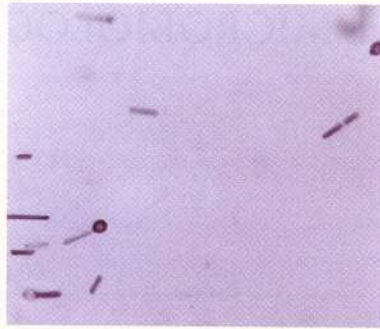
Identification :

- Spores ⊕ : **Culture microscopy**.
- E.g. : *Clostridium tetani* → Terminal spores (Drumstick appearance).
- Spores ⊖ : Perform **culture tests**.
- E.g. : Coagulase +ve → *Staphylococcus aureus*.

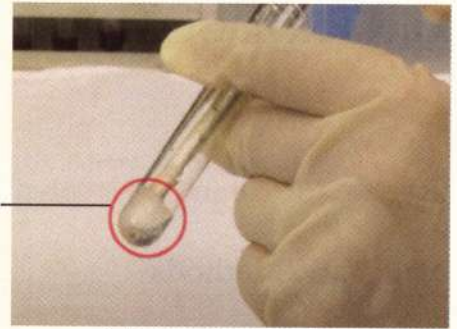


Colonies

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



C. tetani on microscopy



Coagulum

+ve coagulase test

molecular methods :

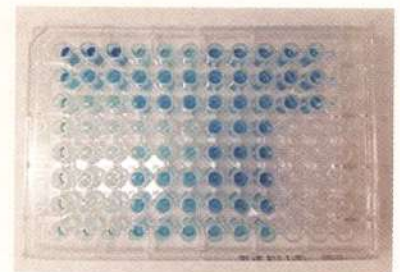
- Identify the genetic composition of organism.
- m/c : **Polymerase chain reaction (PCR)**.
- E.g. : Spike receptor gene → Coronavirus.



PCR

Immunological methods :

- Detect antigens/antibodies.
- m/c : **ELISA**.
- Detecting antigen :
Identify organism (**Antigens are unique**).
 - E.g. :
 - p24 Ag → HIV.
 - rk39 Ag → Leishmania.



ELISA

Antimicrobial Susceptibility Testing (AST)

00:16:07

Objective : To treat infectious diseases.

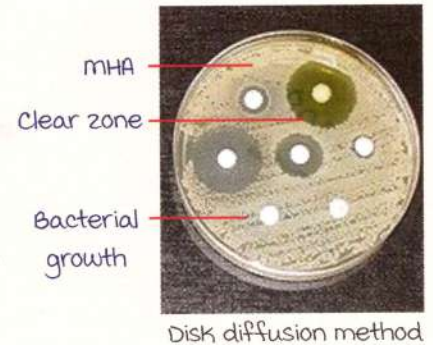
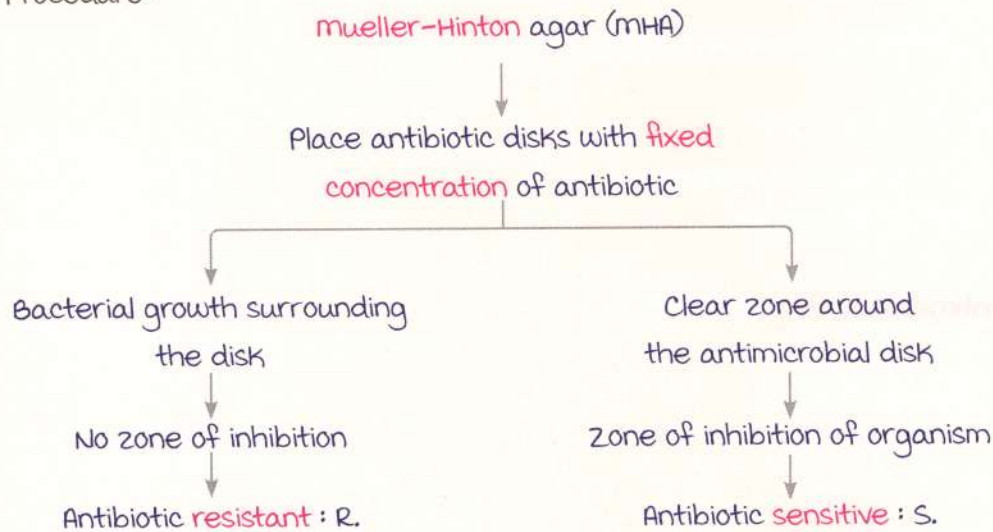
methods :

1. Phenotypic method/culture method :
 - Kirby-Bauer's disk diffusion.
 - Broth/agar dilution.
 - E-strip.
2. Genotypic method/molecular method : **PCR**.

