TOPIC 1

General Microbiology, Ethics & Communication

Number of competencies: 13 Competencies requiring certification: 2

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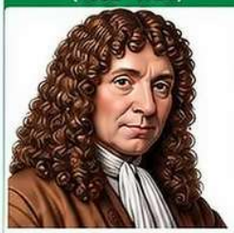
HISTORY OF MICROBIOLOGY

From Observation to Molecular Era – Milestones, Pioneers and Discoveries

1. IMPORTANT MILESTONES IN THE HISTORY OF MICROBIOLOGY

Year	Event / Discovery
1665	**Robert Hooke** – observed "cells" in cork.
1674	**Antonie van Leeuwenhoek** – first to see living microorganisms.
1796	**Edward Jenner** – first vaccine (smallpox).
1838	**Schleiden & Schwann** – cell theory.
1847	**Ignaz Semmelweis** – hand washing prevents puerperal fever.
1857	**Louis Pasteur** – microbial fermentation.
1861	**Louis Pasteur** – disproved spontaneous generation (swan neck flask).
1867	**Joseph Lister** – antiseptic surgery (carbolic acid).
1876	**Robert Koch** – proved specific causative agents of diseases.
1881	**Koch** – solid media (agar), pure culture.
1884	**Koch** – Koch's postulates.
1882	**Paul Ehrlich** – chemotherapy (Salvarsan for syphilis).
1928	**Alexander Fleming** – penicillin.
1935	**Domagk** – sulfonamides.
1944	**Avery, MacLeod & McCarty** – DNA is genetic material.
1953	**Watson & Crick** – DNA double helix.
1970	**Temin & Baltimore** – reverse transcriptase.
1977	**Sanger** – DNA sequencing.
1983	**Mullis** – PCR (polymerase chain reaction).
2000	Human Genome Project.

2. ANTONIE VAN LEEUWENHOEK (1632–1723)



- First to observe living microorganisms using simple microscopes (200–300×).
- Described bacteria, protozoa, algae, spermatozoa (e.g., "animalcules").
- Improved single-lens microscopes.



Leeuwenhoek's microscope (reconstruction)

Bacteria seen by Leeuwenhoek (artistic view)

"First to see the invisible world."

3. LOUIS PASTEUR (1822–1895)



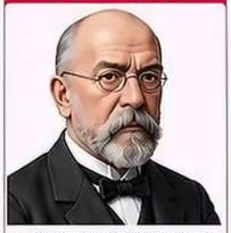
- Disproved spontaneous generation (swan neck flask).
- Microbial fermentation.
- Pasteurization.
- Attenuated vaccines (rabies, anthrax, fowl cholera).
- Germ theory of disease.
- Major contributions to microbiology and immunology.



Pasteur's swan neck flask (experiment)

"Chance favours only the prepared mind."

4. ROBERT KOCH (1843–1910)



- Discovered *Bacillus anthracis* (1876), *Mycobacterium tuberculosis* (1882), *Vibrio cholerae* (1883).
- Developed solid media (agar).
- Pure culture techniques.
- Koch's postulates.
- Introduced staining techniques.
- Nobel Prize in 1905.



Bacillus anthracis (discovered by Koch)

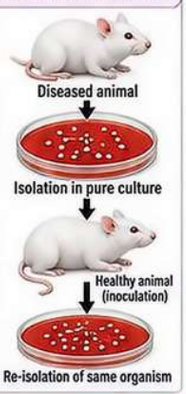


Pure culture on agar (Koch's method)

5. KOCH'S POSTULATES

To establish a microorganism as the cause of a specific infectious disease

1. The organism must be present in all cases of the disease and absent in healthy persons.
2. The organism must be isolated from the diseased host and grown in pure culture.
3. The cultured organism must produce the same disease when inoculated into a healthy, susceptible host (experimental animal).
4. The same organism must be re-isolated from the experimentally infected host and shown to be identical to the original organism.



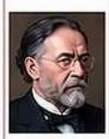
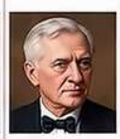
6. EXAMPLES OF KOCH'S POSTULATES

Disease	Causative organism
Anthrax	*Bacillus anthracis*
Tuberculosis	*Mycobacterium tuberculosis*
Cholera	*Vibrio cholerae*
Typhoid fever	*Salmonella Typhi*
Plague	*Yersinia pestis*
Diphtheria	*Corynebacterium diphtheriae*

7. EXCEPTIONS / LIMITATIONS OF KOCH'S POSTULATES

Situation	Reason / Example
Human-only pathogens	No suitable animal model (e.g., *Treponema pallidum* – syphilis; HIV; *Mycobacterium leprae* – leprosy).
Cannot be cultured in vitro	Obligate intracellular organisms (e.g., viruses, *Chlamydia*, *Rickettsia*).
Carrier state	Organism may be present in healthy carriers (e.g., *V. cholerae*, *S. Typhi*).
Multiple etiologies	Diseases with multiple causes (e.g., pneumonia, diarrhoea).
Specific host requirement	Narrow host range (e.g., *Bordetella pertussis*).
Chronic/latent infections	Organism may not be detectable in all stages (e.g., tuberculosis).
Ethical constraints	Cannot satisfy by experimental infection in humans.