Kirby-Bauer's Disk Diffusion method :

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

Procedure :

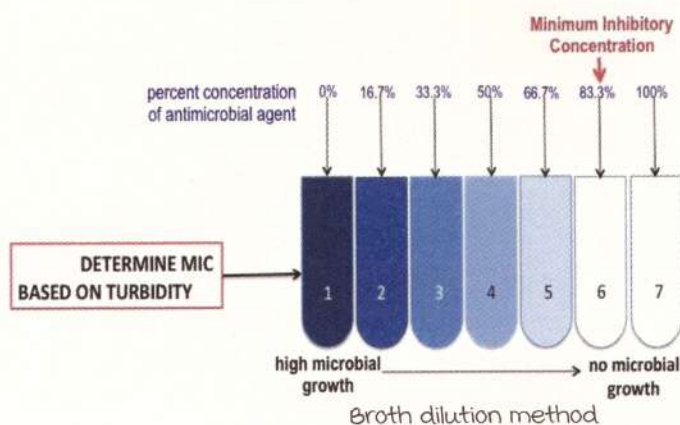
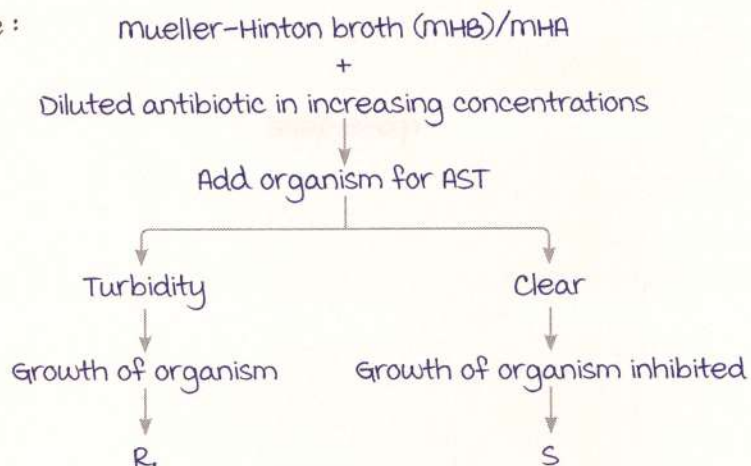


Disadvantage :

Minimum inhibitory concentration : Cannot be found.

Broth/Agar Dilution method :**Gold standard** method for AST : **Broth dilution method.**

Procedure :



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

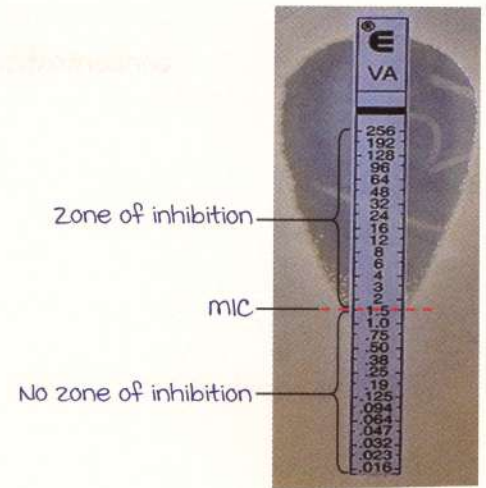
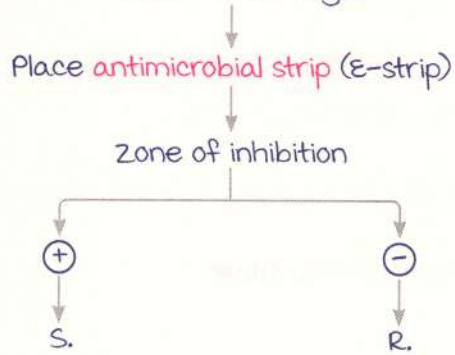
minimum inhibitory concentration (MIC):

minimum % of antibiotic at which organism is inhibited.

Advantage : Determine minimum inhibitory concentration.