8. NOBEL LAUREATES IN MICROBIOLOGY (Selected)

1905 Robert Koch	1908 Ilya Mechnikov	1928 Alexander Fleming	1939 Gerhard Domagk	1944 Oswald Avery	1965 François Jacob & André Lwoff	1970 Howard Temin & David Baltimore	1983 Kary Mullis	1908 Harald zur Hausen	2015 William Campbell & Satoshi Ōmura	2020 Harvey Alter, Michael Houghton & Charles Rice
										
Studies on tuberculosis	Phagocytosis and immunity	Discovery of penicillin	Sulfonamides (antibacterial drugs)	DNA is the genetic material	Genetic control of viral replication	Reverse transcriptase	PCR	Human papillomavirus and cancer	Avermectins (antiparasitic drugs)	Hepatitis C virus



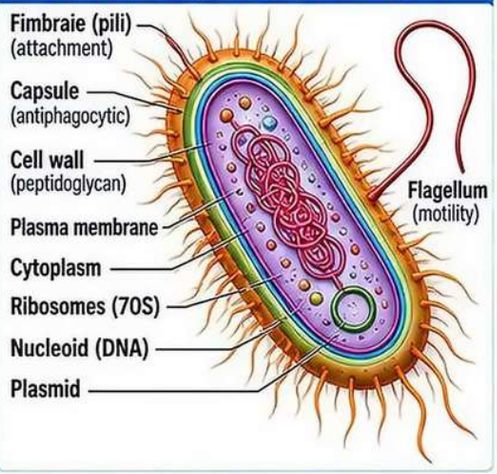
"Great minds, tiny organisms, a healthier world."

Microbes may be invisible, but their impact is immense.

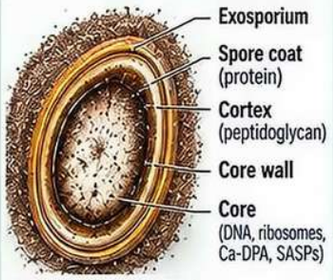
BACTERIAL CELL, SPORE, CLASSIFICATION AND GENETIC EXCHANGE

Structure • Comparisons • Cell Division • Gram Differentiation • Horizontal Gene Transfer • Examples

1. STRUCTURE OF A BACTERIAL CELL



2. BACTERIAL ENDOSPORE



Endospore of *Bacillus* (SEM)

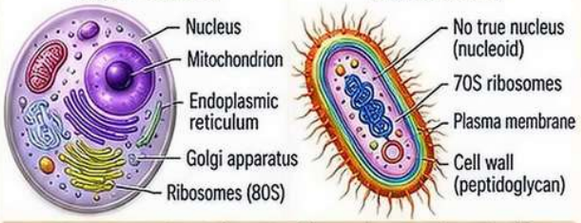
Key features

- Thick protective layers
- Highly resistant to heat, chemicals, UV and desiccation
- Metabolically inactive
- Germinates to vegetative cell in favourable conditions

3. VEGETATIVE CELL vs ENDOSPORE

Feature	Vegetative cell	Endospore
Metabolic activity	Active	Dormant
Size	Larger	Smaller
Water content	High	Very low
Heat/chemical resistance	Less resistant	Highly resistant
Structure	Cell wall + membrane	Multiple protective layers
Function	Growth and multiplication	Survival in adverse conditions
Staining	Stains easily	Requires special spore stain (malachite green, heat)
Examples	*E. coli, Staphylococcus*	*Bacillus, Clostridium*

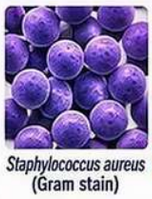
4. EUKARYOTIC vs PROKARYOTIC CELL



Feature	Eukaryotic cell	Prokaryotic cell
Size	10–100 µm	0.5–5 µm
Nucleus	True nucleus	No true nucleus
DNA	Linear, multiple chromosomes	Circular, single chromosome (+ plasmids)
Membrane-bound organelles	Present	Absent
Ribosomes	80S	70S
Cell wall	Absent (except fungi)	Present (peptidoglycan)
Cell division	Mitosis	Binary fission
Examples	Human, fungi, protozoa	Bacteria, archaea

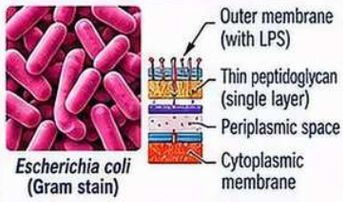
5. GRAM POSITIVE vs GRAM NEGATIVE BACTERIA

Gram Positive (G⁺)



Staphylococcus aureus
(Gram stain)

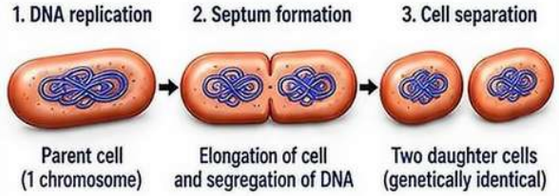
Gram Negative (G⁻)



Escherichia coli
(Gram stain)

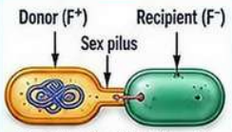
Feature	Gram Positive	Gram Negative
Gram stain colour	Purple (retains crystal violet)	Pink (takes safranin)
Peptidoglycan layer	Thick (20–80 nm)	Thin (2–7 nm)
Teichoic acids	Present	Absent
Outer membrane	Absent	Present (with LPS, porins)
Lipid content	Low	High
Periplasmic space	Minimal	Well developed
Sensitivity to penicillin	Generally more sensitive	Less sensitive (due to outer membrane)
Examples	*Staphylococcus, Streptococcus, Enterococcus, Bacillus, Clostridium*	*Escherichia coli, Klebsiella, Pseudomonas, Salmonella, Neisseria*

6. BACTERIAL CELL DIVISION (BINARY FISSION)



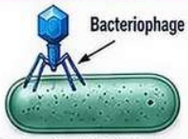
7. HORIZONTAL GENE TRANSFER IN BACTERIA

A. CONJUGATION



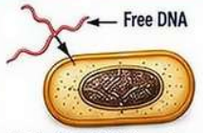
- Direct transfer of DNA (plasmid) through sex pilus
- Requires cell-to-cell contact.
- Example: Transfer of R plasmid (antibiotic resistance).

B. TRANSDUCTION



- Transfer of bacterial DNA by phages Generalized or specialized transduction.
- Example: Transfer of toxin genes in *Corynebacterium diphtheriae.*

C. TRANSFORMATION



- Uptake of naked DNA from environment Natural (e.g., *Streptococcus pneumoniae*) or artificial (laboratory)
- Example: Uptake of capsular gene in *S. pneumoniae* (Griffith's experiment).

8. EXAMPLES OF BACTERIAL SPECIES

Category	Examples
Spore-forming bacteria	*Bacillus anthracis, Bacillus subtilis, Clostridium tetani, Clostridium botulinum*
Non-spore-forming G⁺ cocci	*Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis*
Non-spore-forming G⁻ bacilli	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella Typhi*
Other	*Mycobacterium tuberculosis* (acid-fast), *Vibrio cholerae* (curved), *Neisseria gonorrhoeae* (diplococci)

*"Small organisms,
Big discoveries,
Bigger impact on humanity."*

NUTRITION OF BACTERIA

Bacteria obtain nutrients from their environment for energy, carbon, nitrogen and growth factors.

1. CARBON SOURCE

- **Autotrophs:** Use CO_2 (rare in medical bacteria, e.g., *Nitrosomonas*)
- **Heterotrophs:** Use organic carbon compounds (most pathogenic bacteria)



Escherichia coli (heterotroph)

2. ENERGY SOURCE

- **Phototrophs:** Light energy (e.g., some non-pathogenic bacteria)
- **Chemotrophs:** Chemical energy from oxidation of organic or inorganic compounds (most pathogenic bacteria)



Staphylococcus aureus (chemoheterotroph)

3. NITROGEN SOURCE

- **Organic nitrogen:** Amino acids, peptides, proteins (most bacteria)
- **Inorganic nitrogen:** Ammonia (NH_3), nitrate (NO_3^-), nitrite (NO_2^-) (some bacteria)



Salmonella enterica (uses organic and inorganic nitrogen)

4. SULFUR, PHOSPHORUS AND MINERAL IONS

- Required for synthesis of proteins, nucleic acids and cell wall.
- **Sources:** SO_4^{2-}, PO_4^{3-}, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}/Fe^{2+}, Mn^{2+}, Zn^{2+}, etc.



Helicobacter pylori (requires multiple ions)

5. GROWTH FACTORS (VITAMINS)

- Require preformed growth factors such as:
  - B-complex vitamins
  - NAD (V factor)
  - Haemin (X factor)
  - Amino acids
  - Purines and pyrimidines



Haemophilus influenzae (requires X and V factors)

6. WATER

- Essential for all metabolic reactions.
- Optimum water activity (a_w): 0.98–0.99.



PATHOGENESIS OF BACTERIAL DISEASES

Bacteria cause disease by colonization, invasion and production of toxins, leading to tissue damage and host immune response.

1. ENTRY AND COLONIZATION

- Entry through skin, respiratory tract, GI tract or urogenital tract.
- Adherence to epithelial cells using adhesins (fimbriae, pili, MSCRAMMs).