Epsilometer-strip method:

- Similar to Kirby Bauer's method:
mueller-Hinton agar



ϵ -strip method

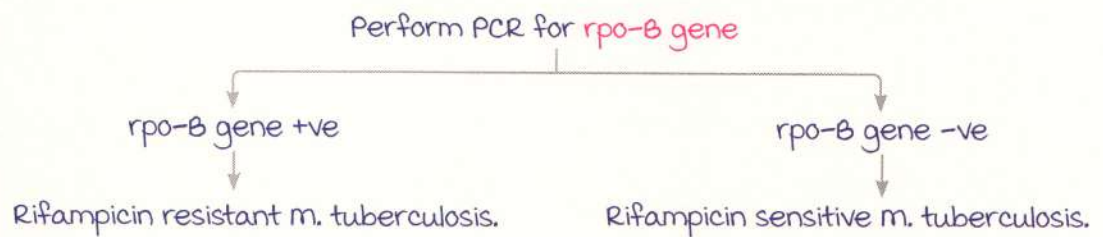
- MIC : Detected with increasing concentration.

PCR:

- Resistant gene

Detected : R.

Not detected : S.
- Example :



PIONEERS IN MICROBIOLOGY

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

Leeuwenhoek, Pasteur & Koch

00:03:18

Antonie Van Leeuwenhoek :

Father of microscopy/microbiology :
Invented simple microscope.



Antonie Van
Leeuwenhoek



Louis Pasteur

Louis Pasteur :

Father of microbiology/germ theory.

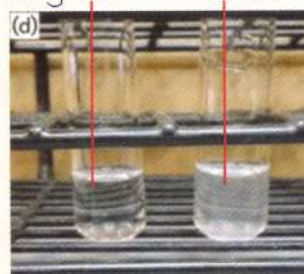
1. Germ theory of disease :
Diseases are caused by micro-organisms.

2. Liquid culture media (Broth) :
Demonstrated culture growth :
Indicated by turbid liquid.

3. Vaccines : CAR.

- a. Cholera.
- b. Anthrax.
- c. Rabies.

Clear : No growth Turbid : Growth



Liquid culture media.

Robert Koch :

Father of bacteriology & microbiology.

Solid culture media (Agar) :
Visualisation of colonies (Group of organisms
derived from one bacterium).



Colonies : Solid culture media.

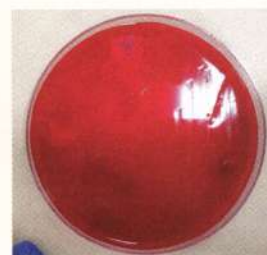
Staining :

Advantages :

↑ visibility of organism → morphology visualised better.

Hanging drop method :

To visualise characteristic motility.



Solid culture media

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

Discovered bacteria responsible for :

CAT :

- **C**holera : *Vibrio cholerae*.
- **A**nthrax : *Bacillus anthracis*.
- **T**B : *Mycobacterium tuberculosis*.

Ehrlich, Lister & Semmelweis

00:11:55

Paul Ehrlich :

Father of chemotherapy.

Salvarsan : Rx of syphilis.

Acid fast staining :

Later modified by Ziehl & Neelsen.

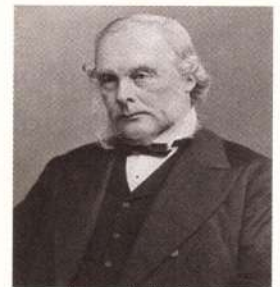


Paul Ehrlich

Joseph Lister :

Father of antiseptic surgery :

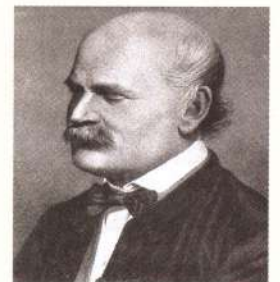
- Introduced surgical aseptic precautions.
- using disinfectant : Phenol (Carbolic acid).



Joseph Lister

Ignaz Semmelweis :

Father of infection control :

Introduced **surgical hand-washing**.

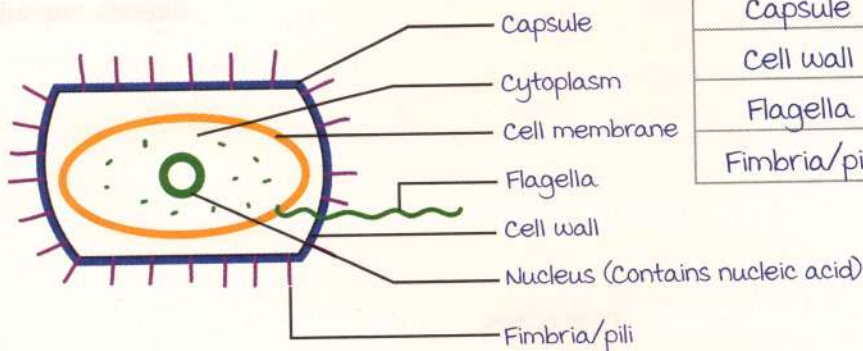
Ignaz Semmelweis

MORPHOLOGY & GROWTH

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

morphology of Bacteria :

Unicellular organisms.



Structure	Antigens
Capsule	K
Cell wall	O
Flagella	H
Fimbria/pili	F

Capsule

00:02:25

Compactly arranged glycocalyx.

Types :

Polysaccharide :

- Meningococcus.
- Pneumococcus.
- Klebsiella.
- Pseudomonas.
- Haemophilus influenzae-b.

Polypeptide :

Bacillus anthracis.

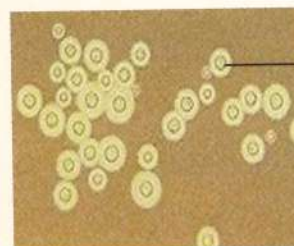
Hyaluronic acid :

Streptococcus pyogenes.

Demonstration :

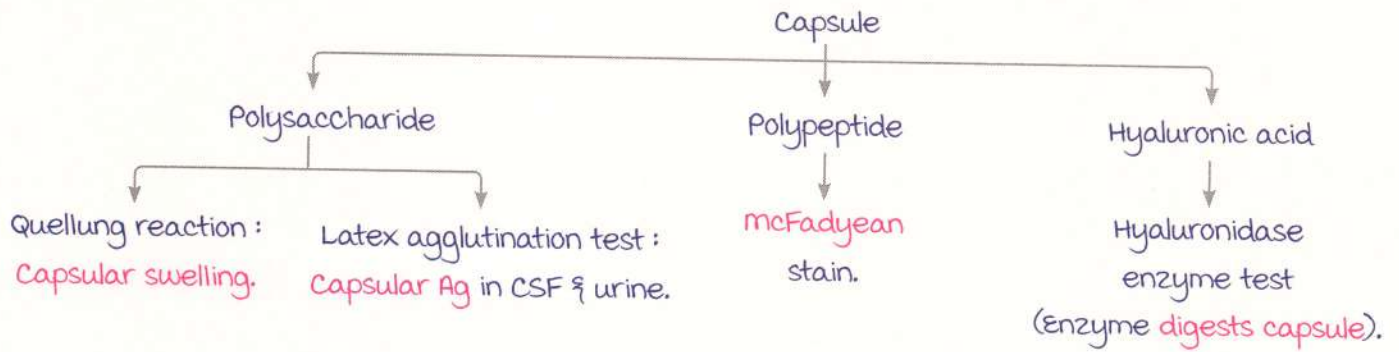
Screening test :

- For all types of capsules.
- m/c : Negative staining with India ink.
 - Stains background.
 - Low specificity.



Negative staining

----- Active space ----- Specific tests :



Cell Wall

00:09:31

Function : Protection of inner bacterial contents from external stress factors.

Cell wall deficient forms :

All bacteria have cell wall except : **mollicutes** → mycoplasma family.

Identification :

Organism	Composition
Gram positive bacteria	Peptidoglycan + lipoteichoic acid
mycobacteria	Peptidoglycan + mycolic acid
Gram negative bacteria	Peptidoglycan + lipopolysaccharide
Neisseria	Peptidoglycan + lipooligosaccharide
Fungi	Chitin

Demonstration :

- Electron microscope.
- microdissection.

Fimbria & Flagella

00:13:00

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

Fimbria/Pili :

Function :

- Organ of **attachment** :
All UTI causing bacteria : **E.coli** (m/c).
- Locomotion : Twitching motility.
E.g. : Pseudomonas, Neisseria.
- Conjugation : Using sex pili.

Demonstration :

- Hemagglutination : Fimbria → Agglutination of RBC.
- Electron microscope.

Note :

Streptococcus : Attaches to pharyngeal cells using **lipoteichoic acid**.

Flagella :

Function : Organ of **locomotion**.

Seen in : All motile bacteria showing true swimming motility.

Demonstration :

Direct :
Electron microscope.

Indirect :

Demonstration of motility :

- Bright field/light microscopy.
 - Dark field microscopy.
 - Phase contrast microscope.
- } visualisation of spirochaetes
(Thin, **corkscrew motility**) :
- Treponema.
 - Borrelia.
 - Leptospira.

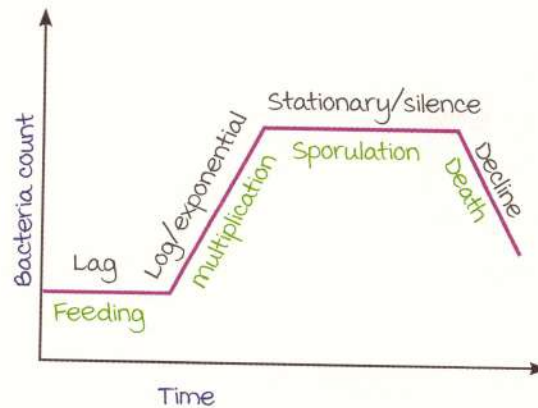
Note :

Spirochaetes (Spiral-shaped, thin bacteria) : Treponema, Borrelia & Leptospira.