Adherence of bacteria to epithelial cells

2. INVASION

- Enzymes (hyaluronidase, collagenase, coagulase, streptokinase) help in tissue spread.
- Some bacteria survive and multiply intracellularly (e.g., *Mycobacterium*, *Listeria*).



Tissue invasion by bacteria

3. TOXIN PRODUCTION

- **Exotoxins:** secreted proteins causing specific effects.
- **Endotoxin:** LPS of Gram-negative bacteria causing inflammation, fever, shock.



Release of toxins (exotoxin)

4. HOST RESPONSE

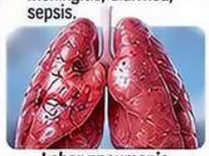
- **Innate immunity:** complement activation, phagocytosis, cytokines.
- **Inflammation:** redness, heat, swelling, pain.



Neutrophil phagocytosing bacteria

5. TISSUE DAMAGE AND CLINICAL DISEASE

- Direct cytotoxic effect of toxins.
- Tissue necrosis and organ dysfunction.
- **Examples:** pneumonia, meningitis, diarrhea, sepsis.



Lobar pneumonia (tissue damage)

6. SPREAD AND COMPLICATIONS

- Local spread (abscess, cellulitis).
- Hematogenous spread (septicemia, metastatic infections).
- Toxin-mediated systemic effects (toxic shock, DIC, organ failure).

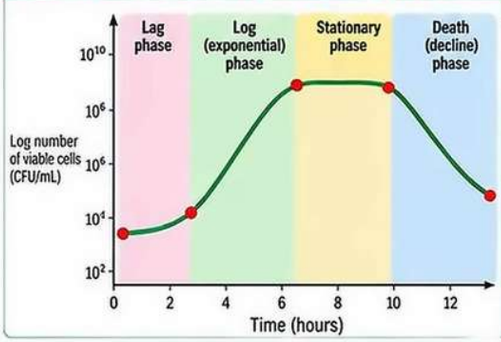


Bacteria in bloodstream (septicemia)

EXOTOXIN vs ENDOTOXIN

Feature	EXOTOXIN	ENDOTOXIN
Chemical nature	Protein (polypeptide)	Lipid A component of LPS
Produced by	Mainly Gram-positive bacteria (some Gram-negative)	Only Gram-negative bacteria
Location	Secreted outside the bacterial cell	Part of outer cell wall; released on cell lysis or shedding of outer membrane vesicles
Potency	Highly potent (active in very small amounts)	Less potent (requires larger amounts)
Heat stability	Heat labile (destroyed at 60–80°C)	Heat stable (withstands 100°C for 1 hour)
Antigenicity	Antigenic (induces neutralizing antibodies → antitoxin)	Weakly antigenic (does not induce neutralizing antibodies)
Specific effects	Specific effects (neurotoxic, enterotoxic, cytotoxic, etc.)	Non-specific effects (fever, inflammation, shock, DIC)
Specid formation	Can be converted to toxoids (e.g., diphtheria, tetanus vaccines)	Cannot be converted to toxoids
Examples	Diphtheria toxin, Tetanus toxin, Botulinum toxin, Cholera toxin, Shiga toxin, Streptococcal pyrogenic exotoxins	LPS of *E. coli*, *Salmonella*, *Shigella*, *Neisseria*, *Pseudomonas*, etc.
Clinical effects	Specific clinical syndromes	Endotoxic effects: fever, hypotension, septic shock, DIC

BACTERIAL GROWTH CURVE Phases, Characteristics and Examples



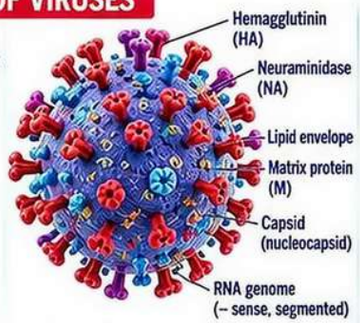
Phase	Characteristics	Physiological state	Examples / Salient features
Lag phase	• No increase in cell number Adaptation to new environment • Synthesis of enzymes and essential metabolites	Metabolically active but not dividing	• *E. coli* in fresh medium needs 1–2 hours. • Duration depends on inoculum size and medium.
Log (exponential) phase	• Rapid and constant doubling time • Maximum metabolic activity • Cells are most susceptible to antibiotics	Active cell division	• *E. coli* doubling time ~20 min. • Best phase for biochemical studies and antibiotic sensitivity testing.
Stationary phase	• Rate of growth = rate of death • Nutrient depletion and waste accumulation • Production of secondary metabolites (e.g., toxins, pigments, spores)	Stress response	• Production of diphtheria toxin (*C. diphtheriae*), antibiotics (e.g., *Streptomyces*), sporulation (*Bacillus*, *Clostridium*).
Death (decline) phase	• Decrease in viable count (log linear) • Cell lysis due to toxic products and nutrient exhaustion	Non-viable cells increase	• After prolonged incubation; spore formers may survive longer.