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

Growth

00:20:44

Bacterial Growth Curve :**1. Lag phase :**

- Feeding $\rightarrow$ $\uparrow$ in size.
- Antimicrobial susceptibility testing (AST) done in log/exponential phase.

2. Log/exponential phase : multiplication $\rightarrow$ $\uparrow$ Bacterial numbers.**3. Stationary/silence phase :**

Unfavourable conditions (Nutrients depleted)

Sporulation : Spore producing bacteria (Clostridium & Bacillus).

OR

- Replication slows down.
- Newly forming cells = Dying cells.
- Bacterial number : **Constant**.

Unfavourable conditions persist

4. Decline phaseDeath of bacteria : $\downarrow$ in count.

MICROSCOPY & STAINING

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

Types of microscopes :

1. Bright field/light microscope (m/c).
2. Darkfield microscope.
3. Phase contrast microscope.
4. Fluorescent microscope.
5. Electron microscope.

Bright Field/Light Microscope

00:01:05

- Background : Bright.
- Drawback : Thin organisms are difficult to see.

methods of visualisation :

1. Wet mount :

- Advantage : morphology & motility seen but limited.
- Limitation : Characteristic motility not seen (Coverslip sandwiches the organism).



Light microscope

2. Staining :

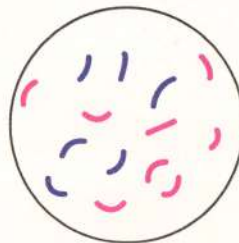
- Advantage : morphology better appreciated.
- Limitation : Organism is killed, motility not appreciated.

3. Hanging drop :

- Drop on coverslip hangs into concavity on slide.
- Advantage : **Characteristic motility** can be appreciated.



Wet mount



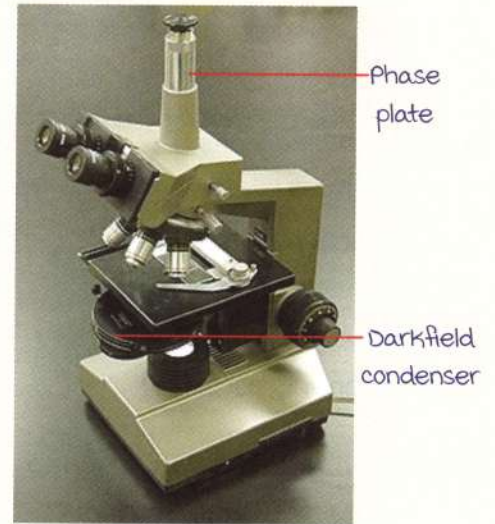
Staining

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

Darkfield & Phase Contrast Microscope

00:04:50

	Darkfield microscope	Phase contrast microscope
Feature of microscope	Darkfield condenser	Phase plate
Organism	Bright ?	Dark ?
Background	Dark	Bright
Advantage	- No staining done - Visualise thin organisms & motility - Thin organisms : <i>Spirochaetes</i> - Treponema - Borrelia - Leptospira } Corkscrew motility	



Darkfield & phase contrast microscope

Fluorescent & Electron Microscope

00:07:30

Fluorescent microscope :

Feature of microscope : uv light source.

Stain used : Fluorescent stains (*Acridine/Acidic stains*).

mechanism :



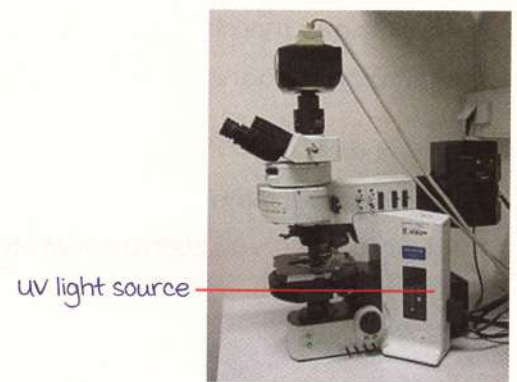
uv light (Short wavelength) → Fluorescent stained organism → Emits visible light (Long wavelength)

Advantage : Small number of organisms identified.

electron microscope :Stain used : Heavy metal stain (*Lead citrate*).

Advantage :

Very high magnification → Visualise small/thin organisms.



Fluorescent microscope