"Small organisms, big discoveries, bigger impact on humanity."

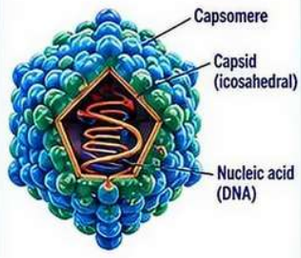


1. GENERAL PROPERTIES OF VIRUSES

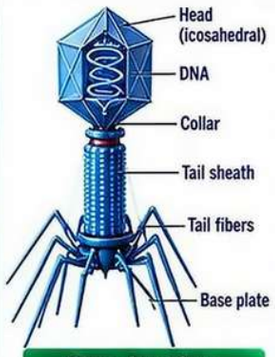
- **Ultramicroscopic:** 20–300 nm
- **Acellular:** no cytoplasm, ribosomes or organelles
- **Obligate intracellular parasites**
- **Genome:** either DNA or RNA (single or double stranded, + or – sense)
- **Protein coat:** capsid (made of capsomeres)
- **Nucleocapsid:** genome + capsid
- **Envelope:** present in some viruses with lipid bilayer and spikes
- **Metabolically inert** outside host cell
- **Replicates** by synthesis of components and assembly (not by binary fission).
- Show host range, tissue tropism and cellular receptors
- Can be crystallized
- Sensitive to heat, UV light, desiccation, solvents and disinfectants
- **High mutation rate** (especially RNA viruses)
- **Antigenic variation** (drift and shift)
- Adaptation and recombination common
- Cause cytopathic effects and inclusion bodies



Enveloped virus (Influenza)



Non-enveloped virus (Adenovirus)



Complex virus (Bacteriophage T4)

2. BALTIMORE CLASSIFICATION OF VIRUSES (DAVID BALTIMORE)

Group	Genome type	mRNA synthesis	Replication strategy (brief)	Representative viruses
I	dsDNA	Transcription	DNA → mRNA (host/viral RNA pol)	Adenovirus, Herpesvirus, Poxvirus, Papillomavirus, Polyomavirus
II	ssDNA	→ dsDNA	ssDNA → dsDNA → mRNA	Parvovirus, Circovirus
III	dsRNA	Transcription	Viral RdRp makes mRNA	Reovirus, Rotavirus
IV	(+) ssRNA	Acts as mRNA	Genome itself acts as mRNA; translated to RdRp for replication	Picornavirus, Flavivirus, Coronavirus, Togavirus, Calicivirus
V	(–) ssRNA	Transcription	Viral RdRp synthesizes mRNA from (–) RNA genome	Orthomyxovirus (Influenza), Paramyxovirus, Rhabdovirus, Filovirus
VI	(+) ssRNA-RT	Reverse transcription	RNA → DNA (reverse transcriptase) → integrated as provirus	Retrovirus (HIV, HTLV)
VII	dsDNA-RT	Reverse transcription	Partially dsDNA → RNA (pregenomic) → reverse transcription	Hepadnavirus (HBV)

3. ENVELOPED vs NON-ENVELOPED VIRUSES

Enveloped viruses (Examples)

- Influenza virus (Orthomyxovirus)
- Coronavirus (SARS-CoV-2)
- HIV (Retrovirus)
- Herpes simplex virus (Herpesvirus)
- Hepatitis B virus (Hepadnavirus)
- Measles virus (Paramyxovirus)
- Mumps virus (Paramyxovirus)
- Rabies virus (Rhabdovirus)
- Ebola virus (Filovirus)
- Respiratory syncytial virus (Paramyxovirus)



Influenza virus (enveloped)



SARS-CoV-2 (enveloped)

Non-enveloped viruses (Examples)

- Adenovirus
- Rhinovirus
- Poliovirus
- Norovirus
- Rotavirus
- Hepatitis A virus
- Parvovirus
- Reovirus
- Human papillomavirus (HPV)
- Enterovirus
- Calicivirus



Adenovirus (non-enveloped)

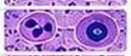
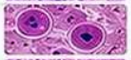
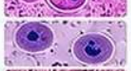


Poliovirus (non-enveloped)

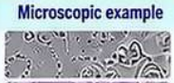
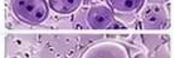
4. INTERFERONS: TYPES AND PROPERTIES

Type	Main source	Receptors	Key properties	Examples / Use
Type I (α, β, ω, ε, κ)	Most nucleated cells (α – leukocytes, β – fibroblasts)	IFNAR (IFN-α/β receptor)	Induce antiviral state, increase MHC I, activate NK cells, inhibit viral replication	IFN-α (leukocyte), IFN-β (fibroblast) Used in HBV, HCV, some cancers
Type II (γ)	Activated T cells (Th1), NK cells	IFNGR (IFN-γ receptor)	Activates macrophages, increases MHC I & II, enhances antigen presentation, anti-viral and immunomodulatory	IFN-γ Used in chronic granulomatous disease and some cancers
Type III (λ1, λ2, λ3)	Epithelial cells (mucosal surfaces)	IFNLR (IL-28/29 receptor)	Antiviral effect at mucosal surfaces (respiratory, gut), less systemic toxicity	IFN-λ (pegylated) Under study/used in HCV

5. INCLUSION BODIES IN VIRAL INFECTIONS

Inclusion body	Site	Associated virus(es)	Microscopic image
Negri bodies	Cytoplasm (neurons)	Rabies virus	
Cowdry type A	Nucleus	Herpes simplex virus, Varicella zoster virus	
Cowdry type B	Nucleus	Adenovirus	
Guarnieri bodies	Cytoplasm	Poxvirus (Variola, Vaccinia)	
Henderson–Patterson bodies	Cytoplasm	Molluscum contagiosum virus	
"Owl's eye" inclusions	Nucleus	Cytomegalovirus (CMV)	
Happy face (HP)	Nucleus	Parainfluenza virus	

6. CYTOPATHIC EFFECTS (CPE) OF VIRUSES

CPE type	Description	Associated virus(es)	Microscopic example
Cell rounding	Cells become rounded and refractile	Enterovirus, Rhinovirus	
Syncytia	Multinucleated giant cells	Measles virus, RSV, HSV	
Ballooning degeneration	Swollen, vacuolated cells	Varicella zoster virus	
Lysis and cell death	Destruction of infected cells	Adenovirus	
Plaque formation	Clear zones due to cell lysis	Many viruses (e.g. HSV, Influenza)	

7. LABORATORY DIAGNOSIS OF VIRUSES

Method	Details
Specimens	Respiratory samples (nasal/throat swab, aspirate), blood/serum, CSF, stool, urine, vesicle/lesion swab, tissue biopsy/autopsy
Direct detection	Nucleic acid detection (RT-PCR, real-time PCR, multiplex PCR, NGS), Antigen detection (rapid test, ELISA, immunofluorescence), Electron microscopy
Virus isolation (culture)	Cell lines: Vero, HeLa, MDCK, HEp-2, MRC-5; Look for CPE (rounding, syncytia, ballooning, lysis); Embryonated egg (e.g., influenza); Shell vial culture (rapid)
Serology	IgM and IgG antibodies (ELISA, CLIA), Paired serum (fourfold rise), Neutralization test, Hemagglutination inhibition (e.g., influenza), Complement fixation test, Virus-specific rapid antibody tests
Other methods	Immunohistochemistry, In situ hybridization, Electron microscopy (negative staining), Sequencing and genotyping, Viral load estimation

CLASSIFICATION OF FUNGI

Based on Morphology, Reproduction and Clinical Relevance

1. KEY FEATURES OF FUNGI

- Eukaryotic, non-photosynthetic, heterotrophic organisms.
- Rigid cell wall containing chitin, glucans and mannans.
- Exist as moulds (filamentous) or yeasts (unicellular).
- Reproduce by asexual and sexual spores.
- Ubiquitous in nature — soil, water, decaying organic matter, on plants and animals.
- Can cause superficial, cutaneous, subcutaneous and systemic mycoses.
- Opportunistic fungi cause disease in immunocompromised hosts.

2. MORPHOLOGICAL FORMS

Yeast (unicellular)



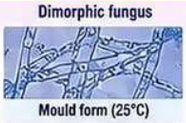
Budding yeast cells (*Candida albicans*)

Mould (filamentous)



Septate hyphae (*Aspergillus* spp.)

Dimorphic fungus



Mould form (25°C)



Yeast form (37°C) *Histoplasma capsulatum*

3. REPRODUCTION IN FUNGI

Asexual reproduction

- Conidia (exogenous)
- Sporangiospores (endogenous)
- Arthrospores
- Blastoconidia (budding)
- Chlamydospores

Sexual reproduction

- Zygospores
- Ascospores
- Basidiospores
- Oospores

Special structures

- Sclerotia (survival structures)
- Appressoria (for host penetration)

4. CLASSIFICATION OF FUNG (Medically Important)

Group (Phylum)	Main Features	Examples	Diseases
Chytridiomycota	• Aseptate or few septate hyphae • Motile zoospores (single posterior flagellum)	*Batrachochytrium dendrobatidis*	Chytridiomycosis (mainly amphibians)
Zygomycota	• Aseptate hyphae • Sporangiospores in sporangia • Zygospores (sexual)	*Rhizopus, Mucor, Lichtheimia (Absidia), Cunninghamella, Rhizomucor*	Mucormycosis (zygomycosis)
Ascomycota	• Septate hyphae • Ascospores in ascus (sexual) • Conidia (asexual)	*Aspergillus, Penicillium, Histoplasma, Coccidioides, Blastomyces, Paracoccidioides, Candida* (teleomorphs)	Aspergillosis, Penicilliosis, Histoplasmosis, Coccidioiidmycosis, Blastomycosis, Paracoccidioidomycosis
Basidiomycota	• Septate hyphae • Basidiospores on basidium	*Cryptococcus neoformans*	Cryptococcosis
Deuteromycota (Fungi imperfecti)	• Asexual reproduction only (conidia) • Many important pathogens	*Trichophyton, Microsporum, Epidermophyton, Fusarium, Alternaria, Curvularia, Acremonium,*	Dermatophytosis, keratitis, onychomycosis, mycetoma, opportunistic infections

5. IMPORTANT MORPHOLOGICAL STRUCTURES



Sporangium with sporangiospores (*Rhizopus*)



Conidiophore with with conidia (*Aspergillus*)



Brush-like conidia (*Penicillium*)



Arthroconidia (*Coccidioides*)



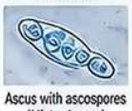
Chlamydospores (*Candida*)



Zygospore (*Rhizopus*)



Basidium with basidiospores (*Cryptococcus*)



Ascus with ascospores (*Histoplasma*)



Sclerotic bodies (*Sclerotium*)

LABORATORY DIAGNOSIS OF MEDICALLY IMPORTANT FUNGI

1. SPECIMENS

- Skin scrapings, hair, nail clippings
- Pus, tissue biopsy, ulcer swab
- Sputum, BAL, bronchial washings
- CSF, blood, bone marrow
- Urine, other sterile body fluids
- Use sterile, leak-proof containers



Skin scrapings (scalp/nail)



Sputum



Tissue biopsy

2. DIRECT MICROSCOPY

- 10–20% KOH mount (with/without calcofluor white)
- Fungal elements: hyphae, pseudohyphae, yeasts, spores
- Gram stain (yeasts)
- India ink (Cryptococcus)
- Lactophenol cotton blue (LPCB) for morphology



KOH mount Septate hyphae (*Aspergillus*)



KOH mount Broad hyphae (*Rhizopus*)



India ink Encapsulated yeast (*Cryptococcus*)



Gram stain Budding yeast cells (*Candida*)

3. CULTURE

- Sabouraud Dextrose Agar (SDA) (pH 5.6) with antibiotics (chloramphenicol, cycloheximide)
- Incubate at 25–30°C (moulds) and 37°C (dimorphic/yeasts)
- Observe colony morphology, reverse pigment
- Subculture for purity
- For dimorphic fungi: incubate at both 25°C and 37°C



Candida Creamy colonies | Aspergillus Green colony | Rhizopus Rapid, fluffy colony | Trichophyton Powdery colony

4. HISTOPATHOLOGY

- H&E: tissue reaction, fungal elements
- Special stains: PAS (magenta), GMS (black). Gridley, mucicarmine (Cryptococcus)



PAS stain (*Aspergillus* hyphae)



GMS stain (Fungal elements)

5. ANTIGEN / ANTIBODY DETECTION

- Cryptococcal antigen (CSF/serum) – latex agglutination or lateral flow assay
- Galactomannan (Aspergillus) – serum/BAL
- (1→3)-β-D-glucan – panfungal marker
- Histoplasma antigen (urine/serum) – EIA
- Coccidioides antibody – serology (ID, CF, EIA)



Cryptococcal Ag (latex test) | Negative



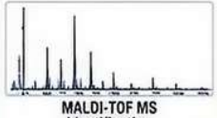
Aspergillus Galactomannan (ELISA)

6. MOLECULAR METHODS

- PCR for species identification (e.g., *Candida, Aspergillus, Mucorales*)
- Real-time PCR for early diagnosis
- Useful in invasive and disseminated infections
- MALDI-TOF MS from culture



PCR result (*Candida* spp.)



MALDI-TOF MS identification

7. COMMON LABORATORY FINDINGS

Fungus	Key microscopic / culture finding
Candida	Budding yeast cells, pseudohyphae; creamy colonies
Aspergillus	Septate hyphae, acute angle branching (~45°); colored colonies
Mucorales (Rhizopus/Mucor)	Broad, aseptate hyphae, right angle branching; fluffy colonies
Cryptococcus	Encapsulated yeast (India ink +); mucoid colonies
Histoplasma	Small intracellular yeasts (2–4 µm); dimorphic
Coccidioides	Spherules with endospores
Dermatophytes	Septate hyphae with arthroconidia; slow growing, powdery colonies

8. KEY POINTS

- Correlation of clinical, microbiological and histopathological findings is essential.
- Use multiple methods for definitive diagnosis.
- Molecular and antigen tests help in early and non-invasive diagnosis.
- Repeat sampling may be needed.
- Species identification is important for appropriate therapy.

9. BIOSAFETY

- Handle cultures in Biosafety Cabinet (Class II).
- Use PPE (gloves, mask, lab coat).
- Some moulds produce airborne spores — risk of laboratory-acquired infection.
- Proper waste disposal and decontamination.

Early and accurate laboratory diagnosis of fungal infections guides timely and appropriate antifungal therapy.


Ascaris lumbricoides
(intestinal nematode)

CLASSIFICATION OF NEMATODES

Based on Habitat, Reproduction, Periodicity, Tail-tip Nuclei, Sheath and Common Examples


Wuchereria bancrofti
(tissue/blood nematode)

1. ACCORDING TO SITE OF HABITAT (LOCATION IN HOST)

Type	Site	Examples	Common diseases	Representative image
Intestinal nematodes	Live in intestine	*Ascaris lumbricoides* *Enterobius vermicularis* *Trichuris trichiura* *Ancylostoma duodenale*	Ascariasis Enterobiasis Trichuriasis Hookworm infection	
Tissue nematodes	Live in tissues or organs other than intestine	*Wuchereria bancrofti* *Loa loa* *Onchocerca volvulus* *Dracunculus medinensis*	Filariasis Loiasis Onchocerciasis Dracunculiasis	
Blood & lymph nematodes	Live in blood or lymphatic system	*Wuchereria bancrofti* *Brugia malayi* *Loa loa*	Lymphatic filariasis Loiasis	
Subcutaneous nematodes	Live in subcutaneous tissue	*Loa loa* *Onchocerca volvulus*	Loiasis Onchocerciasis	
Cavity nematodes	Live in body cavities	*Dracunculus medinensis*	Dracunculiasis	

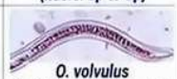
2. ACCORDING TO MODE OF REPRODUCTION

Type	Description	Examples	Illustration
Oviparous (Egg laying)	Females lay eggs which are passed in faeces or deposited outside the body.	*Ascaris lumbricoides* *Enterobius vermicularis* *Trichuris trichiura*	 Ascaris egg
Ovoviviparous	Eggs hatch inside female; larvae are born alive and passed.	*Strongyloides stercoralis* *Trichinella spiralis*	 Larva (Strongyloides)
Viviparous	Larvae develop in uterus and are born alive. No egg stage in peripheral blood.	*Wuchereria bancrofti* *Brugia malayi* *Loa loa*	 Microfilariae (*W. bancrofti*)

3. ACCORDING TO PERIODICITY OF MICROFILARIAE IN BLOOD

Type	Periodicity	Features	Vectors	Examples	Illustration
Diurnal (periodic)	Present in peripheral blood during daytime	Daytime	Aedes Culex Chrysops	*Loa loa*	 *Loa loa* (day blood)
Nocturnal (periodic)	Present in peripheral blood during night	Night	Mansonia Anopheles Culex	*Wuchereria bancrofti* *Brugia malayi*	 *W. bancrofti* (night blood)
Non-periodic (aperiodic)	Present throughout 24 hours	24 hours	Simulium Culicoides	*Onchocerca volvulus* *Mansonella spp.*	 *O. volvulus* (aperiodic)

4. ACCORDING TO TAIL-TIP NUCLEI (IN MICROFILARIAE)

Type	Tail tip appearance	Examples	Illustration
1. Sheathed, nuclei absent at tip	Sheathed	*Wuchereria bancrofti* *Brugia malayi*	 *W. bancrofti* (tail tip without nuclei)
2. Sheathed, nuclei reach end	Sheathed	*Loa loa*	 *Loa loa* (nuclei up to tip)
3. Unsheathed, nuclei absent	Unsheathed	*Onchocerca volvulus*	 *O. volvulus* (tail tip without nuclei)
4. Unsheathed, nuclei present to end	Unsheathed	*Mansonella perstans* *M. ozzardi* *M. streptocerca*	 *Mansonella perstans* (tail tip with nuclei)

5. TAIL-TIP NUCLEI OF IMPORTANT MICROFILARIAE (DETAILED)

Species	Sheath	Tail tip nuclei	No. of nuclei at tail tip	Vector	Illustration
W. bancrofti	Present	Absent	0	Culex	
B. malayi	Present	Present	2	Mansonia	
Loa loa	Present	Present (reach end)	Many	Chrysops	
O. volvulus	Absent	Absent	0	Simulium	
M. perstans	Absent	Present	Many	Culicoides	
M. ozzardi	Absent	Absent	0	Culicoides / Simulium	
M. streptocerca	Absent	Present	Many	Culicoides	

6. ACCORDING TO PRESENCE OR ABSENCE OF SHEATH

Sheathed microfilariae

- Body covered by a delicate proteinaceous sheath.
- **Examples:**
  - Wuchereria bancrofti
  - Brugia malayi
  - Loa loa


W. bancrofti (sheathed)

Unsheathed microfilariae

- Body without sheath.
- **Examples:**
  - *Onchocerca volvulus*
  - *Mansonella perstans*
  - *Mansonella ozzardi*
  - *Mansonella streptocerca*


O. volvulus (unsheathed)

Understand the Classification • Identify the Key Features • Correlate with Morphology • Helpful for MBBS Exams